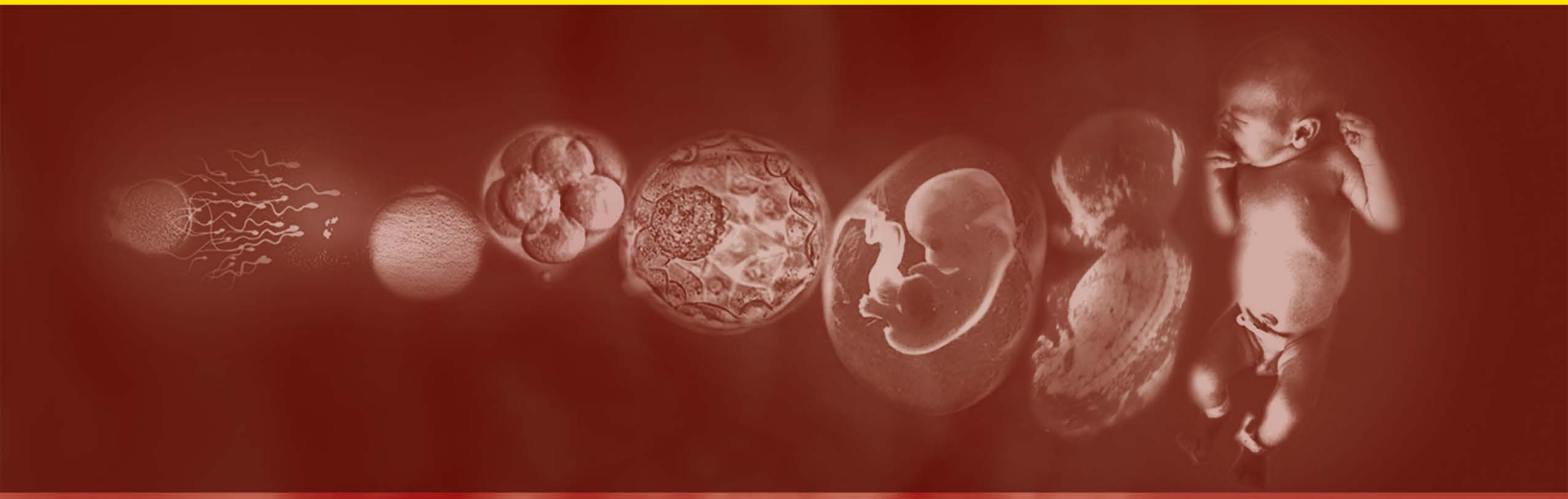
FIFTH EDITION

TEXTBOOK OF ASSISTED REPRODUCTIVE TECHNIQUES

VOLUME 2: CLINICAL PERSPECTIVES



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Textbook of Assisted Reproductive Techniques

Fifth Edition

Volume 2: Clinical Perspectives

The editors would like to dedicate this edition to the late Professor Robert G. Edwards and the late Queenie V. Neri.

The editors (from left to right: Ariel Weissman, David K Gardner, Zeev Shoham, Colin M Howles) at the annual meeting of ESHRE, Geneva, July 2017.

Textbook of Assisted Reproductive Techniques

Fifth Edition

Volume 2: Clinical Perspectives

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The beginnings of human *in vitro* fertilization

ROBERT G. EDWARDS

In vitro fertilization (IVF) and its derivatives in preimplantation diagnosis, stem cells, and the ethics of assisted reproduction continue to attract immense attention scientifically and socially. All these topics were introduced by 1970. Hardly a day passes without some public recognition of events related to this study, and clinics spread ever further worldwide. Now that we must be approaching 1.5 million IVF births, it is time to celebrate what has been achieved by so many investigators, clinical, scientific, and ethical.

While much of this "Introduction" chapter covers the massive accumulation of events between 1960 and 2000, it also briefly discusses new perspectives emerging in the twenty-first century. Fresh advances also increase curiosity about how these fields of study began and how their ethical implications were addressed in earlier days. As for me, I am still stirred by recollections of those early days. Foundations were laid in Edinburgh, London, and Glasgow in the 1950s and early 1960s. Discoveries made then led to later days in Cambridge, working there with many PhD students. It also resulted in my working with Patrick Steptoe in Oldham. Our joint opening of Bourn Hall in 1980, which became the largest IVF clinic of its kind at the time, signified the end of the beginning of assisted human conception and the onset of dedicated applied studies.

INTRODUCTION

First of all, I must express in limited space my tributes to my teachers, even if inadequately. These include investigators from far-off days when the fundamental facts of reproductive cycles, surgical techniques, endocrinology, and genetics were elicited by many investigators. These fields began to move in the twentieth century, and if one pioneer of these times should be saluted, it must be Gregory Pincus. Famous for the contraceptive pill, he was a distinguished embryologist, and part of his work dealt with the maturation of mammalian oocytes *in vitro*. He was the first to show how oocytes aspirated from their follicles would begin their maturation *in vitro*, and how a number matured and expelled a first polar body. I believe his major work was done in rabbits, where he found that the 10–11-hour timings of maturation *in vitro* accorded exactly with those occurring *in vivo* after an ovulatory stimulus to the female rabbit.

Pincus et al. also studied human oocytes (1). Extracting oocytes from excised ovaries, they identified chromosomes in a large number of oocytes and interpreted this as evidence of the completion of maturation *in vitro*. Many oocytes possessed chromosomes after 12 hours, with the proportion remaining constant over the next 30 hours and longer. Twelve hours was taken as the period of maturation. Unfortunately, chromosomes were not classified for their meiotic stage. Maturing oocytes would be expected to display diakinesis or metaphase I chromosome pairs. Fully mature oocytes would display metaphase II chromosomes, signifying they were fully ripe and ready for fertilization. Nevertheless, it is well known that oocytes can undergo atresia in the ovary, involving the formation of metaphase II chromosomes in many of them. These oocytes complicated Pincus' estimates, even in controls, and were the source of his error, which led later workers to inseminate human oocytes 12 hours after collection and culture *in vitro* (2,3). Work on human fertilization *in vitro*, and indeed comparable studies in animals, remained in abeyance from then and for many years. Progress in animal IVF had also been slow. After many relatively unsuccessful attempts in several species in the 1950s and 1960s, a virtual dogma arose that spermatozoa had to spend several hours in the female reproductive tract before acquiring the potential to bind to the zona pellucida and achieve fertilization. In the late 1960s, Austin and Chang independently determined the need for sperm capacitation, identified by a delay in fertilization after spermatozoa had entered the female reproductive tract (4,5). This discovery was taken by many investigators as the reason for the failure to achieve fertilization *in vitro*, and why spermatozoa had to be exposed to secretions of the female reproductive tract. At the same time, Chang reported that rabbit eggs that had fully matured *in vitro* failed to produce normal blastocysts, with none of them implanting normally (6).

MODERN BEGINNINGS OF HUMAN IVF, PREIMPLANTATION GENETIC DIAGNOSIS, AND EMBRYO STEM CELLS

My PhD began at the Institute of Animal Genetics, Edinburgh University, in 1952, encouraged by Professor Conrad Waddington, the inventor of epigenesis, and supervised by Dr. Alan Beatty. At the time, capacitation was gaining in significance. My chosen topic was the genetic control of early mammalian embryology, specifically the growth of preimplantation mouse embryos with altered chromosome complements.

Achieving these aims included a need to expose mouse spermatozoa to x-rays, ultraviolet light, and various chemicals *in vitro*. This would destroy their chromatin and prevent them from making any genetic contribution

to the embryo, hopefully without impairing their capacity to fertilize eggs *in vivo*. Resulting embryos would become gynogenetic haploids. Later, my work changed to exposing ovulated mouse oocytes to colchicine *in vivo* in order to destroy their second meiotic spindle *in vivo*. This treatment freed all chromosomes from their attachment to the meiotic spindle, and they then became extruded from the egg into tiny artificial polar bodies. The fertilizing spermatozoon thus entered an empty egg, which resulted in the formation of androgenetic haploid embryos with no genetic contribution from the maternal side. For three years, my work was concentrated in the mouse house, working at midnight to identify mouse females in estrus by vaginal smears, collecting epididymal spermatozoa from males, and practicing artificial insemination with samples of treated spermatozoa. This research was successful, as mouse embryos were identified with haploid, triploid, tetraploid, and aneuploid chromosomes. Moreover, the wide range of scientific talent in the Institute made it a perfect place for fresh collaborative studies. For example, Julio Sirlin and I applied the use of radioactive DNA and RNA precursors to the study of spermatogenesis, spermiogenesis, fertilization, and embryogenesis, and gained knowledge unavailable elsewhere.

An even greater fortune beckoned. Allen Gates, who was newly arrived from the United States, brought commercial samples of Organon's pregnant mares' serum (PMS) rich in follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG) with its strong luteinizing hormone (LH) activity to induce estrus and ovulation in immature female mice. Working with Mervyn Runner (7), he had used low doses of each hormone at an interval of 48 hours to induce oocyte maturation, mating, and ovulation in immature mouse females. He now wished to measure the viability of three-day embryos from immature mice by transferring them to an adult host to grow to term (8). I was more interested in stimulating adult mice with these gonadotropins to induce estrus and ovulation at predictable times of the day. This would help my research, and I was by now weary of taking mouse vaginal smears at midnight. My future wife, Ruth Fowler, and I teamed up to test this new approach to superovulating adult mice. We chose PMS to induce multifolliculation and hCG to trigger ovulation, varying the doses and times from those utilized by Allen Gates. PMS became obsolete for human studies some time later, but its impact has stayed with me from that moment, even until today.

Opinion in those days was that exogenous hormones such as PMS and hCG would stimulate follicle growth and ovulation in immature female mammals, but not in adults because they would interact badly with an adult's reproductive cycles. In fact, they worked wonderfully well. Doses of 1–3 IU of PMS induced the growth of numerous follicles, and similar doses of hCG 42 hours later invoked estrus and ovulation a further 6 hours later in almost all of them. Often, 70 or more ovulated oocytes crowded the ampulla, most of them being fertilized and developing to blastocysts (9). Oocyte maturation, ovulation, mating, and fertilization were each closely timed in all adults, another highly unusual aspect of stimulation (10). Diakinesis was identified as the germinal vesicle regressed, with metaphase I a little later and metaphase II—expulsion of the first polar body—and ovulation at 11.5–12 hours after hCG. Multiple fertilization led to multiple implantation and fetal growth to full term, just as similar treatments in anovulatory women resulted in quintuplets and other high-order multiple pregnancies a few years later. Years afterward, germinal vesicle breakdown and diakinesis were to prove equally decisive in identifying meiosis and ovulation in human oocytes *in vivo* and *in vitro*. Even as these results were gained, Ruth and I departed in 1957 from Edinburgh to the California Institute of Technology, where I switched over to immunology and reproduction, a topic that was to dominate my life for five or six years on my return to the United Kingdom.

The Institute at Edinburgh had given me an excellent basis not only in genetics, but equally in reproduction. I had gained considerable knowledge about the endocrine control of estrus cycles, ovulation, and spermatozoa; the male reproductive tract; artificial insemination; the stages of embryo growth in the oviduct and uterus; superovulation and its consequences; and the use of radiolabeled compounds. Waddington had also been deeply interested in ethics and the relationships between science and religion, and instilled these topics in his students. I had been essentially trained in reproduction, genetics, and scientific ethics, and all of this knowledge was to prove to be of immense value in my later career. A visit to the California Institute of Technology widened my horizons into the molecular biology of DNA and the gene, a field then in its infancy.

After a year in California, London beckoned me to the National Institute for Medical Research to work with Drs. Alan Parkes and Colin (Bunny) Austin. I was fortunate indeed to have two such excellent colleagues. After two intense years in immunology, my curiosity returned to maturing oocytes and fertilization *in vitro*. Since they matured so regularly and easily *in vivo*, it should be easy to stimulate maturation in mouse oocytes *in vitro* by using gonadotropins. In fact, to my immense surprise, when liberated from their follicles into culture medium, oocytes matured immediately in vast numbers in all groups, with exactly the same timing as those maturing *in vivo* following an injection of hCG. Adding hormones made no difference. Rabbit, hamster, and rat oocytes also matured within 12 hours, each at their own species' specific rates. But to my surprise, oocytes from cows, sheep, and rhesus monkeys, and the occasional baboon, did not mature *in vitro* within 12 hours. Their germinal vesicles persisted unmoved, arrested in the stage known as diffuse diplotene. Why had they not responded like those of rats, mice, and rabbits? How would human oocytes respond? A unique opportunity emerged to collect pieces of human ovary and to aspirate human oocytes from their occasional follicles. I grasped it with alacrity.

MOVING TO HUMAN STUDIES

Molly Rose was a local gynecologist in the Edgware and District Hospital who delivered two of our daughters. She agreed to send me slivers or wedges of ovaries such as those removed from patients with polycystic disease, as recommended by Stein and Leventhal, or with myomata or other disorders demanding surgery. Stein–Leventhal wedges were the best sources of oocytes, with their numerous small Graafian follicles lined up in a continuous rim just below the ovarian surface. Though samples were rare, they provided enough oocytes to start with. These oocytes responded just like the oocytes from cows, sheep, and pigs, their germinal vesicles persisting and diakinesis being absent after 12 hours *in vitro*.

This was disappointing, and especially so for me, since Tjio, Levan, and Ford had identified 46 diploid chromosomes in humans, while studies by teams in Edinburgh and France had made it clear that many human beings were heteroploid. This was my subject, because chromosomal variations mostly arose during meiosis, and this would be easily assessed in maturing oocytes at diakinesis. Various groups also discovered monosomy or disomy in many men and women. Some women were XO or XXX; some men were XYY and XYYY. Trisomy 21 proved to be the most common cause of Down's syndrome, and other trisomies were detected. All this new information reminded me of my chromosome studies in the Edinburgh mice.

For human studies, I would have to obtain diakinesis and metaphase I in human oocytes, and then continue this analysis to metaphase II when the oocytes would be fully mature, ready for fertilization. Despite being disappointed at the current failure with human oocytes, it was time to write my findings for *Nature* in 1962 (11). There was so much to write regarding the animal work and in describing the new ideas then taking shape in my mind. I had heard Institute lectures on infertility, and realized that fertilizing human oocytes *in vitro* and replacing embryos into the mother could help to alleviate this condition. It could also be possible to type embryos for genetic diseases when a familial disposition was identified. Pieces of tissue, or one or two blastomeres, would have to be excised from blastocysts or cleaving embryos, but this did not seem to be too difficult. There were few genetic markers available for this purpose in the early 1960s, but it might be possible to sex embryos by their XX or XY chromosome complement by assessing mitoses in cells excised from morulae or blastocysts. Choosing female embryos for transfer would avert the birth of boys with various sex-linked disorders such as hemophilia. Clearly, I was becoming totally committed to human IVF and embryo transfer.

While looking in the library for any newly published papers relevant to my proposed *Nature* manuscript, I discovered those earlier papers of Pincus and his colleagues. They had apparently succeeded 30 years earlier in maturing human oocytes cultured for 12 hours where I had failed. My *Nature* paper (11) became very different from that originally intended, even though it retained enough for publication. Those results of Pincus et al. had to be repeated. After trying hard, I failed completely to repeat them, despite infusing intact ovaries *in vitro* with gonadotropin solutions, using different culture media to induce maturation, and using joint cultures of maturing mouse oocytes and newly released human oocytes. Adding hormones to culture media also failed. It began to seem that menstrual cycles had affected oocyte physiology in a different manner than in non-menstruating mammalian species. Finally, another line of inquiry emerged after two years of fruitless research on the precious few human oocytes available. Perhaps the timing of maturation in mice and rabbits differed from that of those oocytes obtained from cows, baboons, and humans. Even as my days in London were ending, Molly Rose sent a sliver of human ovary. The few oocytes were placed in culture just as before. Their germinal vesicles remained static for 12 hours as I already knew, and then, after 20 hours *in vitro*, three oocytes remained, and I waited to examine them until they had been *in vitro* for 24 hours. The first contained a germinal vesicle, and so did the second. There was one left and one only. Its image under the microscope was electrifying. I gazed down at chromosomes in diakinesis and at a regressing germinal vesicle. The chromosomes were superb examples of human diakinesis with their classical chiasmata. At last, I was on the way to human IVF, to completion of the maturation program and the onset of studies on fertilization *in vitro*.

This was the step I had waited for, a marker that Pincus had missed. He never checked for diakinesis, and apparently confused atretic oocytes, which contained chromosomes, with maturing oocytes. Endless human studies were opening. It was easy now, even on the basis of one oocyte in diakinesis, to calculate the timing of the final stages of maturation because the post-diakinesis stages of maturation were not too different from normal mitotic cycles in somatic cells. This calculation provided me with an estimate of about 36 hours for full maturation, which would be the moment for insemination. All these gaps in knowledge had to be filled. But now, my research program was stretching far into the future.

At this wonderful moment, John Paul, an outstanding cell biologist, invited me to join him and Robin Cole at Glasgow University to study differentiation in early mammalian embryos. This was exciting, to work in biochemistry with a leading cell biologist. He had heard that I was experimenting with very early embryos, trying to grow cell lines from them. He also wanted to grow stem cells from mammalian embryos and study them *in vitro*. This began one of my most memorable 12 months of research. John's laboratory had facilities unknown anywhere else, with CO_2 incubators, numerous cell lines in constant cultivation, cryopreservation facilities, and the use of media droplets held under liquid paraffin. We decided to start with rabbits. Cell lines did not grow easily from cleaving rabbit embryos. In contrast, stem cells migrated out

in massive numbers from cultures of rabbit blastocysts, forming muscle, nerves, phagocytes, blood islands, and other tissues *in vitro* (12). Stem cells were differentiating *in vitro* into virtually all the tissues of the body. In contrast, dissecting the inner cell mass from blastocysts and culturing it intact or as disaggregated cells produced lines of cells that divided and divided, without ever differentiating. One line of these embryonic stem cells expressed specific enzymes, diploid chromosomes, and a fibroblastic structure as it grew over 200 and more generations. Another was epithelioid and had different enzymes but was similar in other respects. The ability to make whole-embryo cultures producing differentiating cells was now combined with everlasting lines of undifferentiated stem cells that replicated over many years without changing. Ideas of using stem cells for grafting to overcome organ damage in recipients began to emerge. My thoughts returned constantly to growing stem cells from human embryos to repair defects in tissues of children and adults.

Almost at my last moment in Glasgow, with this new set of ideas in my mind, a piece of excised ovary yielded several oocytes. Being placed *in vitro*, two of them had reached metaphase II and expelled a polar body at 37 hours. This showed that another target on the road to human IVF had been achieved as the whole pattern of oocyte maturation continued to emerge but with increasing clarity.

Cambridge University, my next and final habitation, is an astonishing place. Looking back on those days, it seems that the Physiological Laboratory was not the ideal place to settle in that august university. Nevertheless, a mixture of immunology and reproduction remained my dominant theme as I rejoined Alan Parkes and Bunny Austin there. I had to do immunology to obtain a grant to support my family, but thoughts of human oocytes and embryos were never far away. One possible model of the human situation was the cow and other agricultural species, and large numbers of cow, pig, and sheep oocytes were available from ovaries given to me by the local slaughterhouse. Each species had its own timing, all of them longer than 12 hours (13). Pig oocytes were closest to humans, requiring 37 hours. In each species, maturation timings *in vitro* were exactly the same as those arising *in vivo* in response to an hCG injection. This made me suspect that a woman ovulated 36–37 hours after an injection of hCG. Human oocytes also trickled in, improving my provisional timings of maturation, and one or two of them were inseminated, but without signs of fertilization.

More oocytes were urgently needed to conclude the timings of oocyte meiosis. Surgeons in Johns Hopkins Hospital, Baltimore, performed the Stein–Leventhal operation, which would allow me to collect ovarian tissue, aspirate oocytes from their follicles, and retain the remaining ovarian tissues for pathology if necessary.

I had already met Victor McKusick, who worked in Johns Hopkins, at many conferences. I asked for his support for my request to work with the hospital gynecologists for six weeks. He found a source of funds, made laboratory space available, and gave me a wonderful invitation that introduced me to Howard and Georgeanna Jones. This significant moment was equal to my meeting with Molly Rose. The Joneses proved to be superb and unstinting in their support. Sufficient wedges and other ovarian fragments were available to complete my maturation program in human oocytes. Within three weeks, every stage of meiosis was classified and timed (14). We also undertook preliminary studies on inseminating human oocytes that had matured *in vitro*, trying to achieve sperm capacitation by using different media or adding fragments of ampulla to the cultures, and even attempting fertilization in rhesus monkey oviducts. Two nuclei were found in some inseminated eggs, resembling pronuclei, but sperm tails were not identified, so no claims could be made (15). During those six weeks, however, oocyte maturation was fully timed at 37 hours, permitting me now to predict with certainty that women would ovulate at 37 hours after an hCG injection.

A simple means of access to the human ovary was now essential in order to identify human ovarian follicles *in vivo* and to aspirate them 36 hours after hCG, just before the follicular rupture. Who could provide this? And how about sperm capacitation? Only in hamsters had fertilization *in vitro* been achieved, using *in vivo*-matured oocytes and epididymal spermatozoa (16). I met Victor Lewis, my third clinical colleague, and we noticed what seemed to be anaphase II in some inseminated eggs. Again, no sperm tails were seen within the eggs.

An attempt to achieve human capacitation in Chapel Hill, North Carolina, working with Robert McGaughey and his colleagues, also failed (17). A small intrauterine chamber lined with porous membrane was filled with washed human spermatozoa, sealed, and inserted overnight into the uterus of human volunteers at mid-cycle. Molecules entering it could react with the spermatozoa. No matured human eggs were fertilized. Later evidence indicated that the chamber contained inflammatory proteins, perhaps explaining the failure.

DECISIVE STEPS TO CLINICAL HUMAN IVF

Back in the United Kingdom, my intention to conceive human children *in vitro* had grown even stronger. So many medical advantages could flow from it. A small number of human embryos had been flushed from human oviducts or uteri after sexual intercourse, providing slender information on these earliest stages of human embryology. It was time to attain human fertilization *in vitro*, in order to move close to working with infertile patients. Ethical issues and moral decisions would emerge, one after the other, in full public view. Matters such as cloning and sexing embryos, the risk of abnormalities in the children, the clinical use of embryo stem cells, the ethics of oocyte donation and surrogate pregnancy, and the right to initiate human embryonic life *in vitro* would never be very far away. These issues were all acceptable, since I was confident that studies of human conception were essential for future medicine, and correct ethically, medically, and scientifically. The increasing knowledge of genetics and embryology could assist many patients if I could achieve

human fertilization and grow embryos for replacement into their mothers.

Few human oocytes were available in the United Kingdom. Despite this scarcity, one or two of those matured and fertilized *in vitro* possessed two nuclei after insemination. But there were no obvious sperm tails. I devised a cow model for human fertilization, using *in vitro*-matured oocytes and insemination *in vitro* with selected samples of highly active, washed bull spermatozoa extracted from neat semen. It was a pleasure to see some fertilized bovine eggs, with sperm tails and characteristic pronuclei, especially using spermatozoa from one particular bull. Here was a model for human IVF and a prelude to a series of events that implied that matters in my research were suddenly changing. A colleague had stressed that formalin fixatives were needed to detect sperm tails in eggs. Barry Bavister joined our team to study for his PhD and designed a medium of high pH, which gave excellent fertilization rates in hamsters. We decided to collaborate by using it for trials on human fertilization *in vitro*.

Finally, while browsing in the library of the Physiological Laboratory, I read a paper in *The Lancet* that instantly caught my attention. Written by Dr. P.C. Steptoe of the Oldham and District General Hospital (18), it described laparoscopy, with its narrow telescope and instruments and its minute abdominal incisions. He could visualize the ampulla and place small amounts of medium there, in an operation lasting 30 minutes or less and maybe even without using anesthesia. This is exactly what I wanted, because access to the ampulla was equivalent to gaining access to ovarian follicles. Despite advice to the contrary from several medical colleagues, I telephoned him about collaboration and stressed the uncertainty in achieving fertilization *in vitro*. He responded most positively, just as Molly, Howard and Georgeanna, and Victor had done. We decided to get together.

Last but by no means least, Molly Rose sent a small piece of ovary to Cambridge. Its dozen or more oocytes were matured *in vitro* for 37 hours, then Barry and I added washed spermatozoa suspended in his medium. We examined them a few hours later. To our delight, spermatozoa were pushing through the zona pellucida, into several of the eggs. Maternal and paternal pronuclei were forming beautifully. We saw polar bodies and sperm tails within the eggs. That evening in 1969, we watched in delight virtually all the stages of human fertilization *in vitro* (Figure I.1). One fertilized egg had fragments, as Chang had forecast from his work on oocyte maturation and fertilization *in vitro* of rabbit eggs. This evidence strengthened the need to abandon oocyte maturation *in vitro* and replace it with stimulating maturation by means of exogenous hormones. Our 1969 paper in *Nature* surprised a world unaccustomed to the idea of human fertilization *in vitro* (19).

Incredibly fruitful days followed in our Cambridge laboratory. Richard Gardner, another PhD candidate, and I excised small pieces of trophectoderm from rabbit blastocysts and sexed them by staining the sex chromatin body. Those classified as female were transferred into adult females and were all correctly sexed at term. This work transferred my theoretical ideas of a few years earlier into the practice of preimplantation diagnosis of inherited disease, in this case for sex-linked diseases (20). Alan Henderson, a cytogeneticist, and I analyzed chiasmata during diakinesis in mouse and human eggs, and explained the high frequencies of Down's syndrome in offspring of older mothers as a consequence of meiotic errors arising in oocytes formed last in the fetal ovary, which were then ovulated last at later maternal ages (21). Dave Sharpe, a lawyer from Washington, joined forces with me to write an article in *Nature* (22) on the ethics of IVF, the first ever paper in the field. I followed this up with a detailed analysis of ethics and law in IVF covering scientific possibilities, oocyte donation, surrogacy by embryo transfer, and other matters (22). So the first ethical papers were written by scientists and lawyers and not by philosophers, ethicists, or politicians.

THE OLDHAM YEARS

Patrick and I began our collaboration six months later in the Oldham and District General Hospital, almost 200 miles north of Cambridge. He had worked closely with two pioneers, Palmer in Paris (23) and Fragenheim in Germany (24). He improved the pneumoperitoneum to gain working space in the abdominal cavity and used carbon fibers to pass cold light into the abdomen from an external source (25). By now, Patrick was waiting in the wings, ready to begin clinical IVF in distant Oldham. We had a long talk about ethics and found our stances to be very similar.

Work started in the Oldham and District General Hospital and moved later to Kershaw's Hospital, set up by my assistants, especially Jean Purdy. We knew the routine. It was based on my Edinburgh experiences with mice. Piero Donini from Serono Laboratories in Rome had purified urinary human menopausal gonadotropin (hMG) as a source of FSH and the product was used clinically to stimulate follicle growth in anovulatory women by Bruno Lunenfeld (26). It removed the need for PMS, thus avoiding the use of nonhuman hormones. We used low dosage levels in patients; that is, two to three vials (a total of 150–225 IU) given on days 3 and 5, and 5000–7000 IU of hCG on day 10. Initially, the timing of oocyte maturation *in vitro* was confirmed by performing laparoscopic collections of oocytes from ovarian follicles at 28 hours after hCG to check that they were in metaphase I (27). We then moved to 36 hours to aspirate mature metaphase II oocytes for fertilization. Those beautiful oocytes were surrounded by masses of viscous cumulus cells and were maturing exactly as predicted. We witnessed follicular rupture at 37 hours through the laparoscope. Follicles could be classified from their appearance as ovulatory or nonovulatory, this diagnosis being confirmed later by assaying several steroids in the aspirated follicular fluids (Figure I.2).

It was a pleasure and a new duty to meet the patients searching for help to alleviate their infertility. We did our best, driving from Cambridge to Oldham and arriving at

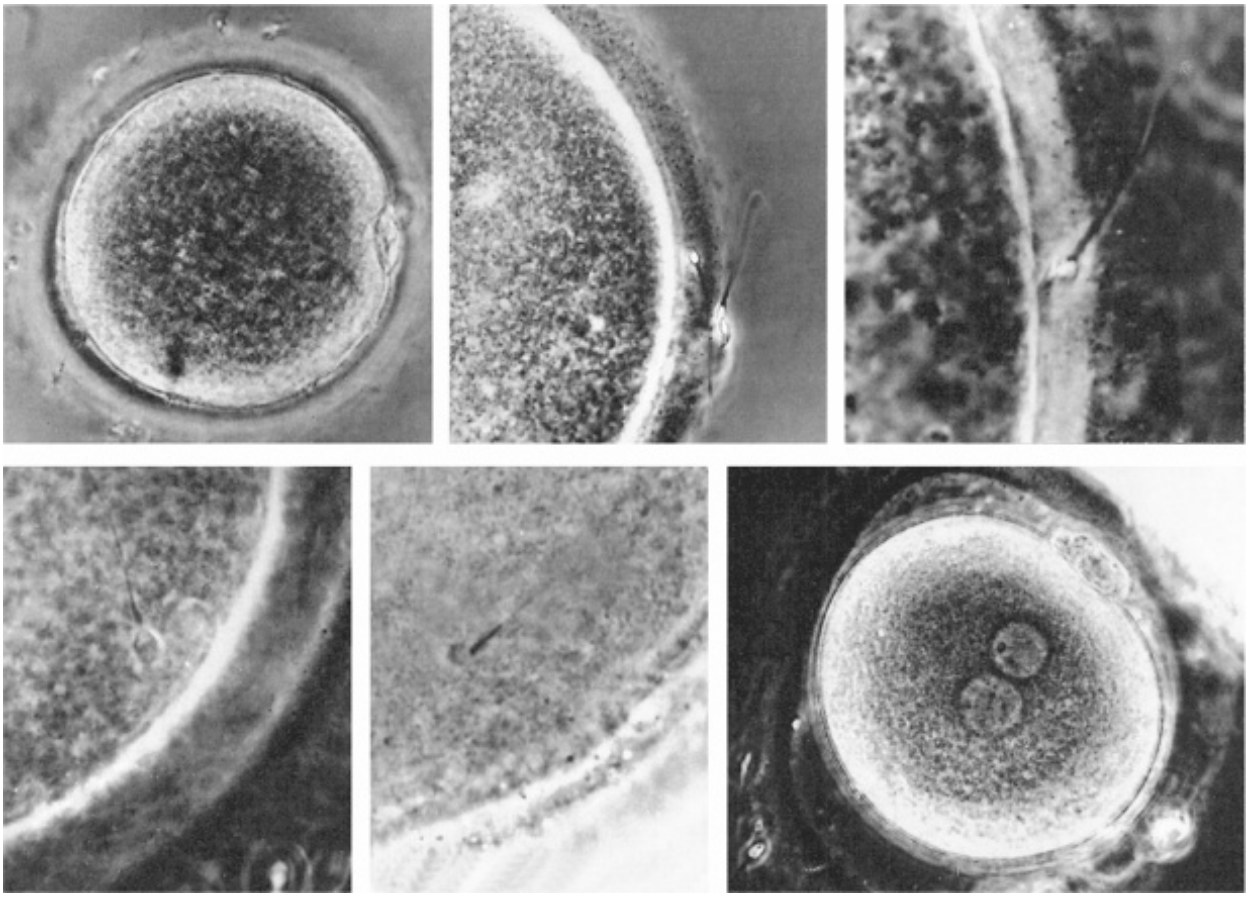

Figure I.1 A composite picture of the stages of fertilization of the human egg. (*Upper left*) An egg with a first polar body and spermatozoa attached to the outer zona pellucida. (*Upper central*) Spermatozoa are migrating through the zona pellucida. (*Upper right*) A spermatozoon with a tail beating outside the zona pellucida is attaching to the oocyte vitelline membrane. (*Lower left*) A spermatozoon in the ooplasm, with enlarging head and distinct mid-piece and tail. (*Lower central*) Further development of the sperm head in the ooplasm. (*Lower right*) A pronucleate egg with two pronuclei and polar bodies. Notice that the pronuclei are apparently aligned with the polar bodies, although more dimensions must be scored to ensure that polarity has been established in all axes.

noon to prepare the small laboratory there. Patrick had stimulated the patients with hMG and hCG, and he and his team led by Muriel Harris arrived to prepare for surgery.

Patrick's laparoscopy was superb. Ovarian stimulation, even though mild, produced five or six mature follicles per patient, and ripe oocytes came in a steady stream into my culture medium for insemination and overnight incubation. The next morning, the formation of two pronuclei and sperm tails indicated fertilization had occurred, even in simple media, now with a near-neutral pH. Complex culture media, Ham's F10 and others, each with added serum or serum albumin, sustained early and later cleavages (28), and even more fascinating was the gradual appearance of morulae and then light, translucent blastocysts (Figure I.3) (29). Here was my reward—growing embryos was now a routine, and examinations of many of them convinced me that the time had come to replace them into the mother's uterus. I had become highly familiar with the teratologic principles of embryonic development, and knew many teratologists. The only worry I had was the chance of chromosomal monosomy or trisomy, on the basis of our mouse studies, but these conditions could be detected later in gestation by amniocentesis. Our human studies had surpassed work on all animals, a point that was highlighted even more when we grew blastocysts to day 9 after they had hatched from their zona pellucida (Figure I.4) (30). This beautifully expanded blastocyst had a large embryonic disc that was shouting that it was a potential source of embryonic stem cells.

When human blastocysts became available, we tried to sex them using the sex chromatin body as in rabbits. Unfortunately, they failed to express either sex chromatin or the male Y body so we were unable to sex them as female or male embryos. Human preimplantation genetic diagnosis would have to wait a little longer.

During these years there were very few plaudits for us, as many people spoke against IVF. Criticism was mostly aimed at me, as usual when scientists bring new challenges to society. Criticism came not only from the Pope and archbishops, but also from scientists who should have known better, including James Watson (who testified to a U.S. Senate Committee that many abnormal babies would

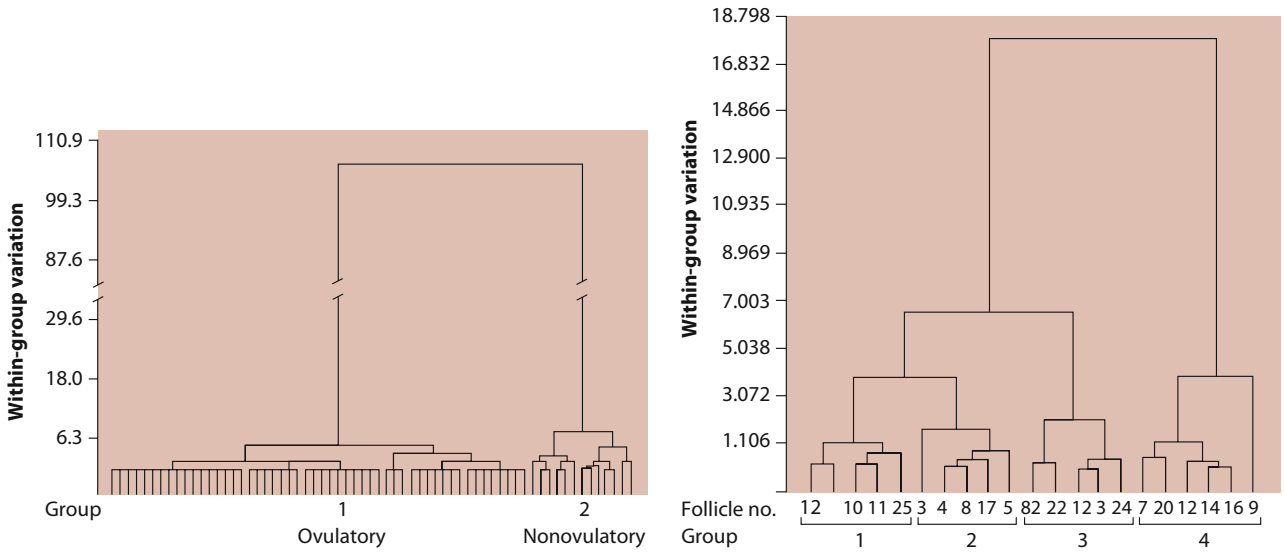

Figure I.2 Eight steroids were assayed in fluids extracted from human follicles aspirated 36–37 hours after the human chorionic gonadotropin (hCG) shot. The follicles had been classified as ovulating or non-ovulating by laparoscopic examination *in vivo*. Data were analyzed by cluster analysis, which groups follicles with similar features. The upper illustration shows data collected during the natural menstrual cycle. Note that two sharply separated groups of follicles were identified, each with very low levels of within-group variance. Attempting to combine the two groups resulted in a massive increase of within-group variation, indicating that two sharply different groups had been identified. These different groups accorded exactly with the two groups identified by means of steroid assays. The lower figure shows the same analysis during stimulated cycles on fluids collected 36–37 hours after injecting hCG. With this form of stimulation, follicle growth displays considerable variation within groups. Attempts to combine all the groups result in a moderately large increase in variation. This evidence suggests that follicles vary considerably in their state of development in simulated cycles using human menopausal gonadotropin (hMG) and hCG.

be born), and Max Perutz, who supported him. These scientist critics knew virtually nothing about my field, so who advised them to make such ridiculous charges? Cloning football teams or intelligentsia was always raised by ethicists, which clearly dominated their thoughts rather than the intense hopes of our infertile patients. Yet one theologian, Gordon Dunstan, who became a close friend, knew all about IVF from us, and wrote an excellent book on its ethics. He was far ahead of almost every scientist in my field of study. Our patients also gave us their staunch support, and so did the Oldham Ethical Committee, Bunny Austin back home in Cambridge, and Elliott Philip, a colleague of Patrick's.

Growing embryos became a routine, so we decided to transfer one each to several patients. Here again we were in untested waters. Transferring embryos via the cervical canal, the obvious route to the uterus, was virtually a new and untested method. We would have to do our best. From now on, we worked with patients who had seriously distorted tubes or none whatsoever. This step was essential, since no one would have believed we had established a test-tube baby in a woman with near-normal tubes. This had to be a condition of our initial work. Curiously, it led many people to make the big mistake of believing that we started IVF to bypass occluded oviducts. Yet we already knew that embryos could be obtained for men with oligozoospermia or antibodies to their gametes, and for women in various stages of endometriosis.

One endocrinological problem did worry me. Stimulation with hMG and hCG shortened the succeeding luteal phase, leaving only a very short time for embryos to implant before the onset of menstruation. Levels of urinary pregnanediol also declined soon after oocyte collection. This condition was not a result of the aspiration of granulosa and cumulus cells, and luteal support would be needed, preferably progesterone. Csapo et al. stressed how this hormone was produced by the ovaries for the first 8–10 weeks before the placenta took over this function (31). Injections of progesterone in oil given over that long period of time seemed unacceptable since it would be extremely uncomfortable for patients. While mulling over this problem, my attention turned to those earlier endocrinologists who believed that exogenous hormones would distort the reproductive cycle, although I doubt they even knew anything about a deficient luteal phase.

This is how we unknowingly made our biggest mistake in the early IVF days. Our choice of Primolut® (Sigma Chemical Co., St. Louis, Missouri) depot, a progestogen, meant it should be given every five days to sustain pregnancies, since it was supposed to save threatened abortions. So, we began embryo transfers to patients in stimulated cycles, giving this luteal phase support. Even though our work was slowed down by having to wait to see whether pregnancies arose in one group of patients before stimulating the next, enough patients had accumulated after two to three years. None of our patients was pregnant, and

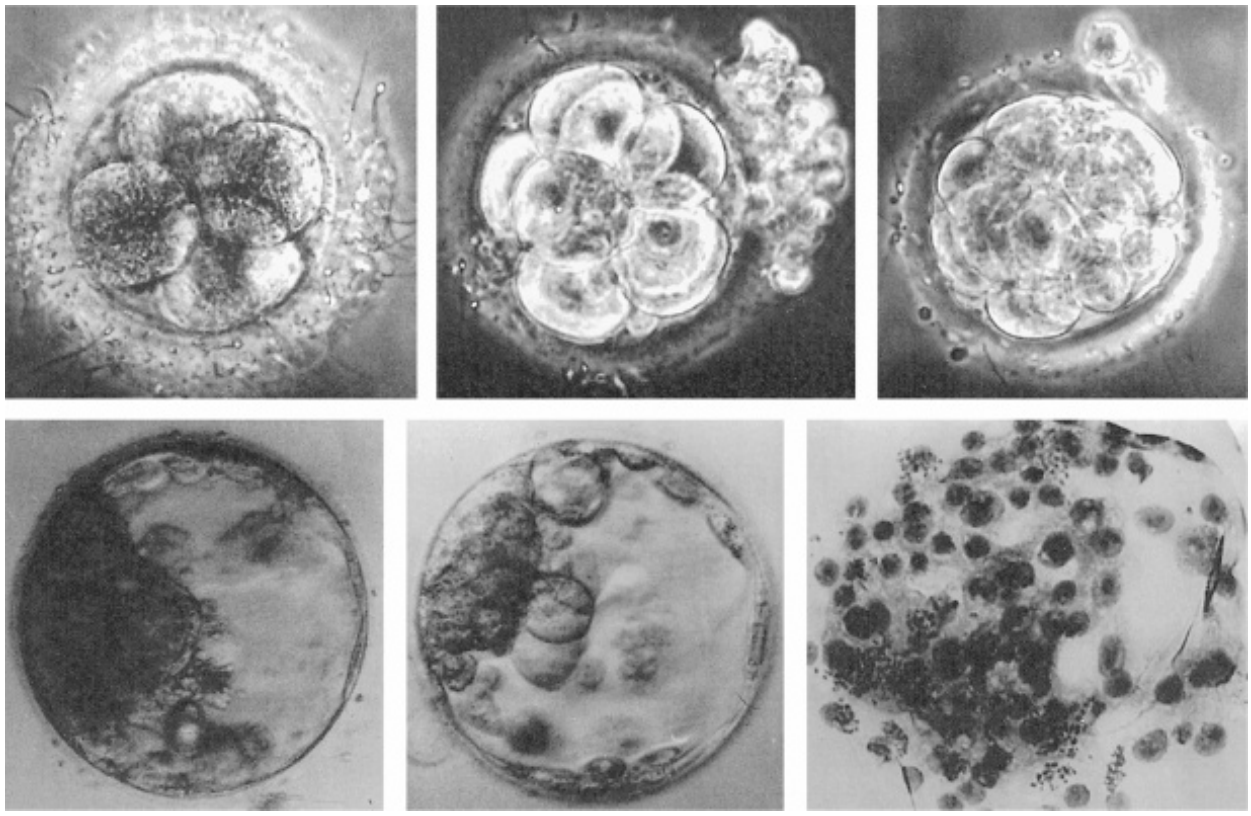

Figure I.3 Successive stages of human preimplantation development *in vitro* in a composite illustration made in Oldham in 1971. (*Upper left*) Four-cell stage showing the crossed blastomeres typical of most mammals. (*Upper middle*) Eight-cell stage showing the even outline of blastomeres and a small piece of cumulus adherent to the zona pellucida. (*Upper right*) A 16–32-cell stage showing the onset of compaction of the outer blastomeres. Often, blastocelic fluid can be seen accumulating between individual cells to give a "stripy" appearance to the embryo. (*Lower left and middle*) Two living blastocysts showing a distinct inner cell mass, single-celled trophectoderm, blastocelic cavity, and thinning zona pellucida. (*Lower right*) A fixed preparation of a human blastocyst at five days, showing more than 100 even-sized nuclei and many mitoses.

disaster loomed. Our critics were even more vociferous as the years passed, and the mutual support between Patrick and I had to pull us through.

Twenty or more different factors could have caused our failure; for example, cervical embryo transfers, abnormal embryos, toxic culture dishes or catheters, inadequate luteal support, incompatibility between patients' cycles and that imposed by hMG and hCG, inherent weakness in human implantation, and many others. We had to glean every scrap of information from our failures. I knew Ken Bagshawe in London, who was working with improved assay methods for gonadotropic hormones. He offered to measure blood samples taken from our patients over the implantation period using his new hCG assay. He telephoned: three or more of our patients previously undiagnosed had actually produced short-lived rises of hCG over this period. Everything changed with this information. We had established pregnancies after all, but they had aborted very early. We called them biochemical pregnancies, a term that still remains today. It had taken us almost three years to identify the cause of our failure, and the finger of suspicion pointed straight at Primulot. I knew it was luteolytic, but it was apparently also an abortifacient, and our ethical decision to use it had caused much heartache, immense loss of work and time, and despair for some of our patients. The social pressures had been immense, with critics claiming our embryos were dud and our whole program was a waste of time; but we had come through it and now knew exactly what to do next.

We accordingly reduced the levels of Primulot depot, and utilized hCG and progesterone as luteal aids. Suspicions were also emerging that human embryos were very poor at implanting. We had replaced single embryos into most of our patients, rarely two. Increasingly we began to wonder whether more should be replaced, as when we replaced two in a program involving transfer of oocytes and spermatozoa into the ampulla so that fertilization could occur *in vivo*.

This procedure was later called gamete intrafallopian transfer (GIFT) by Ricardo Asch. We now suspected that single embryo transfers could produce a 15%–20% chance of establishing pregnancy, just as our first clinical pregnancy arose after the transfer of a single blastocyst in a patient stimulated with hMG and hCG (32). Then came the fantastic news—a human embryo fertilized and grown *in vitro* had produced a pregnancy. Everything seemed fine, even with ultrasound images. My culture protocols

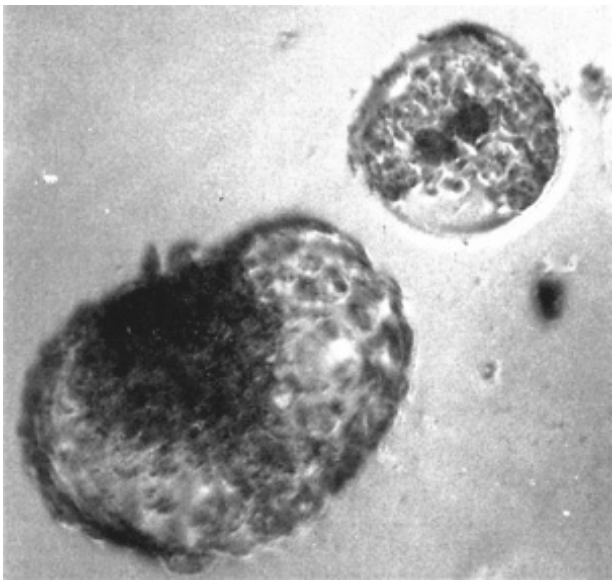

Figure I.4 A hatched human blastocyst after nine days in culture. Notice the distinct embryonic disc and the possible bilaminar structure of the membrane. The blastocyst has expanded considerably, as shown by comparing its diameter with that of the shed zona pellucida. The zona contains dying and necrotic cells and its diameter provides an estimate of the original oocyte end embryo diameters.

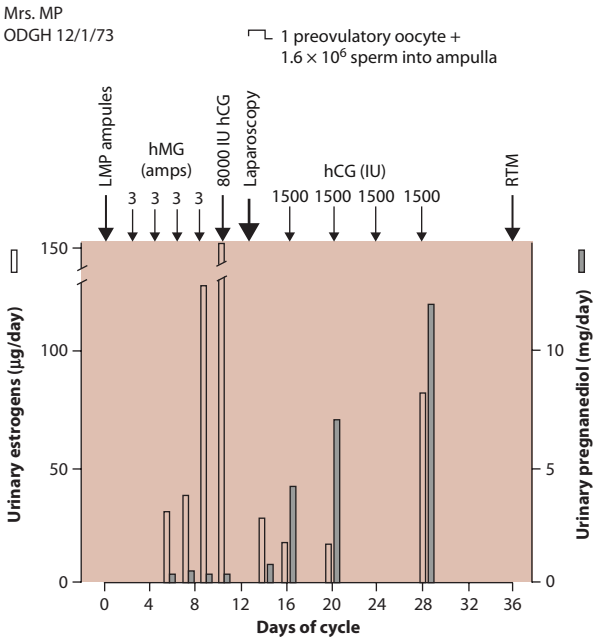

Figure I.5 The first attempts at gamete intrafallopian transfer (GIFT) were called oocyte recovery with tubal insemination (ORTI). In this treatment cycle, using human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG), including additional injections of hCG for luteal support, a single preovulatory oocyte and 1.6 million sperm were transferred into the ampulla. Return to menstruation (RTM) indicates stages of the menstrual cycle. *Abbreviations*: LMP, last menstrual period; ODGH, Oldham and District General Hospital.

were satisfactory after all. Patrick rang: he feared the pregnancy was ectopic and he had to remove it sometime after 10 gestational weeks. Every new approach we tested seemed to be ending in disaster, yet we would not stop, since the work itself seemed highly ethical, and conceiving a child for our patients was perhaps the most wonderful thing anyone could do for them. In any case, ectopic pregnancies are now known to be a regular feature with assisted conception.

I sensed that we were entering the final phase of our Oldham work, seven years after it began. We had to speed up, partly because Patrick was close to retiring from the National Health Service. Four stimulation protocols were tested in an attempt to avoid problems with the luteal phase: hMG and hCG; clomiphene, hMG, and hCG to gain a better luteal phase; bromocriptine, hMG, and hCG because some patients had high prolactin concentrations; and hCG alone at mid-cycle. We also tested what came to be known as GIFT, calling it oocyte recovery with tubal insemination [ORTI] by transferring one or two eggs and spermatozoa to the ampulla) (Figure I.5). Natural-cycle IVF was introduced, based on collections of urine samples at regular intervals eight times daily, to measure exactly the onset of the LH surge, using a modified HiGonavis assay (Figure I.6). Cryopreservation was also introduced by freezing oocytes and embryos that looked to be in good condition when thawed. A recipient was given a donor egg fertilized by her husband's spermatozoa, but pregnancy did not occur.

Lesley and John Brown came as the second entrants for natural-cycle IVF. Lesley had no oviducts. Her egg was aspirated in a few moments and inseminated simply and efficiently. The embryo grew beautifully and was transferred an hour or so after it became eight cells. Their positive pregnancy test a few days after transfer was another milestone—surely nothing could now prevent their embryo developing to full term in a normal reproductive cycle, but those nine months lasted a very long time. Three more pregnancies were established using natural-cycle IVF as we abandoned the other approaches. A triploid embryo died *in utero*—more bad luck. A third pregnancy was lost through premature labor on a mountain walking holiday, two weeks after the mother's amniocentesis (32,33). It was a lovely, well-developed boy. Louise Brown's birth, and then Alistair's, proved to a waiting world that science and medicine had entered human conception. Our critics declared that the births were a fake, and advised against attending our presentation on the whole of the Oldham work at the Royal College of Obstetricians and Gynaecologists.

IVF WORLDWIDE

The Oldham period was over. Good facilities were now needed, with space for a large IVF clinic. Bourn Hall was an old Jacobean house in lovely grounds near Cambridge (Figure I.7). The facilities on offer for IVF in Cambridge were far too small, so we purchased it mostly with venture capital. It was essential to conceive 100 or 1000 IVF babies

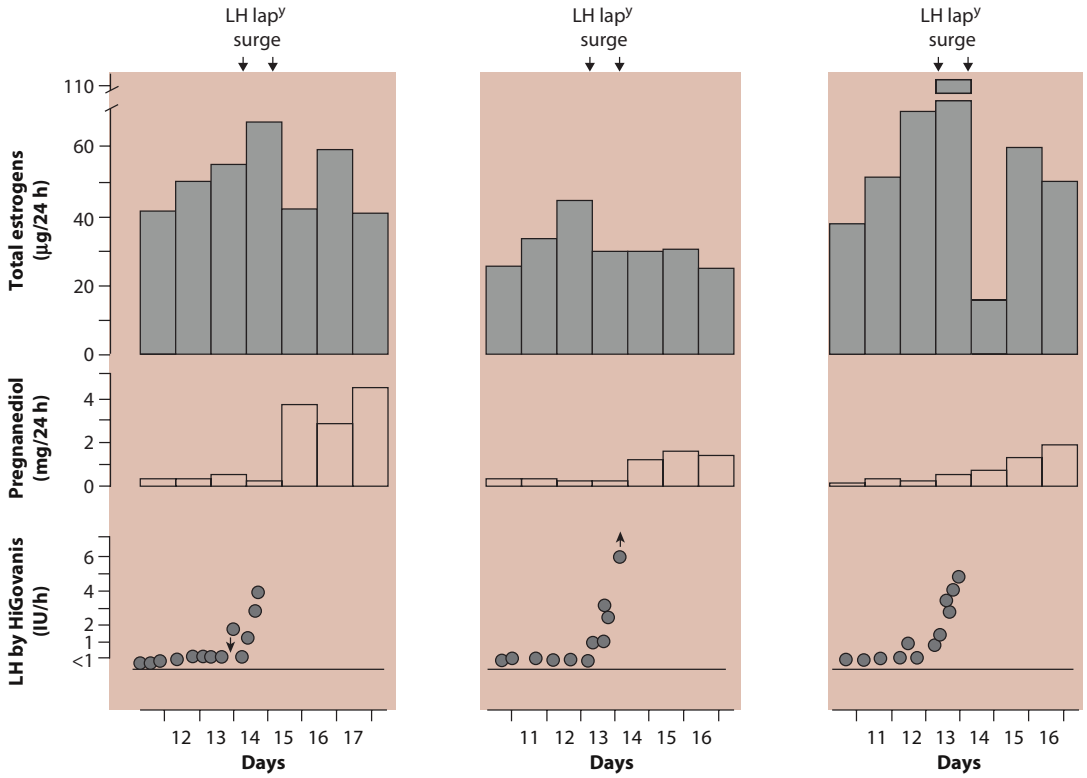

Figure I.6 Recording the progress of the human natural menstrual cycle for *in vitro* fertilization (IVF). Three patients are illustrated. All three displayed rising 24-hour urinary estrogen concentrations during the follicular phase and rising urinary pregnanediol concentrations in the luteal phase. Luteinizing hormone (LH) levels were measured several times daily and the data clearly reveal the exact time of onset of the LH surge.

to ensure that the method was safe and effective clinically. The immense delays in establishing Bourn Hall delayed our work by two years after Louise's birth. Finally, on minimal finance, Bourn Hall was opened in September 1980 on a shoestring, supported by our own cash and loans. The delay gave the rest of the world a chance to join in IVF. Alex Lopata delivered an IVF baby in Australia, and one or two others were born elsewhere. Natural-cycle IVF was chosen initially at Bourn Hall since it had proved successful in Oldham, and we became experts in it. Pregnancies flowed, at 15% per cycle. An Australian team of Alan Trounson and Carl Wood announced the establishment of several IVF pregnancies after stimulation by clomiphene and hCG and replacing two or three embryos (34), so they had moved ahead of us

Figure I.7 Bourn Hall (courtesy of Dr. P Brinsden).

during the delayed opening of Bourn Hall. Our own effort now expanded prodigiously. Thousands of patients queued for IVF. Simon Fishel, Jacques Cohen, and Carol Fehilly joined the embryology team among younger trainees, and new clinicians joined Patrick and John Webster. Patients and pregnancies increased rapidly, and the world was left standing far behind. Howard and Georgeanna Jones began in Norfolk using gonadotropins for ovarian stimulation. Jean Cohen began in Paris, Wilfred Feichtinger and Peter Kemeter in Vienna, Klaus Diedrich and Hans van der Venn in Bonn, Lars Hamberger and Matts Wikland in Sweden, and Andre van Steirteghem and Paul Devroey in Brussels. IVF was now truly international.

The opening of Bourn Hall had not deterred our critics. They put up a fierce rearguard action against IVF, alongside LIFE, Society for the Unborn Child, individual gynecologists, and others.

Objections raised against IVF included low rates of pregnancy (no one mentioned the similar low rates of pregnancy with natural conception), the possibilities of oocyte and embryo donation, surrogate mothers, unmarried parents, one-sex parents, embryo cryopreservation, cloning, and endless other objections.

LIFE issued a legal action against me for the abortion of an embryo grown for 14 days and longer *in vitro*. Their action was rejected by the U.K. Attorney General since the laws of pregnancy began after implantation. We fully respected the intense ethical nature of our proceedings. We also recognized the need for research, and the necessity to protect or cryopreserve the best embryos for later replacement into their mothers. Those not replaced had to be used for research under strict controls, combined with open publication and discussion of our work.

Each year, 1000—rising to almost 2000—patients passed through Bourn Hall. Different stimulation regimens or new procedures could be tested in very little time.

Clomiphene/hMG was reintroduced. Bourn babies increased: 20, 50, 100–1000 after five to six years. This was far more than half of the world's entire IVF babies, including the first born in the U.S.A., Germany, Italy, and many other countries. Detailed studies were performed on embryo culture, implantation, and abortion. We even tried aspirating epididymal spermatozoa for IVF, without achieving successful fertilization.

Among the immense numbers of patients, people with astonishingly varied conditions of infertility emerged. Some were poor responders in whom immense amounts of endocrine priming were essential, some were women with a natural menstrual cycle that was not as it should have been, some had previous misdiagnoses that had laid the cause of infertility on the wife when the husband had never even been investigated, and some were men bringing semen samples that we discovered had been obtained from a friend. The collaboration between nurses, clinicians, and scientists was remarkable. Yet trouble—ethical trouble—was never far away. I purchased a freezing machine to resume our Oldham work, but, unknown to me, Patrick talked to officers of the British Medical Association (BMA) and for some reason agreed to delay embryo cryopreservation. Apparently, the BMA felt it would be an unwelcome social development. I did not approve of these reservations: David Whittingham had shown how low-temperature cryostorage was successful with mouse embryos, without causing genetic damage. "Freezing and cloning" became a term of intense approbation at this time. I unwillingly curtailed our cryopreservation program.

One weekend, major trouble erupted as a result of this difference between Patrick and me. My duties in Bourn Hall prevented me from attending a conference in London. Trying to be helpful, I telephoned my lecture to London. Reception at the other end was apparently so poor as to lead to misinterpretations of what I had said. Next morning, the press furor about my supposed practice of cryopreserving embryos after IVF was awful; so bad, indeed, that legal action had to be taken. Luckily, my lecture had been recorded, and listening to the tapes with a barrister revealed nothing contentious. I had said nothing improper in my lecture or during the question-and-answer session. That day, I issued seven libel actions against the cream of British society: the BMA and its secretary, the BBC, *The Times*, and other leading newspapers. There were seven in one day and another one later! If only one was lost, I could be ruined and disgraced. However, they were all won, even though it took several years with the BMA and its secretary. These legal actions had inhibited our research, with the cryopreservation program being shut down for more than a year. Every single embryological note of mine from those days in Oldham and from Bourn Hall was examined in detail for my opponents by someone who was clearly an embryologist. Nothing was found to incriminate me.

That wretched period passed. The number of babies kept on growing, embryo cryopreservation was resumed, and Gerhard Zeilmaker in The Netherlands beat us and the world to the first "ice" baby (35). Colin Howles and Mike McNamee joined us in endocrinology and Mike Ashwood-Smith and Peter Holland's joined us in embryology as the old team faded away. Fascinating days had returned. Working with barristers, we designed consent forms that were far in advance of those used elsewhere. Oocyte donation and surrogacy by embryo transfer were introduced. The world's first paper on embryo stem cells appeared in *Science* in 1984, sent from Bourn Hall, and the world's first paper on human preimplantation diagnosis in 1987 appeared in *Human Reproduction*. However, embryo research faltered as all normal embryos were cryopreserved for their parents, so almost none were available for study. Alan Handyside, one of our Cambridge PhDs, joined Hammersmith Hospital in London to make major steps in introducing preimplantation genetic diagnosis (36). As we reached 1000 pregnancies, our data showed the babies to be as normal as those conceived *in vivo*.

Test-tube babies (an awful term) were no longer unique and were accepted worldwide, exactly as Patrick and I had hoped. Our work was being recognized (Figure I.8). Clinics sprang up everywhere. Ultrasound was introduced to detect follicles for aspiration by the Scandinavians (37),

Figure I.8 A happy picture of Patrick and I, standing in our robes after being granted our Hon. DSc by Hull University.

making laparoscopy for oocyte recovery largely redundant. Artificial cycles were introduced in Australia and intracytoplasmic sperm injection (ICSI) was introduced in Belgium (38), and gonadotropin-releasing hormone agonists were used to inhibit the LH surge. Ian Craft in London showed how postmenopausal women aged 52 or more could establish pregnancies using oocyte donation and endocrine support. Women over 60 years of age conceived and delivered children. This breakthrough was especially welcome to me, since older women surely have the right to have children at ages almost the same as those possible for men.

Ethics continued side by side with advancing science and medicine. The U.K. governmental Warnock report recommended permitting embryo research and proposed a Licensing Authority for IVF. A year or so later, the U.K. House of Lords, in all its finery, responded with a 3:1 vote in favor, decisive support for all we had done in Mill Hill, Cambridge, and Oldham. What a wonderful day! The British House of Commons passed a liberal IVF law after intense debate, and so did the Spanish government, although elsewhere things were not so liberal. Ten years after the birth of Louise Brown, the British Parliament had therefore accepted IVF, research on human embryos until day 14, and establishing research embryos. Cloning and embryo stem cells still bothered the politicians of 1988, only to re-emerge in 1998, gray shadows of my earlier times in Glasgow. IVF had also become fundamental to establishing embryonic stem cells for organ repair, or cloning. During all this activity, tragedy struck all of us in Bourn Hall. Jean Purdy died in 1986 and Patrick Steptoe in 1988. They at least saw IVF come of age.

By the 1990s, burgeoning medical science was digging deeper into endless aspects of human conception *in vitro*. The intracytoplasmic injection of a single spermatozoon into an oocyte to achieve fertilization, ICSI, was one of the greatest advances since IVF was introduced. It transformed the treatment of male infertility, enabling severely oligozoospermic men to father their own children. It did not stop there, since epididymal spermatozoa and even those aspirated from the testis could be used for ICSI. Spermatids have also been used. ICSI became so simple that many clinics reduced IVF to fewer and fewer cases. New gonadotropin-releasing hormone antagonists introduced novel ways to control the cycle, enabling many oocytes to be stimulated by hMG and, subsequently, by using recombinant human FSH. Treatment in the natural cycle could be improved, since these antagonists control LH levels and prevent premature LH surges. My own interests were returning to embryology, as the molecular biology revolution influenced our thinking. I am convinced that the oocyte and egg must be highly programmed, timewise, in embryonic polarities and integrating genetic systems such that the tight systems place every new gene product in its right place in the one-cell egg and cleaving embryo. This must be right; there can surely be no other explanations for the fabulous modification in embryonic growth in the first week or two of embryonic life. I have been delighted to work with Chris Hansis on identifying a gene (for hCG-^{e}) in one blastomere of four- and eight-cell human embryos, providing evidence of blastomere differentiation at this early stage of embryogenesis (39).

This topic returns me to my scientific origins studying mouse embryos in the Institute of Animal Genetics in Edinburgh, where Waddington reported the amazing story of the gene *Aristapedia* in *Drosophila*, which he had induced to grow legs in place of eyes. These unusual flies then bred true, showing he had uncovered a gene that had been silenced for millions of years and how this could be an essential component of normal differentiation. He called it epigenesis, and we fear today that some aspects of IVF may lead to deleterious epigenetic changes in children such as Angelman or Beckwith–Wiedemann syndrome. Risks of epigenetic changes in cattle embryos and those of other species may be heightened by adding serum to media used to culture embryos to cause, for example, large-calf syndrome. It would be wise to be well aware of these findings when practicing human IVF; for example, by assessing the role of sera in human culture media.

IVF OUTLOOK

In one sense, opening up human conception *in vitro* was perhaps among the first examples of applied science in modern "hi tech." Human IVF has since spread throughout the world, with apparently more than 3.5 million such babies born worldwide by 2008—yet Louise Brown is only just 30 years of age. The need for IVF and its derivatives is greater than ever, since up to 10% of couples may suffer from some form of infertility. Major advances in genetic technologies now identify hundreds of genes in a single cell, and diagnosing genetic disease in embryos promises to help avoid desperate genetic diseases in newborn children. Indeed, the ethics of this field have now become even more serious, since the typing of embryo genotypes provides detailed predictions of future life and health.

IVF has now combined closely with genetics to eliminate disease or disability genes, or lengthen the life span.

But most of all, practicing IVF teaches a wider understanding of the desire and love for a child and a partner, the wonderful and ancient joys of parenthood, the pain of failure, and the deep motivation needed in donating and receiving an urgently needed oocyte or a surrogate uterus. Parenthood is more responsible than ever before. Its complex choices are gathered before couples everywhere by the information revolution, placing family responsibilities on patients themselves, where it really matters. And IVF now reveals more and more about miracles preserved in embryogenesis from flies and frogs to humankind, over 600 million years of evolution.

The Human Genome Project is now complete and will inevitably assist IVF since we will soon understand the genetic aspects of early embryo growth and how to detect abnormal genes in embryos. This textbook contains chapters that describe in detail the several advances and developments that have expanded the possibilities of treating diverse causes of human infertility as well as numerous genetic disorders.

Already it is clear that a staggering array of genes operate in preimplantation stages in mammalian including human embryos, and new methods are being introduced to deal with such highly multigenic embryonic systems. We are indeed enmeshed in a field embracing some of the most fundamental evolutionary stages of our existence as we pass from oocyte to blastocyst and to implantation.

REFERENCES

1. Pincus G, Saunders B. The comparative behavior of mammalian eggs *in vivo* and *in vitro*. VI. The maturation of human ovarian ova. *Anat Rec* 1939; 75: 537–45.
2. Menkin MF, Rock J. *Am J Obstet Gynecol* 1949; 55: 440.
3. Hayashi M. Seventh International Conference of the International Planned Parenthood Federation. *Excerpta Medica* 1963: 505.
4. Austin CR. *Adv Biosci* 1969; 4: 5.
5. Chang M. *Adv Biosci* 1969; 4: 13.
6. Chang MC. The maturation of rabbit oocytes in culture and their maturation, activation, fertilization and subsequent development in the fallopian tubes. *J Exp Zool* 1955; 128: 379–405.
7. Runner M, Gates AH. Sterile, obese mothers. *J Hered* 1954; 45: 51–5.
8. Gates AH. Viability and developmental capacity of eggs from immature mice treated with gonadotrophins. *Nature* 1954; 177: 754–5.
9. Fowler RE, Edwards RG. Induction of superovulation and pregnancy in mature mice by gonadotrophins. *J Endocrinol* 1957; 15: 374–84.
10. Edwards RG, Gates AH. Timing of the stages of the maturation divisions, ovulation, fertilization and the first cleavage of eggs of adult mice treated with gonadotrophins. *J Endocrinol* 1959; 19: 292–304.
11. Edwards RG. Meiosis in ovarian oocytes of adult mammals. *Nature (London)* 1962; 196: 446–50.
12. Cole R, Edwards RG, Paul J. Cytodifferentiation and embryogenesis in cell colonies and tissue cultures derived from ova and blastocysts of the rabbit. *Dev Biol* 1966; 13: 385–407.
13. Edwards RG. Maturation *in vitro* of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature* 1965; 208: 349–51.
14. Edwards RG. Maturation *in vitro* of human ovarian oocytes. *Lancet* 1965; 2: 926–9.
15. Edwards RG, Donahue R, Baramki T, Jones H, Jr. Preliminary attempts to fertilize human oocytesmatured *in vivo*. *Am J Obstet Gynecol* 1966; 96: 192–200.
16. Yanagimachi R, Chang MC. *J Exp Zool* 1964; 156: 361–76.
17. Edwards RG, Talbert L, Israestam D et al. Diffusion chamber for exposing spermatozoa to human uterine secretions. *Am J Obstet Gynecol* 1968; 102: 388–96.
18. Steptoe PC. Laparoscopy and ovulation. *Lancet* 1968; 2: 913.
19. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilisation *in vitro* of human oocytes matured *in vitro*. *Nature (London)* 1969; 221: 632–5.
20. Gardner RL, Edwards RG. Control of the sex ratio at full term in the rabbit by transferred sexed blastocysts. *Nature (London)* 1968; 218: 346–8.
21. Henderson SA, Edwards RG. Chiasma frequency and maternal age in mammals. *Nature (London)* 1968; 218: 22–8.
22. Edwards RG, Sharpe DJ. Social values and research in human embryology. *Nature (London)* 1971; 231: 81–91.
23. Palmer R. *Acad Chir* 1946; 72: 363.
24. Fragenheim H. *Geburts Frauenheilkd* 1964; 24: 740.
25. Steptoe PC. *Laparoscopy in Gynaecology*. Edinburgh: Livingstone, 1967.
26. Lunenfeld B. In: Inguilla W, Greenblatt RG, Thomas RB, eds. *The Ovary*. Springfield, IL: CC Thomas, 1969.
27. Steptoe PC, Edwards RG. Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins. *Lancet* 1970; 1: 683–9.
28. Edwards RG, Steptoe PC, Purdy JM. Fertilization and cleavage *in vitro* of preovulatory human oocytes. *Nature (London)* 1970; 227: 1307–9.
29. Steptoe PC, Edwards RG, Purdy JM. Human blastocysts grown in culture. *Nature (London)* 1971; 229: 132–3.
30. Edwards RG, Surani MAH. The primate blastocyst and its environment. *Uppsala J Med Sci* 1978; 22: 39–50.
31. Csapo AI, Pulkkinen MO, Kaihola HL. The relationship between the timing of luteectomy and the incidence of complete abortions. *Am J Obstet Gycecol* 1974; 118: 985–9.
32. Steptoe PC, Edwards RG. Reimplantation of a human embryo with subsequent tubal pregnancy. *Lancet* 1976; 1: 880–2.
33. Edwards RG, Steptoe PC, Purdy JM. Clinical aspects of pregnancies established with cleaving embryos grown *in vivo*. *Br J Obstet Gynaecol* 1980; 87: 757–68.

34. Trounson AO, Leeton JF, Wood C et al. Pregnancies in humans by fertilization *in vitro* and embryo transfer in the controlled ovulatory cycle. *Science* 1981; 212: 681–2.
35. Zeilmaker GH, Alberda T, Gent I et al. Two pregnancies following transfer of intact frozen–thawed embryos. *Fertil Steril* 1984; 42: 293–6.
36. Handyside A, Kontogianni EH, Hardy K, Winston RML. Pregnancies from biopsied human preimplantation embryos sexed by Y-specifi c DNA application. *Nature (London)* 1990; 344: 768–70.
37. Wikland M, Enk L, Hamberger L. Transvesical and transvaginal approaches for the aspiration of follicles by use of ultrasound. *Ann NY Acad Sci* 1985; 442: 182–94.
38. Palermo G, Joris H, Devroey P et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992; 340: 17–18.
39. Hansis C, Edwards RG. Cell differentiation in the preimplantation human embryo. *Reprod BioMed Online* 2003; 6: 215–20.

Robert G. Edwards and the thorny path to the birth of Louise Brown: A history of *in vitro* fertilization and embryo transfer

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INTRODUCTION

Robert G. Edwards was awarded the 2010 Nobel Prize for Physiology or Medicine "for the development of *in vitro* fertilization" (1). There is a variety of accounts of the events leading up to this discovery and its acceptance, most of them by participants (2), but historical scholarship is rarer (3). This article is based on research undertaken partly in preparation for the introductory lecture to the Nobel Symposium celebrating the award of the 2010 Nobel Prize in Physiology or Medicine to Robert G. Edwards, and partly conducted since then. It is based on a paper published originally in 2011 (1), but adds considerably to that paper by use of verifiable sources to produce a historical narrative of the path to *in vitro* fertilization (IVF) and the birth of Louise Brown that differs in a number of places from the conventionally accepted version and adds further detail. It tries to make clear what a difficult birth IVF had, something often overlooked by current practitioners.

Primary sources used were the publications by Edwards and Steptoe between the 1950s and 1980s; the National Archives, the archives of the Royal Society of Medicine, Cambridge University, the British Medical Association, Churchill College, Cambridge, the Physiology Library at Cambridge, the National Institute for Medical Research (NIMR) at Mill Hill and the personal papers of Robert G. Edwards (courtesy of the Edwards family); transcripts of interviews with Robert G. Edwards (unpublished), with K. Elder and R.L. Gardner (available from the British Library Oral History Section), and with Grace MacDonald (4), Noni Fallows, Sandra Corbertt, and John Webster (5); personal recollections from the late 1970s by Edwards and Steptoe as recalled in interviews with Danny Abse for the autobiographical account *A Matter of Life* and on film with Peter Williams; and members of Robert G. Edwards' family and his colleagues and former students and staff members for clarificatory evidence about personal recollections by Edwards, for additional verifiable information and with whom to test some new interpretations.

CHILDHOOD BACKGROUND

Robert G. Edwards was born on September 27, 1925 into a working-class family in the small Yorkshire mill town of Batley. Edwards, who was known by his middle name of Geoff until he was 18, was the second of three brothers, between an older brother, Sammy, and the younger, Harry (2). Sammy was named after his father, Samuel, who was frequently away from home working on the railways, maintaining the track in the Blea Moor tunnel on the Carlisle–Settle line. It was an unhealthy place to work, filled with coal-fired smoke that exacerbated Samuel's bronchitis, a consequence of being gassed in World War I. Edwards' mother, Margaret, was a machinist in a local mill. She came originally from Manchester, to where the family relocated when Edwards was about five, having been offered the relative security of a council house in the suburb of Gorton. It was in Manchester that Edwards received his education: bright working-class children could take a scholarship exam at age 10 or 11 to compete for the few coveted places at a grammar school, the potential pathway out of poverty and even to university. All three brothers passed the exam, but Sammy decided against grammar school, preferring to leave education as soon as he could to earn. His mother was reportedly furious at this wasted opportunity, and so when her two younger sons passed the exam, there was no doubting that they would continue in education, and so it was that that Geoff/Bob progressed in 1937 to Manchester Central Boy's High School, which also claims James Chadwick FRS (1891–1974) as an alumnus. Chadwick, like Edwards, became a Cambridge professor and was awarded the 1935 Nobel Prize in Physics for discovering the neutron (6). The Edwards family's summers were spent in the Yorkshire Dales, to where their mother took her sons to be closer to their father's place of work. There, Edwards labored on the farms and developed an enduring affection for the Dales. These early experiences were formative for Edwards in three ways. Thus, Edwards became a life-long egalitarian, for five years a Labour Party councilor and almost selected as the Labour parliamentary candidate for Cambridge (7), willing to listen to and talk with all and sundry, regardless of class, education, status, and background. Second he also developed an enduring curiosity about agricultural and natural history and especially the reproductive patterns among the Dales' sheep, pigs, and cattle. Finally, he claimed great pride in being a "Yorkshire man," traditionally having attributes of affability and generosity of spirit combined with no-nonsense blunt speaking. Indeed, following his only meeting with Gregory Pincus (1903–1967 [8]) at a conference in Venice in May 1966, at which Edwards, the young pretender, clashed with the "father of the pill" over the timing of egg maturation in humans, he paid

Figure II.1 Edwards on National War Service in the 1940s (courtesy of the Edwards family).

Pincus the biggest compliment he could then imagine, saying, "He would have made a fine Yorkshireman!" (2).

The intervention of World War II provided an unwelcome interruption to Edwards' education, for, after leaving school in 1943, he was conscripted for war service into the British Army for almost four years (Figure II.1). To his surprise, as someone from a working-class family, he was identified as potential officer material and sent on an officer-training course, before being commissioned in 1946. However, his army experiences were broadly negative, the alien lifestyle of the officers' mess reinforcing his socialist ideals. The one positive feature of his war service was the chance to travel overseas; he particularly appreciated his time in the Middle East. The years in the army were broken by nine months' compassionate leave back in the Yorkshire Dales, for which he was released to help run a farm when his farmer friend there fell ill. So engaged did he become in farming life that, after discharge from the army in 1948, he returned home to Gorton, from where he applied to read agricultural sciences at the University College of North Wales at Bangor and gained both a place and a government grant to fund it. However, he was disappointed in the course offered at Bangor, describing it as not "scientific," and he was bored through two tedious years of agricultural descriptions. For his third year, he transferred to zoology, a course much more to his style and led by the more intellectually challenging Rogers Brambell FRS (1901–1970 [9]). However, that year was insufficient to salvage his honors degree, and in 1951, aged 26, he gained a simple pass. Unbeknown to him at the time, he was not alone in this undistinguished academic embarrassment, as neither "Tibby" Marshall FRS (1878–1949 [10]), the founder of the reproductive sciences, nor Sir Alan Parkes FRS (1900–1990 [11]), the first Professor of Reproductive Sciences at Cambridge, who was later to recruit Edwards there, distinguished themselves as undergraduates. In 1951, however, Edwards "was disconsolate. It was a disaster. My grants were spent and I was in debt. Unlike some of the students I had no rich parents … I could not write home, 'Dear Dad, please send me £100 as I did badly in the exams'" (2).

However, his low spirits did not last long. He learnt that John Slee (12), a life-long friend he had made at Bangor, had been accepted on a postgraduate diploma course in animal genetics at Edinburgh University under Conrad Waddington FRS (1905–1975 [13]), who had moved there in 1947 from Christ's College in Cambridge, home also to both Marshall and Parkes. Edwards applied and, despite his pass degree and to his amazement, he was accepted. That summer, he worked harvesting hay, portering bananas, heaving sacks of flour, and in a menial job with a newspaper, all to earn enough to pay his way in Edinburgh (2).

FAMILY LIFE

In Edinburgh, Edwards not only started to map out his scientific career, but importantly also met Ruth Fowler (Figure II.2), who was to become his life-long scientific collaborator and whom he was to marry in 1954, with their five daughters arriving between 1959 and 1964. When they met, Edwards claims that he was initially somewhat overwhelmed, even "intimidated" by Ruth's august family background. Her father, Sir Ralph Fowler FRS (1889–1944 [14]), and her maternal grandfather, Lord Ernest Rutherford FRS (1871–1937 [15]), were not only both "titled," but both also had the most impressive academic credentials imaginable: a world away from a working-class Northern family. Ralph Fowler was an exceptionally talented Plummer Professor of Mathematical Physics in Cambridge from 1932 to 1944 (14). Back in Cambridge in 1919 after World War I, he was stimulated to work with Rutherford, who had recently arrived there to take the chair of Experimental Physics. Rutherford was the first Nobel laureate in Ruth's family, having been awarded the 1908 Nobel Prize for Chemistry "for his investigations

Figure II.2 Ruth Fowler in the laboratory, Edinburgh, in the 1950s (courtesy of the Edwards family).

into the disintegration of the elements, and the chemistry of radioactive substances" (15). Ralph Fowler not only worked under Rutherford, but, in the course of doing so, met his only daughter, Eileen, whom he married in 1921. They had four children, of whom Ruth was the last, born in December 1930. Tragically her mother died shortly afterwards and her father, although himself unwell, undertook such grueling high-security war work, much of it away in North America, that his health deteriorated and he died at the relatively young age of 55 when Ruth was 13. Thus, Ruth was to know only Mrs. Phyllida Cook as her parent (14).

EDWARDS, THE RESEARCH SCIENTIST

The intellectual spirit of scientific enquiry that Edwards experienced in Edinburgh fitted his aptitudes well, for Waddington rewarded his Diploma year with a three-year PhD place (1952–1955), followed by two years of postdoctoral research, and funded it with a salary of the princely sum of £240 per year (2). His chosen field of research was the developmental biology of the mouse. Edwards realized that to understand development involved engaging in an interdisciplinary mix, not just of embryology and reproduction—the conventional view at the time—but also of genetics. Given the increasing scientific and social emphasis on genetics over the last 50 years, it is important to understand how advanced this view was in the 1950s, when genetic knowledge was still rudimentary and largely alien to the established developmental and reproductive biologists of the day, as Edwards himself was later to recall (16). For example, it was in the 1950s that DNA was established as the molecular carrier of genetic information (17–20), that it was first demonstrated that each cell of the body carried a full set of DNA/genes (21–23), and that genes were selectively expressed as mRNA to generate different cell phenotypes (24). Moreover, it was only by the late 1950s that cytogenetic studies led to the accepted human karyotype as 46 chromosomes (25,26), that agreement was reached on the Denver system of classification of human chromosomes (27), and that the chromosomal aneuploidies underlying developmental anomalies such as Down, Turner, and Klinefelter syndromes were described (28–31). The dates of these discoveries make Edwards' research between 1952 and 1957 all the more remarkable. Working under his supervisor Alan Beatty, he generated haploid, triploid, and aneuploid mouse embryos and studied their potential for development. In order to undertake what were, in effect, early attempts at "genetic engineering" in mammals, he needed to be able to manipulate the chromosomal composition of eggs, spermatozoa, and embryos.

In mice, spermatozoa were abundant, and were studied in experiments mostly undertaken with a visiting Argentinean postdoc, Julio Sirlin (Figure II.3) (2). Together they labeled spermatozoa radioactively *in vivo* in order to study the kinetics of spermatogenesis and then to follow the radioactive products post-fertilization, thereby to demonstrate the fate of the male contributions during

Figure II.3 Julio Sirlin with Edwards in the 1950s (courtesy of Julio Sirlin).

early development. They also exposed males and/or their spermatozoa to various chemical mutagens and UV or x-ray irradiation, and examined the effects on sperm-fertilizing capacity and, where it was shown to be present, how the treatments impacted on development. In some cases, sperm activation of the egg was evident, but in the absence of any functional sperm chromatin, and so gynogenetic embryos were formed. These experiments resulted in 14 papers, including four in *Nature*, between 1954 and 1959 (see Gardner and Johnson [32] for a full bibliographic record of Edwards).

Eggs and embryos were not as abundant as spermatozoa, and overcoming this problem led Edwards to two discoveries that proved to be of particular significance for his later IVF work. First, working with his wife Ruth, he devised ways of increasing the numbers of synchronized eggs recoverable from adult female mice through a series of papers, the first published in 1957 (33), on the control of ovulation induced by use of exogenous hormones. In doing so, they overturned the conventional wisdom that superovulation of adults was not possible. Second, working with an American postdoc, Alan Gates (34), Edwards described the remarkable timed sequence of egg chromosomal maturation events that led up to ovulation after injection of the ovulatory hormone, human chorionic gonadotropin.

His six years in Edinburgh, between 1951 and 1957, give an early taste of his prodigious energy, resulting in 38 papers (32). Indeed, so productive was this period that the last of the Edinburgh-based papers did not appear in print until 1963. These papers firmly placed the young Edwards at the forefront of studies on the genetic manipulation of development and started to attract attention. It was also in Edinburgh that Edwards' interest in ethics was first sparked by the interdisciplinary debates among scientists and theologians that Waddington organized, and, as a result, he went on what he describes as a "church crawl," trying the 10 or so variants of Christianity on offer in 1950s Edinburgh. He did not emerge from his consumer testing "God-intoxicated" (2), but convinced that man held his own future in his own hands. Edwards' humanist ethical sympathies and antipathy to the "revealed truths"

of religion were to be developed further in all his later encounters (32).

AN AMERICAN DIVERSION

These 1950s studies in science and ethics were to form the platform on which Edwards' later IVF work was to be based, but before that his interests and life took a diversion to the California Institute of Technology for the year 1957–1958. He describes his year at Caltech as being "a bit of a holiday," but it was a holiday that with hindsight had both distracting and significant consequences. He went there to work with Albert Tyler (1906–1968 [35]), an influential elder statesman of American reproductive science, working on spermatozoon–egg interactions. Caltech was then a hotbed of developmental biology, and Tyler had clustered around him an exciting group of young scientists, which included that year a visit by the then English doyen of fertilization, Lord Victor Rothschild FRS (1910–1990 [36]). Rothschild was later to clash scientifically with Edwards over his IVF work (37), a clash in which the younger man triumphed again (38), just as he had with Pincus. Tyler was exploring the molecular specificity of egg–spermatozoon interactions and had turned for a model to immunology. Immunology was then at an exciting phase in its development, with the engaging Sir Peter Medawar FRS (1915–1987, Nobel laureate in Physiology or Medicine, 1960 [39]), influentially for Bob, extending his ideas on immunological tolerance to the paradox of the "fetus as an allograft": a semi-paternal graft nonetheless somehow protected from maternal immune attack inside the mother's uterus (40). This confluence of reproduction and immunology excited Edwards' restless curiosity and hence the choice of Tyler. Significantly, the subject also offered funding possibilities via the Ford and Rockefeller Foundations and the Population Council, which were increasingly concerned about world population growth and the need for better methods to control fertility (41–43). Immuno-contraception then seemed to offer tantalizingly specific possibilities, alas not much closer to being realized today (44).

So when Edwards returned to the United Kingdom from Caltech in 1958 at Alan Parkes' invitation to join him at the Medical Research Council (MRC) National Institute for Medical Research (NIMR) in north London, it was to work on the science of immuno-contraception (7). This period in the U.S.A. initiated a series of 23 papers on the immunology of reproduction between 1960 and 1976 (32). It also prompted Edwards' first involvement in founding an international society in 1967 in Varna, Bulgaria, when the International Coordinating Committee for the Immunology of Reproduction was created (45). Immuno-reproduction was, in retrospect, to prove a distracting diversion from what was to become Edwards' main work, albeit one that continued to enthuse and stimulate his imagination for many years. Indeed, it was his research into immuno-reproduction that led serendipitously to his first meeting with Patrick Steptoe (see later). The period at Mill Hill, between 1958 and 1962, seems to have been a period of increasing intellectual conflict for him. While being enthusiastic about the science underlying immuno-contraception, his old interests in eggs, fertilization, and, in particular, the genetics of development were gradually reasserting themselves. His day job was therefore increasingly supplemented by evening and weekend flirtations with egg maturation.

THE CRUCIAL EGG MATURATION STUDIES

The stimulus that reawakened Edwards' interest in eggs was provided by the then recent consensus about the number of human chromosomes and, more particularly, the descriptions in 1959 of the pathologies in man that resulted from chromosomal anomalies (28–31). Thus, his 1962 *Nature* paper begins: "Many of the chromosomal anomalies in man and animals arise through non-disjunction or lagging chromosomes during meiosis in the oocyte. Investigation of the origin and primary incidence of such anomalies would be greatly facilitated if meiotic stages etc., were easily available" (46). The idea that these aneuploidies in humans might result from errors in the complex chromosomal dance that he and Gates had observed in maturing mouse eggs drove his thinking. The possible clinical relevance of his work on egg maturation and aneuploidy in the mouse was becoming significant. So Edwards resumed his experimenting with mice, trying to mimic *in vitro* the *in vivo* maturation of eggs, one rationale being that this route would open the possibility of similar studies in humans, in which not even induced ovulation had then been described (47). He tried releasing the immature mouse eggs from their ovarian follicles into culture medium containing the ovulatory hormone human chorionic gonadotropin, to explore whether he could simulate their *in vivo* development. Amazingly, he found it worked first time; the eggs seemed to mature at the same rate as they had *in vivo*. However, they did so whether or not the hormone had been added. The eggs evidently were maturing spontaneously when released from their follicles. The same happened in rats and hamsters. If this were to happen in humans too, then the study of the chromosomal dance during human egg maturation was a realistic practical possibility, as was IVF and thereby studies on the genetics of early human development. Edwards' excitement at seeing eggs mature spontaneously was temporarily blunted by his library discovery that Pincus in the 1930s (48,49) and M.C. Chang (1908–1991 [50,51]) earlier in the 1950s had been there before him, using both rabbit and, Pincus claimed, human eggs.

In order to pursue his cytogenetic studies on the maturation of human eggs, he needed a reliable supply of human ovarian tissue from which to retrieve and mature eggs. This requirement posed difficulties for a scientist with no medical qualification, given the elitist attitudes and lack of scientific awareness then prevalent amongst most of the U.K. gynecological profession (3,52,53). His first breakthrough came with Molly Rose, who was a gynecologist at the Edgware General Hospital, northwest London, near Mill Hill. Edwards was introduced to her through John

Humphrey FRS (1915–1997 [54]), who was the medically qualified Head of Immunology at Mill Hill. Humphrey, notwithstanding his more privileged social background, was a kindred spirit for Edwards, sharing his passion for science, its social application and utility, as well as his left-wing politics; indeed, he had been a Marxist until 1940 and was for many years denied entry to the U.S.A. in consequence. Edwards asked Humphrey if he knew anyone who might be helpful, and he not only suggested Rose, but also offered to arrange an introduction. Rose was to provide biopsied ovarian samples intermittently for the next 10 years.

Between 1960 and 1962, Edwards used human ovarian biopsies provided by Rose to try to repeat and extend Pincus' observations from the 1930s. Given the sporadic supply of human material, he also tried dog, monkey, and baboon ovarian eggs, but in all cases with limited success compared with smaller rodents. In the 1962 *Nature* paper (46), he cautiously interprets the few maturing human (3/67), monkey (10/56), and baboon (13/90) eggs that he had observed as most likely arising from *in vivo* stimulation, rendering them partially matured at the time of their recovery from the biopsy. He suggests that Pincus' observations on human eggs are also likely to be similarly artefactual, the source of his Venice spat with Pincus some four years later (*vide supra*). This 1962 paper ends with the report of an ingenious experimental approach to try and persuade the reluctant human eggs to mature. Thus, the ovarian arteries of patients undergoing ovarian removal were cannulated and perfused with hormones post-removal, perhaps unsurprisingly in retrospect, without success.

However, by this time, his quest for human eggs, and his dreams of IVF and studying the genetics and development of early human embryos, had reached the ears of the then Director of the Institute, Sir Charles Harington FRS (1897–1972 [55]), who, Edwards alleged (2), banned any work on human IVF at NIMR. Alan Parkes was no longer able to defend Edwards, having left in 1961 to take up his chair in Cambridge and, although he had asked Edwards to join him there, funding was not available until 1963. So by the time Edwards left Mill Hill in 1962 for a year in Glasgow, he had encountered a taste of the opposition to human IVF that was to come.

GLASGOW AND STEM CELLS

Edwards had accepted an invitation from John Paul to spend a year in the biochemistry department at Glasgow University. Paul was then the acknowledged master of tissue culture in the U.K. and had got wind of some experiments that Edwards had been doing at NIMR attempting to generate stem cells from rabbit embryo cultures (56,57). The objective of this strategy was to use these stem cells to study early developmental mechanisms, either *in vitro* or *in vivo* after their incorporation into embryos. Paul had proposed that they work together, with fellow Glasgow biochemist Robin Cole, to see what progress might be made. This must have been an attractive invitation, not simply because the challenge was scientifically interesting, but also because Edwards could learn more about culture media for his eggs and hopefully later embryos, then an uncertain prospect, with successful mouse embryo culture only recently having been described (58). However, by this time, the Edwards family was growing, so Ruth remained in north London with their young daughters, while her husband commuted to Glasgow for the working week.

The collaboration was to result in two papers (56,57), remarkable for their prescience. They described the production of embryonic stem cells from both rabbit blastocysts and the inner cell masses dissected from them. The cells were capable of proliferating through over 100 generations and of differentiating into various cell types. These experiments were initiated some 20 years before Evans and Kaufman (59) described the derivation of embryonic stem cells from mice. That this work has largely been ignored by those in the stem cell field is probably mainly attributable to its being too far ahead of its time (60). Thus, reliable molecular markers for different types of cells were not available then, nor were appropriate techniques with which to critically test the developmental potential of the cultured cells.

THE MOVE TO CAMBRIDGE

Edwards arrived in Cambridge from Glasgow in 1963 as a Ford Foundation Research Fellow, and settled with Ruth and his five daughters in a house in Gough Way, off the Barton Road. He had previously visited Cambridge at least once, as "a recently graduated PhD" in the late 1950s for a conference on reproduction held in Trinity College (Figure II.4), where he recalls meeting some of the big names in the subject, including John Hammond, Alan Parkes, M.C. Chang, Thaddeus Mann, Rene Moricard, Bunny Austin, and Charles Thibault (16). Although Edwards was to remain in Cambridge for the rest of his career, in 1963 his initial reactions to the place were mixed. He describes how he immediately reacted against the then extant "misogynist public-school traditions; the exclusivity," "the privileges given to the already privileged." But he set against that the "sheer beauty of the place," "the concern with the truth and high seriousness," "the ambience of scientific excellence … I was surrounded by so many talented young men and women" (2).

Edwards worked in a cluster of seven smallish rooms at the top of the Physiological Laboratory backing onto Downing Place, which were collectively known as the "Marshall laboratory" and were to be shared eventually with two other groups. One group was led initially by Sir Alan Parkes, the first Mary Marshall, and Arthur Walton, Professor of Reproductive Physiology at the University (11), who had arrived in 1961. His group included scientists with mainly zoological or comparative interests, such as his wife Ruth Deansley, Bunny Austin, and Dick Laws FRS, who was often away "in the field" with Parkes collecting material, especially in Uganda at the Nuffield Unit of Tropical Animal Ecology (11). Parkes was also much involved at this time in writing and committee work,

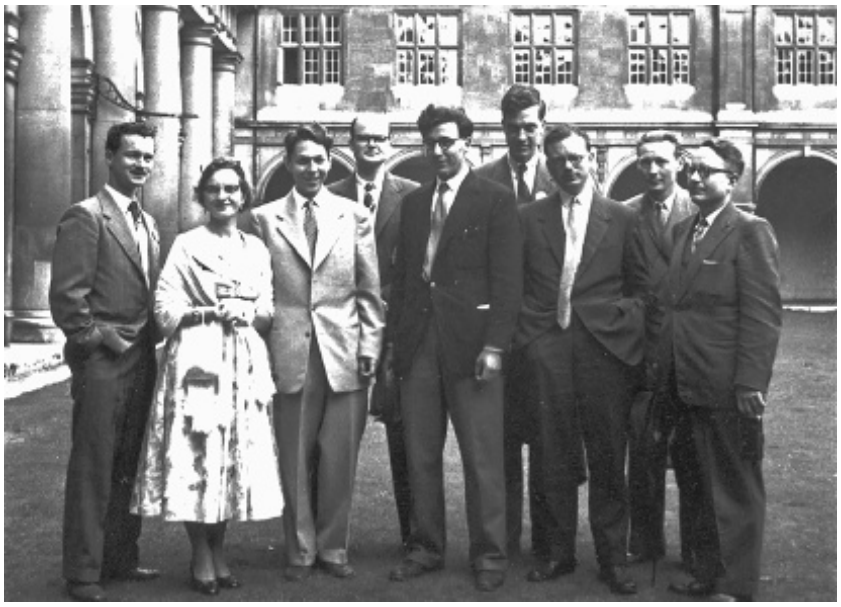


Figure II.4 Edwards as "a very recent PhD student" (center) and Alan Gates (extreme left) at a meeting in Trinity College Cambridge in the late 1950s (courtesy of the Edwards family).

especially with the World Health Organization, which was then becoming concerned about world population growth and ways to curb it (11). Parkes was also acting as an unpaid company secretary to the then fledgling *Journal of Reproduction and Fertility* (called *Reproduction* since 2001 [61–63]). In 1967, Parkes retired. Edwards applied for his chair on January 6, 1966 (64), but was unsuccessful, the chair passing instead to Thaddeus Mann FRS (1908–1993 [65]), who worked on the biochemistry of semen. Mann decided not to relocate to the Physiology Laboratory from his Cambridge base at the Agricultural Research Council Unit of Reproductive Physiology and Biochemistry at Huntingdon Road, where he was Director. Neither was the leadership of the Marshall laboratory to pass to Edwards, as the University appointed as its head his more senior colleague and friend Colin "Bunny" Austin (1914–2004 [66]), who had been in Cambridge intermittently since 1962 (Figure II.5). Austin was elected the first Charles Darwin Professor of Animal Embryology (1967–1981) and began attracting several upcoming reproductive biologists to the Marshall laboratory, including John Marston, David Whittingham, and Matthew Kaufmann. In addition, a new group was formed in 1967 with the arrival from the Strangeways laboratory of Denis New (1929–2010) as university lecturer in histology (67). New built a group comprising initially PhD students Chris Steele and David Cockroft, later joined by postdoc Frank Webb (1976–1977), and visiting scientists such as Joe Daniels Jr, on leave from the University of Colorado.

It was against this varied scientific background that Edwards, who was already 38 when he arrived in Cambridge, began for the first time to assemble his own group. He recruited as his technician Jean Purdy (Figure II.6) in 1968, one of her attractions being her nursing qualification, a sign of the increasing importance that his forays into the use of clinical material was assuming. Purdy was to stay with him until her early death at age 39 in 1985 (68). He also recruited his first two graduate students: Richard Gardner and this author in 1966 (69,70). Gardner studied early mouse embryology from 1966 to 1971 and until 1973 as a postdoctoral worker, before moving to zoology in Oxford. This author worked on immunoreproduction from 1966 to 1969, returning as a postdoc between 1971 and 1974 after two years in the U.S.A., before moving to the Anatomy Department in Cambridge.

From 1969 onwards, Edwards' group increased in size substantially as more accommodation was made available

Figure II.5 Edwards with "Bunny" Austin (1960s) (courtesy of the Edwards family).

Figure II.6 Jean Purdy (1946–1985) (courtesy of Barbara Rankin).

to the Marshall laboratory. David Griffin (now retired from the World Health Organization) was to join as Head Technician between 1970 and 1975, with junior technicians including Sheila Barton (1936–2013) in addition to Jean Purdy. Early graduate students recruited included Roger Gosden (1970–1974), Carol Readhead (1972–1976), and Rob Gore-Langton (1973–1978), all working on follicle growth; Craig Howe (1971–1974) working on immuno-reproduction; and Azim Surani (1975–1979) working on implantation. A "third generation" of graduate students also arrived; for example, Janet Rossant (from 1972) studied with Gardner, and Alan Handyside (from 1974), Peter Braude (from 1975), and Ginny Bolton (from 1976) studied with Johnson. Postdoctoral workers also arrived, including Ginny Papaioannou (1971–1974), and Ruth Fowler-Edwards resumed working in the laboratory, developing hormonal assays and studying the endocrine aspects of follicle development and early pregnancy. Thus, slowly until 1969, and more rapidly thereafter, Edwards built a lively group, its members working in diverse areas of reproductive science that reflected his own broad interests and knowledge. Moreover, Edwards encouraged a spirit of open communication and egalitarianism, which extended across all three groups, with sharing of resources, space, equipment, knowledge, and ideas, as well as social activities.

Through the 1960s and 1970s, Edwards' work was funded by the Ford Foundation via grants first to Parkes and then to Austin (71) to continue work on basic reproductive mechanisms, with an eye to developing new methods of fertility control, and he continued to pursue the immunology of reproduction. However, he also worked on egg maturation, collecting pig, cow, sheep, the odd monkey, and some human eggs. He showed that eggs of all these species would indeed mature *in vitro*, but that the eggs of larger animals simply needed a longer time than those of smaller ones, with human eggs taking up to 36 hours rather than the 12 hours or less erroneously reported by Pincus. These cytogenetic studies were reported in two seminal papers in 1965 (72,73), both of which are primarily concerned with understanding the kinetics of the meiotic chromosomal events during egg maturation. In its discussion, the *Lancet* paper displays a breathtaking clarity of vision as Edwards sets out a program of research that predicted the events of the next 20 years and beyond (Table II.1). Significantly, if not surprisingly given his research interests, the early study and detection of genetic disease is afforded a heavy focus compared with the slight emphasis on infertility

Table II.1 Key points in the program of research laid out in the discussion to Edwards' 1965 *Lancet* paper

1. Studies on non-disjunction of meiotic chromosomes as a cause of aneuploidy in humans[a]
2. Studies on the effect of maternal age on non-disjunction in relation to the origins of trisomy 21[a]
3. Use of human eggs in *in vitro* fertilization (IVF) to study fertilization
4. Study of culture methods for human eggs fertilized *in vitro*
5. Use of priming hormones to increase the number of eggs per woman available for study/use
6. Study of early IVF embryos for evidence of (ab)normality—especially aneuploidies arising prior to or at fertilization[a]
7. Control of some of the genetic diseases in man[a]
8. Control of sex-linked disorders by sex detection at the blastocyst stage and transfer of only female embryos[a]
9. Intracervical transfer of IVF embryos into the uterus
10. Use of IVF embryos to circumvent blocked tubes[b]
11. Avoidance of a multiple pregnancy (as observed after hormonal priming and *in vivo* insemination) by transfer of a single IVF embryo

Source: Edwards RG. *Lancet* 1965; 286: 926–9.

[a] Five aims relating specifically to genetic disease.

[b] One aim relating specifically to infertility relief.

alleviation. This genetic focus continues in his research papers over the next four years. Thus, within three years, working with his graduate student Richard Gardner, he provided proof of principle for preimplantation genetic diagnosis (PGD) in a paper on rabbit embryo sexing published in 1968 (74), a paper that was to anticipate the development of PGD clinically by some 22 years (75). Likewise, working with the Cambridge geneticist Alan Henderson, Edwards was to develop his "production line theory" of egg production to explain the origins of maternal aneuploidy in older women. Thus, the earliest eggs to enter meiosis in the fetal ovary were shown to have more chiasmata and to be ovulated earlier in adult life than those entering meiosis later in fetal life (76,77).

THE PROBLEM OF FERTILIZATION OF THE HUMAN EGG

Notwithstanding his broad range of scientific interests, Edwards' ambitions to achieve IVF in humans remained undiminished. In 1966, this was no trivial task, having been accomplished convincingly only in rabbits and hamsters (78,79). In trying to achieve this aim, he was engaging in two struggles: the first being simply but critically the continuing practical difficulty in obtaining a regular supply of human ovarian tissue. Local Cambridge sources proved unreliable and Molly Rose was now two to three hours' drive away in London; so, during the summer of 1965, Edwards turned to the U.S.A. for help and approached Victor McKusick, a leading American cytogeneticist at the Johns Hopkins University. There he initiated his longstanding contact with Howard and Georgeanna Jones in obstetrics and gynecology (80). The supply of American eggs they generated during his six-week stay allowed him to confirm the maturation timings that were published the same year.

However, it was the second scientific struggle that was then occupying most of his attention, namely that in order to fertilize these *in vitro*-matured eggs, he had to "capacitate" the spermatozoa, a final maturation process that spermatozoa undergo physiologically in the uterus and that is essential for the acquisition of fertilizing competence. The requirement for sperm capacitation had been discovered in the early 1950s by Austin, and independently by M.C. Chang (81,82). Failing to achieve this convincingly at Johns Hopkins, he made a second transatlantic summer journey in 1966 to visit Luther Talbot and his colleagues at Chapel Hill. He tried a variety of ways (83) to overcome the problem of "sperm capacitation," one of the most ingenious of which was to construct a 2.5 cm-long chamber from a nylon tube, plugged at each end, and with holes drilled in the walls that were encased in panels made of Millipore membrane (84). The chamber, which had a short thread attached to it, fitted snugly inside the inserter tube of an intrauterine device and so could be placed into the volunteer woman's uterus intracervically at mid-cycle, where it sat for up to 11 hours before being recovered by gently pulling on the thread, exactly as was being done routinely for the insertion and removal of intrauterine devices. By placing spermatozoa within the chamber, the membrane of which permitted equilibration of its contents with uterine fluid, he hoped to expose them to a capacitating environment. However, this ingenious approach, like the many others, failed—in this case most probably because the chamber itself induced an inflammatory response or a local bleed. For all the ingenuity of his various experimental approaches to achieving capacitation, and despite the occasional evidence of early stages of fertilization using such spermatozoa, no reliable evidence for the completion of the process was forthcoming. Then, in 1968, both struggles began to resolve.

THE MEETING WITH PATRICK STEPTOE

Patrick Steptoe (1913–1988; Figure II.7) had been a consultant obstetrician at Oldham General Hospital since 1951 (85), where for several years he had been pioneering the development and use of the laparoscope in gynecological surgery (85,86). Much to his frustratio-n, his progress had fallen on the largely deaf ears of the conservative gynecological hierarchy, and indeed incited considerable opposition and some outright hostility (87). Edwards' claimed that he was scanning the medical and scientific journals in the library, and in a "eureka" moment occurring in "one autumn day in 1967" (2), came across a paper by Steptoe describing his experiences with laparoscopy (2,85,88). Edwards goes on to describe how he rang Steptoe to discuss a possible collaboration, but was "warned off" Steptoe by London gynecological colleagues (2,89). This warning and the daunting prospect of collaboration in far-away Oldham deterred him from following through. Finally, Edwards reported actually meeting Steptoe the following spring of 1968 at a meeting at the Royal Society of Medicine, at which, ironically, Edwards was talking about his work on immuno-reproduction, not his attempts at IVF.

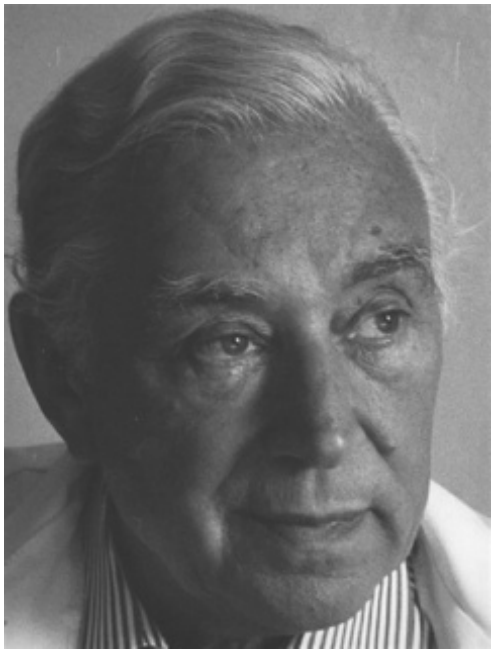
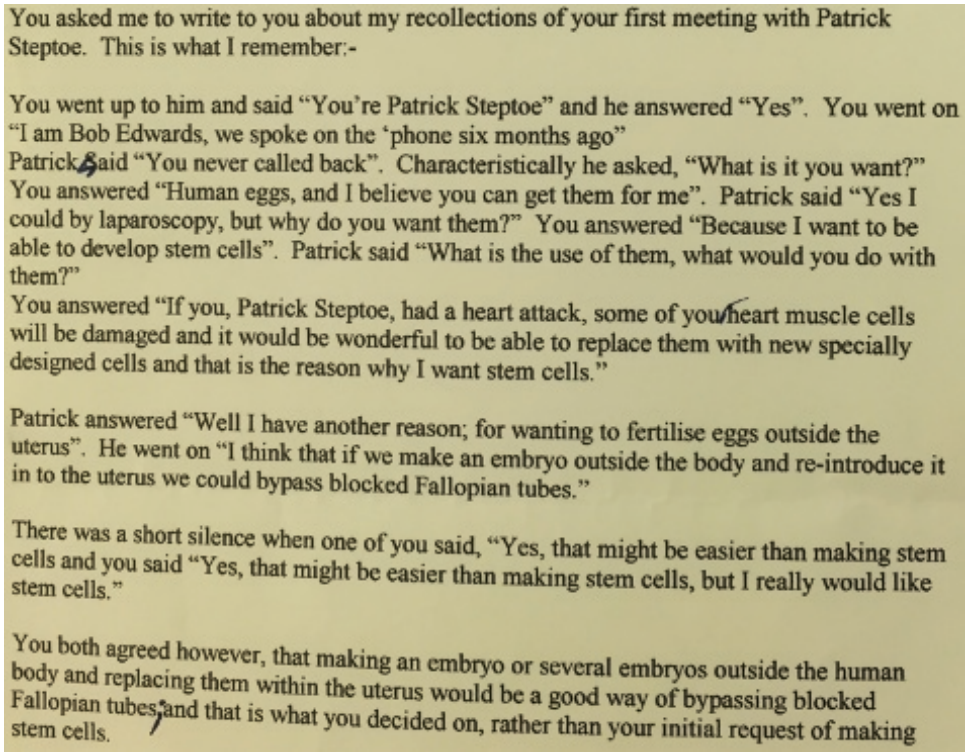


Figure II.7 Patrick Steptoe (1913–1988) (courtesy of Andrew Steptoe).

The Steptoe paper that Edwards found that day in the library was cited in his later tributes to the then deceased Steptoe (85,88) as being a *Lancet* paper entitled "Laparoscopy and ovulation" (90). However, these later recollections do not withstand scrutiny. Thus, the *Lancet* paper cited was published in October 1968, but their first meeting was in fact earlier that year, on Wednesday February 28, 1968, at a joint meeting of the Section of Endocrinology of the Royal Society of Medicine with the Society for the Study of Fertility held at 1 Wimpole Street (1,91). Moreover, according to Steptoe (92), they had already commenced collaborating prior to October 1968; indeed, their first paper together was submitted for publication later that year in December 1968 (see next section). Clearly, the paper read by Edwards must have been another, earlier than October 1968, one that preceded February 1968 by several months. The "paper" by Steptoe that Edwards most likely saw was his book on gynecological laparoscopy (1,86,93,94), and the feature that probably caught his attention, according to two earlier accounts (1,2,89), was his realization that laparoscopy could provide a way of recovering capacitated spermatozoa from the oviduct by flushing with a small volume of medium: "a practical way … of letting spermatozoa be in contact with the secretions of the female tract" (2). Indeed, Edwards says he actually rang Steptoe to ask whether this really was possible and was reassured by him that this was the case. Steptoe explicitly lays out this possibility in his book (86). Thus, on page 27 he reports: "By means of laparoscopy, Sjovall (1964) has carried out extended post-coital tests and has recovered spermatozoa from the fimbriated end of the tubes … "; and on page 70 he writes: "An extended post-coital test can be done by aspirating fluid from the tubal ostium … " Steptoe's book arrived in the Cambridge University library in March 1967 (1) and it is possible that Edwards' attention was drawn to the book by a review of it in the *British Medical Journal* on November 11, 1967 (1,95). This conclusion conflicts with the later memories of Edwards (85,88) that he contacted Steptoe initially because of his ability to recover eggs laparoscopically. However, it is possible that by time they met, some six months later, this had become more of a concern to Edwards, given the emerging reports of the failure of *in vitro*-matured rabbit eggs to produce viable embryos. Indeed, a letter, written admittedly on July 30, 2003, by Eliot Philipp, recalls that at the actual meeting Edwards had said it was eggs that he wanted Steptoe to recover (Figure II.8), albeit for making human stem cells (96), an enduring interest of Edwards.

FERTILIZATION OF THE HUMAN EGG ACHIEVED AT LAST

Despite the initiation of the collaboration with Steptoe, the actual solution to the capacitation problem existed nearer to home than Oldham, in the laboratories shared with Austin. In the early 1950s, Austin had co-discovered the requirement for sperm capacitation (81,82), and after his appointment to the Cambridge chair, Austin's first graduate student (1967–1972) was Barry Bavister, who set



You asked me to write to you about my recollections of your first meeting with Patrick Steptoe. This is what I remember:-

You went up to him and said "You're Patrick Steptoe" and he answered "Yes". You went on "I am Bob Edwards, we spoke on the 'phone six months ago"
Patrick said "You never called back". Characteristically he asked, "What is it you want?"
You answered "Human eggs, and I believe you can get them for me". Patrick said "Yes I could by laparoscopy, but why do you want them?" You answered "Because I want to be able to develop stem cells". Patrick said "What is the use of them, what would you do with them?"
You answered "If you, Patrick Steptoe, had a heart attack, some of your heart muscle cells will be damaged and it would be wonderful to be able to replace them with new specially designed cells and that is the reason why I want stem cells."

Patrick answered "Well I have another reason; for wanting to fertilise eggs outside the uterus". He went on "I think that if we make an embryo outside the body and re-introduce it in to the uterus we could bypass blocked Fallopian tubes."

There was a short silence when one of you said, "Yes, that might be easier than making stem cells and you said "Yes, that might be easier than making stem cells, but I really would like stem cells."

You both agreed however, that making an embryo or several embryos outside the human body and replacing them within the uterus would be a good way of bypassing blocked Fallopian tubes, and that is what you decided on, rather than your initial request of making stem cells.

Figure II.8 Extract from a letter, written on July 30, 2003, by Eliot Philipp to Edwards, recalling his memories of the words used at the first meeting between Edwards and Steptoe (courtesy of the Edwards family).

Table II.2 Summary of data

Egg type	Experimental	Control
Initially assigned	56	17
Survived	54/56	17/17
Matured to metaphase II	34/54	7/17
Evidence of sperm penetration	18/34	
Sperm within the zona pellucida	6/18	
Sperm inside the zona pellucida (~7 hours post-insemination)	5/18	
Evidence of pronuclei (~11 hours post-insemination)	7/18	0/7
With two pronuclei	2/18	

Source: Edwards RG, Bavister BD, Steptoe PC. *Nature* 1969; 221: 632–5.

to work to try and define the factors influencing the capacitation of hamster spermatozoa *in vitro*. By 1968, Bavister had discovered a key role for pH, showing how higher rates of fertilization could be obtained by simply increasing the alkalinity of the medium (97). Edwards seized on this observation and co-opted Bavister to his project. That proved to do the trick, and in December 1968 Edwards, together with Bavister and Steptoe, submitted the paper to *Nature* in which IVF in humans was described convincingly for the first time (38).

This 1969 *Nature* paper makes modest claims. Only 18 of 56 eggs assigned to the experimental group showed evidence of "fertilization in progress," of which only two were described as having the two pronuclei to be expected if fertilization were occurring normally (Table II.2). However, like Edwards' other papers, this one is a model of clarity, describing well-controlled experiments, cautiously interpreted. Despite the relatively small numbers, this paper convinced eventually, although some doubts were expressed at the time (37,98). That this paper convinced where previous claims had failed (99–104) was precisely because the skilled hands and creative intellect that were behind it are so evident from its text.

The provenance of the eggs described in the 1969 paper is not immediately clear from the paper itself. All were obtained by *in vitro* maturation after ovarian biopsy, but in addition to Steptoe's co-authorship, four other gynecologists are thanked in the acknowledgements section of the paper: Molly Rose, Norman Morris (1920–2008; Professor of Obstetrics and Gynecology at Charing Cross Hospital, London from 1958 to 1985 [105]), Janet Bottomley (1915–1995; Consultant Obstetrician and Gynecologist at Addenbrooke's Hospital, Cambridge from 1958 to 1976), and Sanford Markham (b. 1934; Chief of the Section of Obstetrics and Gynaecology at the U.S. Air Force Hospital, South Ruislip, to the northwest of London from 1967 to 1972). An analysis described by Johnson (1) reasonably concludes that those eggs described in the paper as "undergoing fertilization" were provided in roughly equal numbers by Rose and Steptoe.

However, with Steptoe now on board, Rose no longer featured as a supplier of eggs (2). While the initial attraction of laparoscopy for Edwards had been the recovery of capacitated spermatozoa from the oviduct, once working with Steptoe, he rapidly exploited the wider possibilities for the recovery of *in vivo*-matured eggs from the ovary (90). Indeed, the 1969 paper includes the following statement: "Problems of embryonic development are likely to accompany the use of human oocytes matured and fertilized *in vitro*. When oocytes of the rabbit and other species were matured *in vitro* and fertilized *in vivo*, the pronuclear stages appeared normal but many of the resulting embryos had subnuclei in their blastomeres, and almost all of them died during the early cleavage stages … When maturation of rabbit oocytes was started *in vivo* by injecting gonadotropins into the mother, and completed in the oviduct or *in vitro*, full term rabbit fetuses were obtained" (98). The paper goes on to discuss how the use of hormonal priming to stimulate intrafollicular egg maturation might be achieved and reports: "Preliminary work using laparoscopy has shown that oocytes can be recovered from ovaries by puncturing ripening follicles *in vivo* … "

Through these preliminary collaborative studies, Edwards and Steptoe were already building a research partnership. Although both had very different personalities and brought very different skills to the project, they shared energy, commitment, and vision. Both were also marginalized by their professional peers, a marginalization that also perhaps helped to cement their partnership (3). With the paper's publication, announced to the media on St. Valentine's Day (106), all hell was let loose. The impossible tangle of TV cables and pushy reporters trying to force their way up the stairs to the fourth floor laboratories proved a major disruption to the physiological laboratory in general and to the members of the Marshall laboratory in particular. It was something that was to recur episodically over the next 10 years.

THE BATTLES BEGIN

However, 1969 seemed to be a good year for Edwards. Not only did IVF succeed at long last and his partnership with Steptoe seemed set to flourish, but also so impressed were the Ford Foundation with his work that in late 1968 they had established, at Austin's prompting (107), an

endowment fund with the University of Cambridge to cover the salary cost of a Ford Foundation Readership (a halfway step to a professorship [108]). Elated by Edwards' promotion and their achievement, Edwards and Steptoe pressed on, with the latter's laparoscopic skills coming to the fore, first in 1970 with the collection of *in vivo*-matured eggs from follicles after mild hormonal stimulation (4,109), and then achieving regular fertilization of these eggs and their early development through cleavage to the blastocyst stage (110–112). So well was the work going that in late 1970 and early 1971 they confidently applied to the U.K. Medical Research Council (MRC) for long-term funding (2).

However, any illusions that Edwards may have had that their achievements would prove a turning point in his fortunes were soon shattered. The hostility to his work of much of the media coverage in 1969 heralded the dominant pattern of scientific and medical responses for the next 10 years and resulted just two months later in the MRC rejecting the grant application (3). The practical consequences of this rejection were profound—both psychologically and physically—not least that for the next seven years, Edwards and Purdy shuttled on the 12-hour round trip between Cambridge and Oldham, Greater Manchester, paradoxically just north of his schoolboy haunts of Gorton, where the two of them had set up a small laboratory and clinic in Dr. Kershaw's cottage hospital (113), all the while leaving Ruth and his five daughters in Cambridge. The one bright feature in undertaking this heroic task was the unswerving financial support provided by an American heiress—Lillian Lincoln Howell (71)—that at least ameliorated the MRC decision.

The professional attacks on Edwards and his work took a number of forms (3), and one must try to make a mental time trip back to the 1960s and 1970s to understand their basis. Despite the nature of the political and religious battles to come in the 1980s, his scientific and medical colleagues did not then focus on the special status of the human embryo as an ethical issue. Ethical issues were raised professionally, but took quite a different form. It is perhaps difficult now to comprehend the complete absence of infertility from the consciousness of most gynecologists in the U.K. at the time, of whom Steptoe was a remarkable exception (85). Indeed, even Edwards' strong commitment to treating infertility came to the fore only after he had teamed up with Steptoe, with his previous priority being the study and prevention of genetic and chromosomal disorders.

In the several reports from the Royal College of Obstetricians and Gynaecologists (RCOG) and the MRC during the 1960s examining the areas of gynecological ignorance that needed academic attention, infertility simply did not feature (52,53). Overpopulation and family planning were seen as dominant concerns and the infertile were ignored as, at best, a tiny and irrelevant minority and, at worst, as a positive contribution to population control. This was a values system that Edwards did not accept (114), and the many encouraging letters he received from infertile couples spurred him on and provided a major stimulus to his continued work later, despite so much professional and press antagonism. For his professional colleagues, however, the fact that infertility was not seen as a significant clinical issue meant that any research designed to alleviate it was viewed not as experimental treatment, but as using humans in experiments. Given the sensitivity to the relatively recent Nazi "medical experiments," the formal acceptance of the Helsinki Declaration (115,116), and the public reaction and disquiet surrounding the recent publication of "human guinea-pigs" (117), this distinction was critical. The MRC, in rejecting the grant application, took the position that what was being proposed was human experimentation, and so were very cautious, emphasizing risks rather than benefits, of which they saw few if any (3,5).

Edwards and Steptoe were also attacked for their willingness to talk with the media. It is difficult nowadays, when the public communication of science is embedded institutionally, to understand how damaging to them this was. The massive press interest of the late 1960s was unabated in the ensuing years, and so Edwards was faced with a choice: either he could keep his head down and allow press fantasies and speculations to go unanswered and unchallenged, or he could engage, educate, and debate. For him this was no choice, regardless of the consequences professionally (32). His egalitarian spirit demanded that he trust common people's common sense. His radical political views demanded that he fought the corner of the infertile: the underdog with no voice. The Yorkshireman in him relished engagement in the debate and argument. In Edwards and Sharpe (114), he sets out his reasons for public engagement and acknowledges the risk to his own interests:

> Scientists may have to make disclosures of their work and its consequences that run against their immediate interests; they may have to stir up public opinion, even lobby for laws before legislatures (114).

And risky it was. One of the scientific referees on their MRC grant application started his referee's report by declaring the media exposure distasteful:

> Dr. Edwards feels the need to publicise his work on radio and television, and in the press, so that he can change public attitudes. I do not feel that an ill-informed general public is capable of evaluating the work and seeing it in its proper perspective. This publicity has antagonised a large number of Dr. Edwards' scientific colleagues, of whom I am one (3).

Edwards' pioneering role in the public communication of science proved to be disadvantageous to his work.

The Edwards and Sharpe (114) paper is a tour de force in its survey of the scientific benefits and risks of the science of IVF, in the legal and ethical issues raised by IVF, and in the pros and cons of the various regulatory responses to them. It sets out the issues succinctly and anticipates social

Figure II.9 Louise Brown holding the thousandth Bourn Hall baby, 1987 (courtesy of Bourn Hall Clinic).

responses that were some 13–19 years into the future. Edwards built on his strong commitment to social justice based on a social ethic in subsequent years, as he engaged at every opportunity with ethicists, lawyers, and theologians, arguing, playing "devil's advocate" (literally, in the eyes of some), and engaging in what would now be called practical ethics as he hammered out his position and felt able to fully justify his instincts intellectually (118). However, the establishment was, with few exceptions, unwilling to engage seriously in ethical debates (118,119) in advance of the final validation of IVF that was to come in 1978 with the birth of Louise Brown (Figure II.9) (120).

THE BIRTH OF LOUISE BROWN

It is difficult now to comprehend the sheer magnitude of the task facing Edwards, Steptoe, and Purdy in 1969. Not only did they suffer almost complete isolation from their peers, they also faced a massive scientific and clinical mountain to climb to get from a fertilized egg to a baby, given the paucity of knowledge at the time (see Table II.3). In fact, their progress on reaching the point where transfer of embryos was possible (stage 9 in Table II.3) was impressively rapid, with the first embryo transfer being attempted in December 1971, just two years from the start of their collaboration. This rapid progress was achieved probably because the end point of each task from stage 1 to stage 9 was easily measurable in relation to controlled changes made to the protocols (112). However, for the 97 women who underwent laparoscopy between 1969 and December 1971, when egg recovery, fertilization, and *in vitro* culture were being perfected, there was no chance of a pregnancy, and so they were "experimental subjects," as the MRC had claimed. Moreover, 76 of them (27% of the total number of women who volunteered as patients between 1969 and 1978) did not subsequently undergo embryo transfer attempts in Oldham up to 1978 (5). However, all the evidence suggests that these patients were well informed about the risks and benefits (5), but nonetheless they, as much as Edwards, Purdy, and Steptoe, deserve recognition for their pioneering role in the development of IVF.

Table II.3 Some of challenges that had to be overcome before the first successful live births after *in vitro* fertilization and embryo transfer were achieved

1. Technical aspects of follicle aspiration laparoscopically ("new suction gadget")
2. Ovulation induction
3. Timing of laparoscopy in relation to induction of ovulation
4. Ovarian stimulation
5. Cycle monitoring
6. Oocyte culture
7. Sperm preparation
8. Insemination procedure: medium and timing
9. Culture for embryo cleavage: medium and assessment
10. Technical aspects of embryo transfer, including route of transfer, medium, and timing
11. Luteal support
12. Monitoring of early pregnancy

Source: Elder K, Johnson MH. *Reprod Biomed Soc Online* 2015; 1: 19–33.

After the first transfer in December 1971, most viable embryos were transferred, with a total of 112 transfers occurring between that first one and the last in June 1978 (4,112). Once transfer was being attempted, the task became much more difficult, however, and this difficulty was behind the delay in achieving a successful pregnancy. There were essentially two types of problem to be grappled with. The first was that multiple features of the system could have been responsible for the failure to establish a pregnancy: the transfer technique and timing (both of which had proved difficult to get right in cattle) (120), the quality of the embryos, and the receptivity of the endometrium. Moreover, varying the latter two of these systematically was difficult, and, given the absence of a reliable and sensitive test for human chorionic gonadotropin until late in 1977, there was no immediate way of assessing the impact of any changes that were made. The second problem was the suspicion that the endocrine conditions established as being ideal for the production of eggs and embryos may have been deleterious for the receptivity of the endometrium. Indeed, it was this latter suspicion that drove most of their experimental variations to the treatment schedules, and that ultimately resulted in the two successful

Oldham pregnancies, both of which came, heroically, from single egg collections in natural cycles (4,112).

Only with the live births did the U.K. social, scientific, and medical hierarchies, such as the MRC, the RCOG, the British Medical Association, the Royal Society, and Government moved, albeit gradually, from their almost visceral reactions against IVF and its possibilities to serious engagement with the issues (121). Thus, both the MRC and the RCOG started to move to consider funding of work on IVF, perhaps somewhat surprisingly, given that only two live births resulted from a total of 112 transfers (4), although again the MRC declined to fund a second grant proposal from Edwards. Moreover, the National Health Service declined to provide facilities in Cambridge for Edwards and Steptoe to relocate to on Steptoe's retirement from Oldham, hence the setting up of the Bourn Hall clinic in 1982. The Thatcher Government of the time started to seriously consider the issues and set up the Warnock committee of enquiry in 1982 that reported in 1984, to a storm of parliamentary criticism, which Edwards and others had to battle to turn around over the next five years with a fierce campaign of both public and parliamentary education to counter the increasingly shrill voice of the anti-embryo research lobby (121,122). In addition, Edwards' personal battles continued, with legal suites issued during the 1980s against both the British Medical Association and various members of the national press for defamation. Thus, it was not until 1989, 24 years after Edwards' 1965 visionary paper in *The Lancet*, 20 years after IVF had first been described, and 11 years after the birth of Louise Brown that the U.K. Parliament finally gave its stamp of approval to his vision.

However, Edwards' role was realized and recognized professionally at last by the awards of fellowships of the RCOG in 1984 and of the Royal Society in 1983 (and an FRS for Steptoe followed in 1987). But despite being awarded the Albert Lasker prize in 2001, the Nobel Prize and a knighthood did not come his way until 2010—some 40 years after the *Nature* paper that started the whole IVF story in earnest.

DISCUSSION

This chapter describes some of the early years of Edwards' life and work in order to provide a context for the events leading up to the 1969 *Nature* paper describing IVF and the final validation of the claims made in that paper with the birth of Louise Brown in 1978. It is evident even from the earliest stages of his late entry into research that Edwards is a man of extraordinary energy and drive, qualities sustained throughout his long career, as witnessed in his prodigious output of papers between 1954 and 2008 (32). Indeed, several of the referees on the unsuccessful 1971 MRC grant application specifically criticized his "overenthusiasm," doubting that he could achieve the program he sets out therein as "too ambitious" (3). Tenacity of purpose comes through clearly in Edwards' work, a trait he was inclined to attribute to his Yorkshire origins, but that may also have been fueled by his working-class determination to show himself to be as good as the next (wo)man. The influences of Waddington's Edinburgh Institute, of Waddington himself, and of his supervisor, Alan Beatty, on Edwards' interests and values are also clear from the dominant role that developmental genetics played in his thinking, especially until the time he met Steptoe. Indeed, from examination of Edwards' papers and interests, his passionate conversion to the cause of the infertile seems directly attributable to Steptoe's influence. Admittedly, Edwards' forays into immuno-reproduction did involve consideration of immunological causes of infertility, but these were more usually of interest to him as models for developing new contraceptive agents. Indeed, Edwards was as captured as most reproductive biologists of the time by the 1960s' consensus on the need for better methods of world population control. This position was understandable given the reality of those concerns, as is demonstrated now in the problem of global warming that is attributable at least in part to a failure to control population growth. It is a measure of his imagination and empathy that he could grasp so rapidly Steptoe's understanding of the plight of the infertile and so flexibly incorporate this understanding into his plans. That empathy clearly reflects his underprivileged origins, with his espousal of the cause of the junior, the disadvantaged, the ill-informed, and the underdog being a thread running through his career. Edwards can be very critical, but I have found no one who can remember him ever being nasty or vindictive. Even when he disagrees with someone passionately, he never loses his respect for them as people. That Steptoe tapped into this sentiment is clear.

The way in which Edwards met Steptoe has been absorbed into folklore, but an examination of the evidence seems to warrant some revision to commonly held later reminiscences. It remains uncertain exactly which publication(s) by Steptoe it was that Edwards read in 1967, but seems likely that he did read Steptoe's book. Thus, it was spermatozoa, not eggs, that were exercising Edwards in 1967, and it was the problem of sperm capacitation, not egg retrieval, to which Steptoe and his laparoscope seemed to offer a solution in 1967. The book is the only place that this issue is specifically addressed. Their actual meeting at the Royal Society of Medicine in 1968 is also re-evaluated: Edwards was an invited speaker lecturing about his work on immuno-reproduction; so paradoxically, what has been seen as a sidetrack to his main work was, albeit serendipitously, the reason for their actual meeting.

The early collaboration between them involved the recovery of ovarian biopsies, just like those Rose and others had been providing. However, the attractions of pre-ovulatory follicular egg recovery were already clear to them both by 1968, and became, with embryo replacement, the central planks of their partnership. Steptoe and Edwards were in many ways an unlikely partnership. Their personal styles were very different, and there are clear hints in his writings that Edwards found their early days together difficult. But like most successful partnerships, their differences were sunk in a mutual respect for the other's pioneering skills and willingness to take on

Figure II.10 Edwards, Purdy, and Steptoe at Bourn Hall, 1981 (courtesy of Bourn Hall Clinic).

the established conventions. In Jean Purdy, they also had a partner who worked quietly away in the background, smoothing the bumps on the path of their work together (Figure II.10) (113).

However, it remains Edwards' extraordinary foresight that marks him out so distinctively. His combination of vision and intellectual rigor is evident not just in his work on stem cells, PGD, and, with Steptoe, infertility, but also in his pioneering work in the public communication of science, in how ethical discourse about reproduction is conducted, in consideration of regulatory issues, and in the dissemination of IVF internationally, the latter largely a consequence of his key role in both the establishment of the European Society for Human Reproduction and Embryology in 1984 and in the founding of five journals: *Human Reproduction* (in 1986), *Human Reproduction Update* and *Molecular Human Reproduction* (both in 1996), *Reproductive BioMedicine Online* (in 2000) and *Reproductive BioMedicine and Society* (in 2015). The epithet "the father of assisted reproduction" is surely deservedly appropriate.

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REFERENCES

1. Johnson MH. Robert Edwards: The path to IVF. *Reprod Biomed Online* 2011; 23: 245–62.
2. Edwards RG, Steptoe PC. *A Matter of Life: The Story of a Medical Breakthrough*. London: Hutchinson, 1980.
3. Johnson MH, Franklin SB, Cottingham M, Hopwood N. Why the medical research council refused Robert Edwards and Patrick Steptoe support for research on human conception in 1971. *Hum Reprod* 2010; 25: 2157–74.
4. Elder K, Johnson MH. The Oldham Notebooks: An analysis of the development of IVF 1969–1978. II. The treatment cycles and their outcomes. *Reprod Biomed Soc Online* 2015; 1: 9–18.
5. Johnson MH, Elder K. The Oldham Notebooks: An analysis of the development of IVF 1969–1978. IV. Ethical aspects. *Reprod Biomed Soc Online* 2015; 1: 34–45.
6. Massey H, Feather N. James Chadwick: 20 October 1891–24 July 1974. *Biogr Mems Fell R Soc* 1976; 22: 10–70.
7. Ashwood-Smith M. Robert Edwards at 55. *Reprod Biomed Online* 2002; 4(Suppl 1): 2–3.
8. Ingle DJ. Gregory Goodwin Pincus. April 9, 1903–August 22, 1967. *Biogr Mems Natl Acad Sci USA* 1971; 229–70.
9. Oakley CL. Francis William Rogers Brambell. 1901–1970. *Biogr Mems Fell R Soc* 1973; 19: 129–71.
10. Parkes AS. Francis Hugh Adam Marshall. 1878–1949. *Biogr Mems Fell R Soc* 1950; 7: 238–51.
11. Polge C. Sir Alan Sterling Parkes. 10 September 1900–17 July 1990. *Biogr Mems Fell R Soc* 2006; 52, 263–83.
12. Slee J. *RGE* at 25—Personal reminiscences. *Reprod Biomed Online* 2002; 4(Suppl 1): 1.
13. Robertson A. Conrad Hal Waddington. 8 November 1905–26 September 1975. *Biogr Mems Fell R Soc* 1977; 23: 575–622.
14. Milne EA. Ralph Howard Fowler. 1889–1944. *Biogr Mems Fell R Soc* 1945; 5: 60–78.
15. Eve AS, Chadwick J. Lord Rutherford. 1871–1937. *Biogr Mems Fell R Soc* 1938; 2: 394–423.
16. Edwards RG. An astonishing journey into reproductive genetics since the 1950's. *Reprod Nutr Dev* 2005; 45: 299–306.
17. Watson JD, Crick FH. Genetical implications of the structure of deoxyribonucleic acid. *Nature* 1953; 171: 964–7.
18. Watson JD, Crick FH. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. *Nature* 1953; 171: 737–8.
19. Franklin R, Gosling R. Molecular configuration in sodium thymonucleate. *Nature* 1953; 171: 740–1.

20. Wilkins MHF, Stokes AR, Wilson HR. Molecular structure of deoxypentose nucleic acids. *Nature* 1953; 171: 738–40.
21. Gurdon JB. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *Development* 1962a; 10: 622–40.
22. Gurdon JB. Adult frogs derived from the nuclei of single somatic cells. *Dev Biol* 1962b; 4: 256–73.
23. Gurdon JB, Elsdale TR, Fischberg M. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature* 1958; 182; 64–5.
24. Weinberg AM. Messenger RNA: Origins of a discovery. *Nature* 2001; 414: 485.
25. Ford CE, Hamerton JL. The chromosomes of man. *Nature* 1956; 178: 1020–23.
26. Tjio JH, Levan A. The chromosome number of man. *Hereditas* 1956; 42: 1–6.
27. Lejeune J, Levan A, Böök M et al. A proposed standard system of nomenclature of human mitotic chromosomes. *Lancet* 1960; 275: 1063–65.
28. Ford CE, Jones KW, Polani PE, De Almeida JC, Briggs JH. A sex chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet* 1959; 273: 711–13.
29. Ford CE, Polani PE, Briggs JH, Bishop PM. A presumptive human XXY/XX mosaic. *Nature* 1959; 183: 1030–32.
30. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. *Nature* 1959; 183: 302–3.
31. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatiques de neuf enfants mongoliens. *Comptes Rendus Hebd Seances Acad Sci* 1959; 248: 1721–22.
32. Gardner RL, Johnson MH. Bob Edwards and the first decade of *Reproductive BioMedicine Online*. *Reprod Biomed Online* 2011; 22: 106–24.
33. Fowler RE, Edwards RG. Induction of superovulation and pregnancy in mature mice by gonadotrophins. *J Endocr* 1957; 15: 374–84.
34. Edwards RG, Gates AH. Timing of the stages of the maturation divisions, ovulation, fertilization and the first cleavage of eggs of adult mice treated with gonadotrophins. *J Endocr* 1959; 18: 292–304.
35. Horowitz NH, Metz CB, Piatigorsky J, Piko L, Spikes JD, Ycas M. Albert Tyler. *Science* 1969; 163: 424.
36. Reeve S. Nathaniel Mayer Victor Rothschild, G.B.E., G.M. Third Baron Rothschild. 31 October 1910–20 March 1990. *Biogr Mems Fell R Soc* 1994; 39: 364–80.
37. Rothschild D. Did fertilization occur? *Nature* 1969; 221: 981.
38. Edwards RG, Bavister BD, Steptoe PC. Early stages of fertilization *in vitro* of human oocytes matured in vitro. *Nature* 1969; 221: 632–5.
39. Mitchison NA. Peter Brian Medawar. February 1915–2 October 1987. *Biogr Mems Fell R Soc* 1990; 35: 282–301.
40. Medawar PB. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symp Soc Exp Biol* 1953; 7: 320–38.
41. Clarke AE. *Disciplining Reproduction: Modernity, American Life Sciences, and the Problems of Sex*. Berkeley, CA: University of California Press, 1998.
42. Connelly M. *Fatal Misconception: The Struggle to Control World Population*. Cambridge, MA: Harvard University Press, 2008.
43. Marks LV. *Sexual Chemistry: A History of the Contraceptive Pill*. New Haven, CT: Yale University Press, 2001.
44. Naz RK, Gupta SK, Gupta JC, Vyas HK, Talwar GP. Recent advances in contraceptive vaccine development: A mini-review. *Hum Reprod* 2005; 20: 3271–83.
45. Rukavina D. The history of reproductive immunology: My personal view. *Am J Reprod Immunol* 2008; 59: 446–50.
46. Edwards RG. Meiosis in ovarian oocytes of adult mammals. *Nature* 1962; 196: 446–50.
47. Gemzell CA. The induction of ovulation with human pituitary gonadotrophins. *Fertil Steril* 1962; 13: 153–68.
48. Pincus G, Enzmann EV. The comparative behavior of mammalian eggs *in vivo* and *in vitro*: I. The activation of ovarian eggs. *J Exp Med* 1935; 62: 665–75.
49. Pincus G, Saunders B. The comparative behavior of mammalian eggs *in vivo* and *in vitro*: VI. The maturation of human ovarian ova. *Anat Rec* 1939; 75: 537–45.
50. Greep RO. Min Chueh Chang. October 10, 1908–June 5, 1991. *Biogr Mems Natl Acad Sci USA* 2010.
51. Chang MC. The maturation of rabbit oocytes in culture and their maturation, activation, fertilization and subsequent development in the Fallopian tubes. *J Exp Zool* 1955; 128: 379–405.
52. MRC. *Research in Obstetrics and Gynaecology: Report to the Secretary of the Council by Section A1, General Clinical Medicine*. National Archives, FD 7/912, 1969.
53. RCOG. *Macafee Report*. The Training of Obstetricians and Gynaecologists in Britain, and Matters Related Thereto: The Report of a Select Committee to the Council of the Royal College of Obstetricians and Gynaecologists. London: RCOG, 1967.
54. Askonas BA. John Herbert Humphrey. 16 December 1915–25 December 1987. *Biogr Mems Fell R Soc* 1990; 36: 274–300.
55. Himsworth H, Pitt-Rivers R. Charles Robert Harington. 1897–1972. *Biogr Mems Fell R Soc* 1972; 18, 266–308.
56. Cole RJ, Edwards RG, Paul J. Cytodifferentiation in cell colonies and cell strains derived from cleaving ova and blastocysts of the rabbit. *Exp Cell Res* 1965; 37: 501–4.
57. Cole RJ, Edwards RG, Paul J. Cytodifferentiation and embryogenesis in cell colonies and tissue cultures derived from ova and blastocysts of the rabbit. *Dev Biol* 1966; 13: 385–407.

58. McLaren A, Biggers JD. Successful development and birth of mice cultivated *in vitro* as early embryos. *Nature* 1958; 182: 877–8.
59. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981; 292: 154–6.
60. Edwards RG. IVF and the history of stem cells. *Nature* 2001; 413: 349–51.
61. Parkes AS. *Off-beat Biologist*. Cambridge: The Galton Foundation, 1985.
62. Cook B. *JRF*—The first 100 volumes. *Reproduction* 1994; 100: 2–4.
63. Clarke J. The history of three scientific societies: the Society for the Study of Fertility (now the Society for Reproduction and Fertility) (Britain), the Societe 'Francaise pour l'Etude de la Fertilite', and the Society for the Study of Reproduction (USA). *Stud Hist Phil Biol Biomed Sci* 2007; 38: 340–57.
64. Edwards RG. Letter to Cambridge University Registrary, plus supporting documents, applying for the Mary Marshall and Arthur Walton Professorship of the Physiology of Reproduction. Edwards' papers, 6 Jan 1966. Uncatalogued, Churchill College Archive.
65. Polge C. Obituary: Professor Thaddeus Mann. The Independent, 9 December 1993.
66. Short R. Colin Austin. *Aust Acad Sci Newslett* 2004; 60: 11.
67. Arechaga J. Technique as the basis of experiment in developmental biology: An interview with Denis A.T. New. *New Int J Dev Biol* 1997; 41: 139–52.
68. Edwards RG, Steptoe PC. Preface. In: Edwards RG, Purdy JM, Steptoe PC (eds). *Implantation of the Human Embryo*. London: Academic Press, 1985: vii–viii.
69. Gardner RL. Bob Edwards—2010 Nobel laureate in physiology or medicine. *Physiology News* 2011; 82: 18–22.
70. Gardner RL, Johnson MH. Robert Edwards. *Hum Reprod* 1991; 6: iii–iv.
71. Johnson MH, Elder K. The Oldham Notebooks: An analysis of the development of IVF 1969–1978. VI. Sources of support and patterns of expenditure. *Reprod Biomed Soc Online* 2015: 1, 58–70.
72. Edwards RG. Maturation *in vitro* of human ovarian oocytes. *Lancet* 1965; 286: 926–9.
73. Edwards RG. Maturation *in vitro* of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. *Nature* 1965; 208: 349–51.
74. Gardner RL, Edwards RG. Control of the sex ratio at full term in the rabbit by transferring sexed blastocysts. *Nature* 1968; 218: 346–9.
75. Theodosiou AA, Johnson MH. The politics of human embryo research and the motivation to achieve PGD. *Reprod Biomed Online* 2011; 22: 457–71.
76. Edwards RG. Are oocytes formed and used sequentially in the mammalian ovary? *Phil Trans R Soc B* 1970; 259: 103–5.
77. Henderson SA, Edwards RG. Chiasma frequency and maternal age in mammals. *Nature* 1968; 218: 22–8.
78. Chang MC. Fertilization of rabbit ova *in vitro*. *Nature* 1959; 184: 466–7.
79. Yanagimachi R, Chang MC. Fertilization of hamster eggs *in vitro*. *Nature* 1963; 200: 281–2.
80. Jones Jr HW. From reproductive immunology to Louise Brown. *Reprod Biomed Online* 2002; 4(Suppl 1): 6–7.
81. Austin CR. Observations of the penetration of sperm into the mammalian egg. *Aust J Sci Res B* 1951; 4: 581–96.
82. Chang MC. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature* 1951; 168: 697–8.
83. Edwards RG, Donahue RP, Baramki TA, Jones Jr HW. Preliminary attempts to fertilize human oocytes matured *in vitro*. *Am J Obstet Gynecol* 1966; 96: 192–200.
84. Edwards RG, Talbert L, Israelstam D, Nino HN, Johnson MH. Diffusion chamber for exposing spermatozoa to human uterine secretions. *Am J Obstet Gynecol* 1968; 102: 388–96.
85. Edwards RG. Patrick Christopher Steptoe, C. B. E. 9 June 1913–22 March 1988. *Biogr Mems Fell R Soc* 1996; 42: 435–52.
86. Steptoe PC. *Laparoscopy in Gynaecology*. Edinburgh: E and S. Livingstone, 1967.
87. Philipp E. Obituary: P C Steptoe CBE, FRCSED, FRCOG, FRS. *Br Med J* 1988; 296: 1135.
88. Edwards RG. Tribute to Patrick Steptoe: Beginnings of laparoscopy. *Hum Reprod* 1989; 4(Suppl): 1–9.
89. Edwards RG. Interviewed in: To Mrs. Brown a daughter. Peter Williams TV: The Studio, Boughton. Faversham, UK, 1978.
90. Steptoe PC. Laparoscopy and ovulation. *Lancet* 1968; 292: 913.
91. Hunting P. *The History of the Royal Society of Medicine*. London: The Royal Society of Medicine Press Ltd, 2002.
92. Steptoe PC. Laparoscopy: Diagnostic and therapeutic uses. *Proc Roy Soc Med* 1969; 62: 439–41.
93. Steptoe PC. A new method of tubal sterilisation. In: Westin B, Wiqvist N, eds. *Amsterdam: Fertility and Sterility: Proc 5th World Congress June 16–22, 1966*. Stockholm: International Congress Series no. 133, Excerpta Medica Foundation, 1967; 1183–4.
94. Steptoe PC. Laparoscopic studies of ovulation, its suppression and induction, and of ovarian dysfunction. In: Wood C, Walters WAW (eds). *Fifth World Congress of Gynaecology and Obstetrics, held in Sydney, Australia, 1967*. Sydney: Butterworths, 1967; 364.
95. Morrison DL. Laparoscopy. *BMJ* 1967; 4: 34.
96. Letter from Eliot Philipp to Edwards dated July 2003, setting out his memory of the exchange of remarks when Steptoe and Edwards first met in the spring of 1967. Uncatalogued Edwards' papers, Churchill College Archive. *General Letters, file 146*.

97. Bavister BD. Environmental factors important for *in vitro* fertilization in the hamster. *J Reprod Fertil* 1969; 18: 544–5.
98. Edwards RG, Bavister BD, Steptoe PC. Did fertilization occur? *Nature* 1969; 221: 981–2.
99. Hayashi M. Fertilization *in vitro* using human ova. In: *Proceedings of the 7th International Planned Parenthood Federation, Singapore. Excerpta Medica International Congress Series No. 72*. Amsterdam, The Netherlands, 1963.
100. Petrov GN. Fertilization and first stages of cleavage of human egg *in vitro*. *Arkhiv Anatomii Gistologii i Embriologii* 1958; 35: 88–91.
101. Petrucci D. Producing transplantable human tissue in the laboratory. *Discovery* 1961; 22: 278–83.
102. Rock J, Menkin M. *In vitro* fertilization and cleavage of human ovarian eggs. *Science* 1944; 100: 105–7.
103. Shettles LB. A morula stage of human ovum developed *in vitro*. *Fertil Steril* 1955; 9: 287–9.
104. Yang WH. The nature of human follicular ova and fertilization *in vitro*. *J Jpn Obstet Gynecol Soc* 1963; 15: 121–30.
105. Anon, 2008. Morris, Prof. Norman Frederick. In: *Who Was Who 1920–1980*. A & C Black and Oxford University Press. [Available from: http://www.ukwhoswho.com/view/article/oupww/whowaswho/U28190].
106. Anon. New step towards test-tube babies. Nature—Times News Service; *The Times* 14 February, 1969.
107. Holmes RF. Letter to D Kellaway (Sec Fac Board Biol. B), dated 21 May. University of Cambridge Archives, 1968; 731.020.
108. Hankinson GS. Letter (G.B.6812.534) from the General Board of the Faculties, the Old Schools, Cambridge to RG Edwards detailing some aspects of the Ford Foundation endowment fund, 20 December 1968. Edwards' papers uncatalogued, Churchill College Archive.
109. Steptoe PC, Edwards RG. Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins. *Lancet* 1970; 295: 683–9.
110. Edwards RG, Steptoe PC, Purdy JM. Fertilization and cleavage *in vitro* of preovulatory human oocytes. *Nature* 1970; 227: 1307–9.
111. Steptoe PC, Edwards RG, Purdy JM. Human blastocysts grown in culture. *Nature* 1971; 229: 132–3.
112. Elder K, Johnson MH. The Oldham Notebooks: An analysis of the development of IVF 1969–1978. III. Variations in procedures. *Reprod Biomed Soc Online* 2015; 1: 19–33.
113. Johnson MH, Elder K. The Oldham Notebooks: An analysis of the development of IVF 1969–1978. V. The role of Jean Purdy reassessed. *Reprod Biomed Soc Online* 2015; 1: 46–57.
114. Edwards RG, Sharpe DJ. Social values and research in human embryology. *Nature* 1971; 231: 87–91.
115. Rickham PP. Human Experimentation: Code of Ethics of World Medical Association. Declaration of Helsinki. *Br Med J* 1964; 2: 177.
116. Hazelgrove J. The old faith and the new science. The Nuremberg Code and human experimentation ethics in Britain 1946–73. *Soc Hist Med* 2002; 15:109–35.
117. Pappworth MH. *Human Guinea-pigs*. London: Routledge and Keegan Paul, 1967.
118. Edwards RG. Fertilization of human eggs *in vitro*: morals, ethics and the law. *Q Rev Biol* 1974; 49: 3–26.
119. Jones A, Bodmer WF. *Our Future Inheritance: Choice or Chance?* London: Oxford University Press, 1974.
120. Edwards RG, Steptoe PC. Birth after the reimplantation of a human embryo. *Lancet* 1978; 312: 366.
121. Johnson MH, Theodosiou AA. PGD and the making of the genetic embryo as a political tool. In: McLean S (ed.). *Regulating PGD: A Comparative and Theoretical Analysis*. London: Routledge, 2011.
122. Mulkay M. *The Embryo Research Debate: Science and the Politics of Reproduction*. Cambridge: Cambridge University Press, 1997.

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Quality management in reproductive medicine

33

MICHAEL ALPER

INTRODUCTION

Quality management (QM) systems have become integral management tools in many *in vitro* fertilization (IVF) centers around the world. The European Union Tissue Directive, issued in 2004, clearly demands a QM system for any institution handling human gametes/embryos. The primary concerns of any healthcare system will continue to be clinical outcomes. However, if we regard medical facilities as businesses providing a particular service to patients and referring doctors, then other parameters beyond clinical outcomes become important. Governmental agencies and insurance companies will continue to place increasing pressure on documenting that they provide services in a particular fashion. This will mean that strict procedures for documentation of results will be needed and, furthermore, practices could be penalized if not performing adequately. Governmental agencies control some practices through regulations (e.g., certain infection disease protocols). However, beyond these rules, many medical organizations currently develop their own internal standards. These standards are often only informally documented and most of the time are fragmentary. These standards affect and direct the internal workings of the organization and the interactions of various areas within the company. They may also affect the interactions of the company with external partners. For example, if every institution were to use their own internally developed methodology for documenting and handling different procedures, then it would be very difficult to compare and contrast different systems. A customizable single system to follow all the internal workings of the organization is the goal of the QM systems such as that of the International Organization for Standardization (ISO; see below). A very important element to recognize is that an organization has many "customers." Often clinicians feel uncomfortable referring to our patients as customers, but of course patients are our key customers. But other "customers" exist and include referring doctors, insurance companies, regulatory bodies, and students, among others. Another set of key customers consist of our employees—our "internal customers."

The individual elements of a QM system are developed to different degrees, but always according to the tasks and the orientation of the particular institution. They exist in a varied yet well-defined relationship with one another. All of these elements and their interconnections as a whole enable a clinic or private practice to reach the expected and agreed results with the customer on a timely basis, and with an appropriate use of resources. The sum of directive elements and elements that transcend or relate to the process is called the "QM system" of a clinic or a private practice. Compared with other medical specialties, reproductive medicine has led the way (in Europe) with the introduction of QM systems over the past several years. In this chapter, different QM systems are described, the instruments of these systems are discussed, and the question of how QM systems contribute to success in reproductive medicine is addressed.

DIFFERENT QM SYSTEMS

Several industry-specific QM systems have been developed worldwide. In 1964, Good Production Practice (a World Health Organization [WHO] directive) was developed for the pharmaceutical and food industries. Good Laboratory Practice (an Organization for Economic Cooperation and Development [OECD] directive) followed in 1978, as did the Hazard Analysis of Critical Control Points (a National Advisory Committee on Microbiological Criteria for Foods directive) in 1992. The EU with its "Global Concept" (1985) strongly promoted the development of QM systems and expanded them to production and services.

ISO 9001 standards

The systems that followed—the manuals of the ISO (the ISO 9000 series)—became the most widespread worldwide standard. In the 1980s, the ISO created regulations for QM systems with the standard series 9001 through 9004 developed for the production of goods and services. These manuals described the basic elements of the QM system in a relatively abstract manner. Medical institutions were required to adapt these standards to the medical field, which required some interpretation and modification. The introduction of ISO 9000 states: "The demands of the organizations differ from each other; during the creation of quality management systems and putting them into practice, the special goals of the organization, its products and procedures and specific methods of acting must be taken into consideration unconditionally." This means that, for medical applications, the standards state which elements should be considered in the QM system, but the manner in which these elements should be realized in the specific medical organization must be defined individually. Furthermore, specific interpretation of the ISO for IVF centers is limited. The ISO standards have now been adapted to medicine, which is fortunate since there is no QM system specifically designed for hospitals or medical practices. ISO 9001 through 9003 contain the elements that are important for a quality system (Table 33.1). The criteria

Table 33.1 Elements/criteria of the International Organization for Standardization (ISO) standard

Number	Quality element according to ISO 9000 ff.
1	Responsibility
2	Quality management system
3	Contract control
4	Design management
5	Document and data management
6	Measures
7	Management of products provided for customers
8	Designating and retrospective observation
9	Process management
10	Revision
11	Control of the revision resources
12	Evidence of revisions
13	Defective product management
14	Corrections and preventive measures
15	Handling, storage, packaging, conservation, and distribution
16	Quality report management
17	Internal quality audits
18	Training
19	Maintenance
20	Statistical methods

according to which QM systems are applied vary with the type of enterprise. For example, the 9001 standard is applicable to manufacturing and complicated service companies, including hospitals and medical practices. On the other hand, the 9002 standard is more suitable for rehabilitation and foster-care institutions (1). The application of a certified QM system for hospitals can be performed on the basis of ISO 9001 or ISO 9004 (2). More recent publications describing the application of ISO to IVF centers are now available (see the textbook by Carson et al.). As mentioned earlier, IVF units occupy a special place within clinical medicine. This is a highly specialized area involving the interaction of staff in various areas, including the laboratory, ultrasound, administration, physicians, and nurses. Treatment can only be successful when a structured interaction exists between the clinical and laboratory departments. ISO 9001 (3) is very much focused on a process approach and is directed at the outcome of the process (i.e., that the products or services meet the previously determined requirements). Since this does not necessarily ensure that a laboratory will be successful or pregnancy rates will be as good as possible, or that it will achieve the highest level of care for the patients that it serves, assisted reproduction technology (ART) laboratories may also want to consider additional requirements, including standards concerning qualifications and competence. Relevant standards are provided by the ISO/IEC 17025:1999 (4) (IEC being the International Electrotechnical Commission). This standard, entitled "General requirements for the competence of testing and calibration laboratories," replaces both the ISO/IEC Guide 25 (5) and the European standard EN 45001 (6). Compliance with the ISO 17025 standard can lead to accreditation (defined as "a procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks"), which exceeds certification (defined as "a procedure by which a third party gives written assurance that a product, process or service conforms to specific requirements"). ART laboratories may want to consider ISO 17025 accreditation. However, one should realize that both ISO/IEC Guide 25 and EN 45001 are focused more on the technical aspects of competence, and do not cover all areas within clinical laboratories. It has already been stated that although the ISO standards are the most widely accepted standards in the world, there is no appropriate international standard for laboratories in the healthcare sector. To fulfill this need, several professional associations and laboratory organizations have also framed and published standards and guidelines, most of which are confined to a specific clinical laboratory discipline. Some specific and relevant examples of guidelines for ART laboratories that are commonly available are (7–10):

1. Revised guidelines for human embryology and andrology laboratories, the American Fertility Society, 2008
2. Revised guidelines for good practice in IVF laboratories, the European Society of Human Reproduction and Embryology (ESHRE), 2008
3. Reproductive laboratory accreditation standards, College of American Pathology, 2013
4. Accreditation standards and guidelines for IVF laboratories, the Association of Clinical Embryologists, 2000

The above-mentioned guidelines and standards describe the specific requirements for reproductive laboratories, and include various aspects of the implementation of a QM system. These well-defined standards describe the minimum conditions that should be met by laboratories/clinics. Recently, the EU Tissue Directive (11) has been released, which demands a QM system for every medical institution dealing with human gametes or embryos.

Total Quality Management and the Excellence Model of the European Foundation for Quality Management

There is a wide range of QM models and strategies based on continuous improvement. Two of the best-documented models/strategies are Total Quality Management (TQM) and the Excellence Model of the European Foundation for Quality Management (EFQM). TQM is an all-encompassing concept that integrates quality control, assurance, and improvement. It is more of a philosophy than a model. Deming developed the basics of this concept after World War II. Both the TQM and the EFQM models incorporate the objective of continuously striving to improve every aspect of a service, and require continuous scrutiny of all components of the QM system of an organization. Measurement and feedback are crucial elements in QM. This can be illustrated by the so-called Deming cycle (the "Plan–Do–Check–Act" cycle) (Figure 33.1). Important elements of a TQM program are:

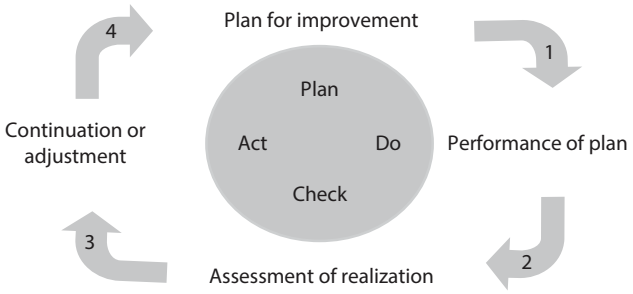


Figure 33.1 Total quality management: the Denning cycle.

1. Appropriately educated and trained personnel with training records
2. Complete listing of all technical procedures performed
3. Housekeeping procedures: cleaning and decontamination procedures
4. Correct operation, calibration, and maintenance of all instruments with manuals and logbook records
5. Proper procedure policy and safety manuals
6. Consistent and proper execution of appropriate techniques and methods
7. Proper documentation, record keeping, and reporting of results
8. Thorough description of specimen collection and handling, including verification procedures for patient identification and chain of custody
9. Safety procedures, including appropriate storage of materials
10. Infection control measures
11. Documentation of suppliers and sources of chemicals and supplies, with dates of receipt/expiry
12. System for appraisal of test performance correction of deficiencies and implementation of advances and improvements
13. Quality materials, tested with bioassays when appropriate
14. Quality assurance programs

QUALITY POLICY

One of the first steps for the implementation of a QM system in medical institutions is to clearly define the quality policy. Quality policies are a group of principles that establish the workings of the institution. Although successful treatment of an existing disease or reduction of discomfort is certainly the highest priority for most medical institutions, it might be an important goal to achieve this in the most efficient manner possible. This means that structure is needed to ensure that diagnostic and therapeutic procedures are performed using as appropriate financial, organizational, or time resources as possible, while still striving for a high quality of treatment. After all, optimum quality is achieved by the "right" balance between cost and quality. The quality policy of a medical institution cannot be defined by a single person (e.g., the owner or Medical Director), but should be developed as a consensus between management and employees. Only in this way will personnel identify with the quality policy of the institution. A quality policy should be formulated in an active manner and the formulation should also be short and simple so that every employee can repeat the quality policy at any time. The most important aspects of the quality policy should be posted in suitable and accessible areas of the institution for employees, patients, and visitors in order to strengthen the employees' knowledge of common goals, improve their identification with their own areas of competence, and communicate these principles to others. It is important to state that quality policies should be reviewed periodically to make sure that the principles are still valid and that management and employees still agree with them. As an organization's perspectives and goals change, the quality policy needs to be modified accordingly. As an example, Boston IVF's quality policy is "CARE," standing for Compassionate, Advanced, Responsive, and Experienced.

MANAGEMENT'S RESPONSIBILITY

In spite of the fact that the responsibility of management (or the governing structure) can be defined differently in various medical institutions, according to ISO standards, certain generally valid aspects can be defined. The hierarchy of the institution has to be defined and outlined clearly. Although larger institutions commonly have clear charts of who reports to whom, the structure might be more challenging to delineate in private centers with multiple partners in equal positions. In such cases, an agreement that describes the division of responsibilities for particular fields among the physicians must be in place. Several possibilities are available; for example, one of the partners could be in charge of research and another could be in a business role. However, for many privately held practices, a model may exist for dividing these tasks on a rotational basis. It is here that clear descriptions of authority for all positions within the organization are required and must be known to everyone, both internally and externally. The more complex the hierarchic structures within a medical institution, the more precisely these structures must be defined for the system to work effectively and robustly at all times and under all (extraordinary) conditions. The "decision maker" of the head of the organization must be available at any time, even if he or she is physically absent. Therefore, it must be absolutely clear to everyone within the organization who has the competence and authority to make decisions. If the "decision maker" is not available, then someone in the organization should be identified to make these decisions in his/her absence. It is also important for customers outside of the company to be aware of who the decision makers are for various tasks. There are various ways of making these structures as transparent as possible. One easy way is the development of an organizational chart (Figure 33.2). This organizational diagram can be placed in a suitable and accessible location, helping employees to understand everyone's roles and responsibilities. Furthermore, making the organizational diagram available to everyone strengthens trust, cooperation, and professionalism within the company. It is also important for communication with patients, interested parties, or cooperating departments. The organizational diagram should be updated frequently. Management

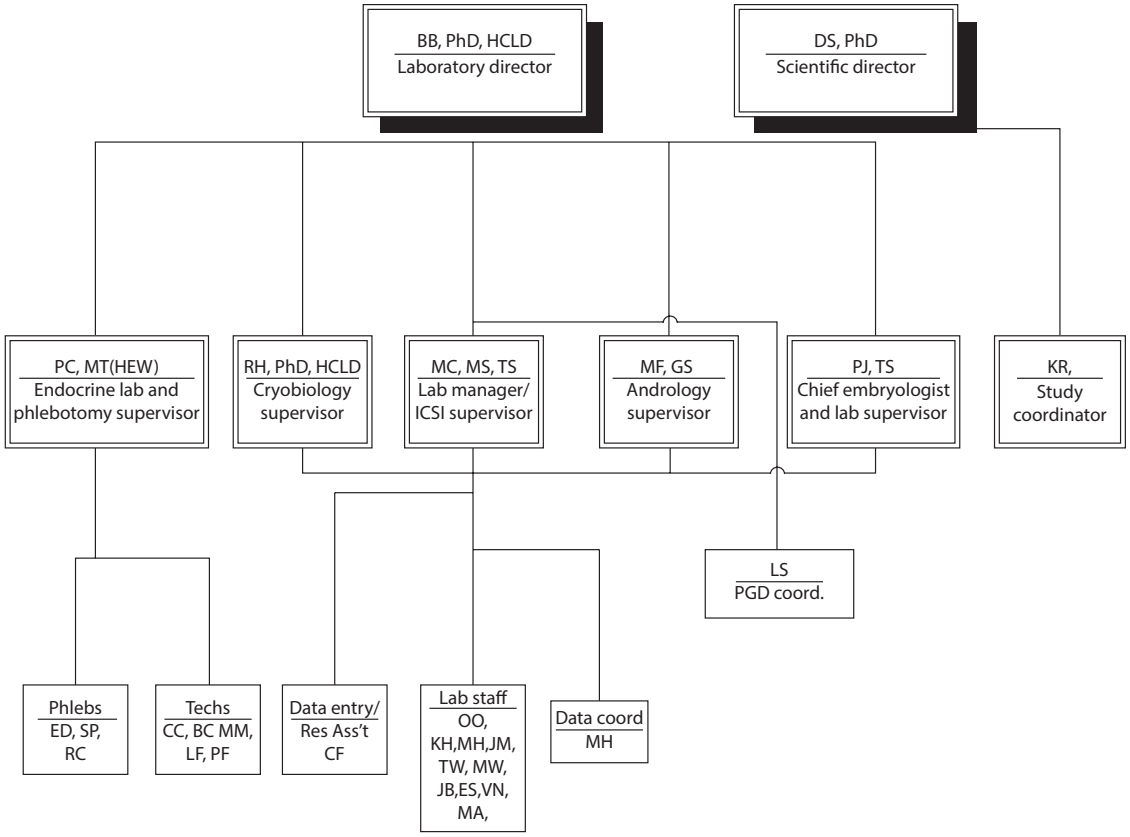


Figure 33.2 Example of an organizational chart.

should strongly support the quality policies of the company and should take an active part in their development and implementation. It is important to lead by example.

MANAGEMENT OF PROCESSES

Processes are all of the procedures that are necessary for the completion of tasks. For medical facilities, the most important processes are those of diagnostic and therapeutic procedures. In addition, many other processes are involved in the care of patients, such as the scheduling of patients for tests, communication, and anything else that may greatly affect the patient's (customer's) perspective. Sometimes poor communication can ruin a patient's experience, despite the best diagnostic procedures within the organization. In fact, it is our observation that it is more likely that a patient will leave a medical facility because of an organizational problem such as a substandard secretarial or administrative problem than a medical deficiency. Even with properly working medical treatment, poor communication with colleagues can endanger or directly destroy the positive result of the treatment. When establishing a QM system, it is necessary to precisely define and describe all relevant processes and to structure them according to QM guidelines. These descriptions are often best realized by flow diagrams that can overlap in various places. These areas of contact between two flow diagrams are called boundaries, interferences, joints, or areas of juncture.

Documentation in a QM system

In addition to defining the processes that are relevant to the system, it is important for everything to be documented. The different levels of documentation are shown in Figure 33.3. One of the most important documents in a QM system is the quality manual. The main purpose of the quality manual is to outline the structure of the documentation used in the quality system (12). It should also include or refer to the standard operating procedures (SOPs). There should be clear definitions of management's areas of responsibility, including its responsibility for ensuring compliance with the international standards on which the system is built. A simple overview of the quality system requirements and the position of the quality manual are shown in Figure 33.4. A good-quality manual should be precise and brief; it should be an easily navigable handbook for the entire quality system. The most important procedures are preferably included in the manual itself, but deeper descriptions should be referred to in the underlying documentation. An easy way to start building a system is to make up a table of contents for the quality manual and to decide which processes should be described in the manual and which should rather be described in the underlying documentation (e.g., SOPs). Whereas the quality manual contains more general information, the individual processes and procedures are described in a more detailed way in handbooks/job instructions or SOPs. These SOPs go through the processes step by step and describe the materials and methods used and

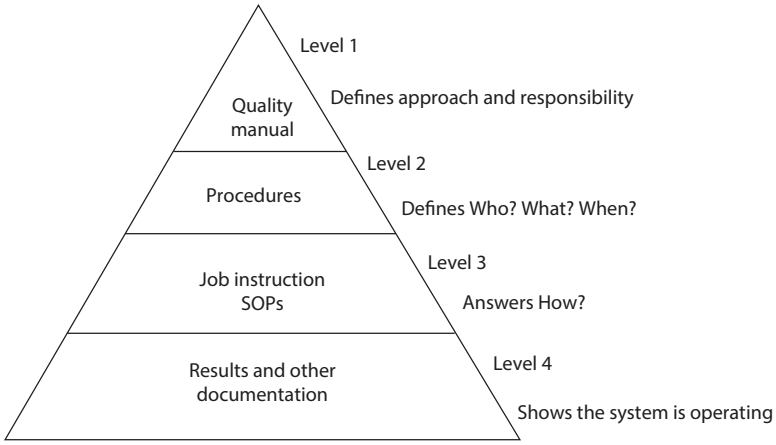
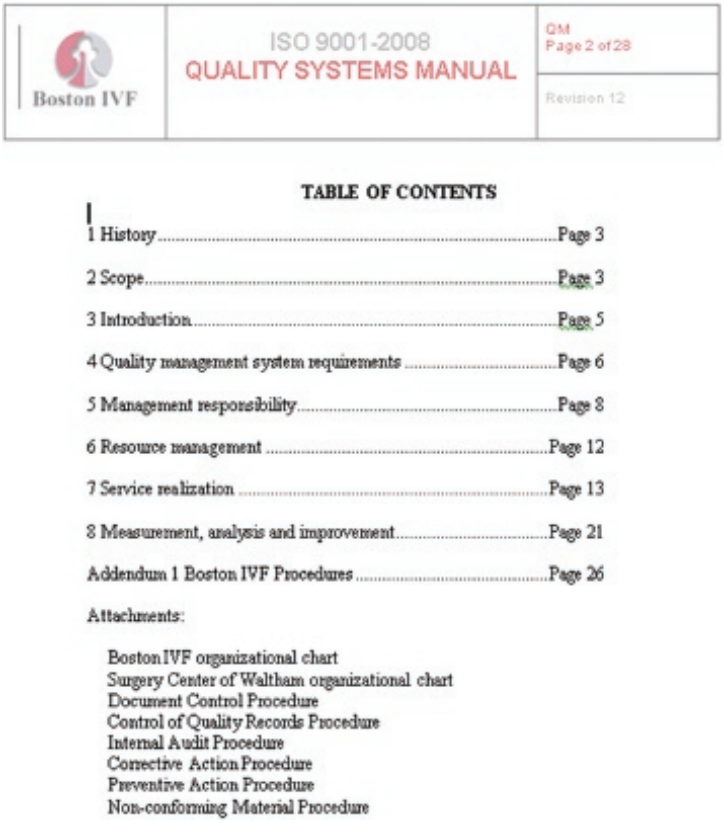


Figure 33.3 Levels of documentation. *Abbreviation*: SOP, standard operating procedure.

the way the process is performed precisely. SOP manuals should be available to all personnel, and every single procedure in these manuals must be fully documented with signature, date, and regular review.

Document control

According to ISO 9001:2008, the clinic should establish and maintain procedures to control all documents that form part of its quality documentation. This includes both internally generated documentation such as SOPs and protocol sheets and externally generated documentation such as law texts, standards, and instruction manuals for equipment. Document handling and control are important parts of the quality system and, if not designed properly, can become enormous burdens for a smooth running system. Since it is something that touches every part of the system, it is important to sit down and think through how this system of paperwork is best handled in your clinic and to ensure that the system you choose covers the demands of the standards. The identification of the documents should be logical, and it is a good suggestion to use numbers as unique identifiers. The same identification number could


Boston IVF

ISO 9001-2008
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Control of Quality Records Procedure
Internal Audit Procedure
Corrective Action Procedure
Preventive Action Procedure
Non-conforming Material Procedure

Figure 33.4 Example of an International Organization for Standardization quality manual.

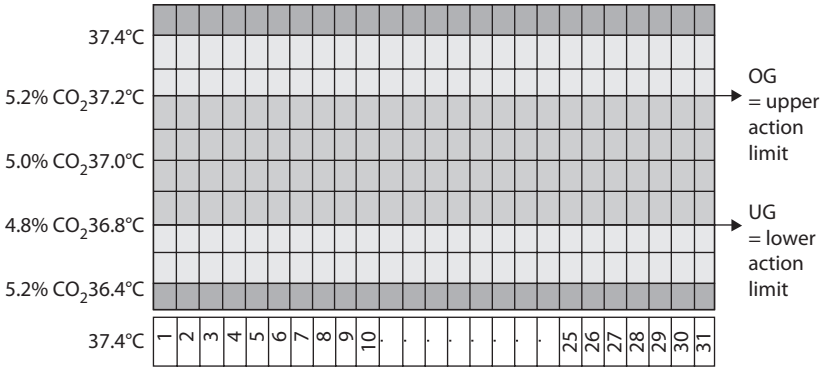

Figure 33.5 Monitoring temperature and CO_2 levels in an incubator.

then be used for the file name within the computerized version. The issue number in parentheses or after a dash could follow this number. Pagination is important. If you choose not to use pagination, you must clearly mark where the document starts and ends. The dates of issue together with information on who wrote the document and who approved it (with an authorized signature) are usually included in the document header. Questions that should have an answer in your document control system include:

1. Is *all* documentation in the laboratory or clinic covered by your document control system?
2. Who writes or changes the document?
3. Who approves and has the authority to issue documents?
4. Does the document have:
   a. Unique identification?
   b. Issue number and current revision status?
   c. Date of latest issue?
   d. Pagination?
5. Where can I find the document: physical location, level in the system, and on computer file?
6. Who ensures that only the latest issue of the document is present in the system, removes outdated issues, and files them?
7. Are amendments to documents clearly marked, initialed, and dated?
8. How are changes in a document implemented with the personnel?

Documentation of results

A very important level of documentation concerns "results." This includes not only the results of treatment such as pregnancy rate per treatment cycle, but also all documents referring to:

1. Control of quality records
2. Internal audits
3. Control of nonconformity
4. Corrective and preventive action

Performance of key indicators is essential, and an example of this in the laboratory is equipment. Incubators are one of the most important pieces of equipment in the IVF laboratory and need to be controlled properly. Two markers of incubator performance are the temperature and the CO_2 level. These two parameters are documented on the control cards, and upper and lower limits of tolerance are defined to determine when corrective actions are needed (Figure 33.5). It is useful to plot results of system checks on a graph, so that there is a clear visual image that can monitor:

1. Dispersion: increased frequency of both high and low numbers
2. Trend: progressive drift of reported values from a prior mean
3. Shift: an abrupt change from the established mean

If nonconformity to the standard is diagnosed, it is important to collect data on:

1. When the problem was realized
2. How often the problem could be identified
3. How conformity to the standards could be reassured

Audits and management reviews

Audits are essential to ensuring that a quality system is working. Audits can be internal, initiated by the organization itself, or external, initiated by a governing body, certification body, or accreditation body. ISO 9001:2008 lays out the rules for internal audits and demands that the clinic undertakes internal audits at planned intervals to determine how well the system is functioning and if it is effectively implemented and maintained. Audits are tools for improving and keeping your system up to date with the standards. The quality manual should include specific instructions covering both how and how often audits should be performed. Management usually chooses internal auditors, and they should be familiar with both the standards and the activities performed in the clinic; auditors are from other departments within the organization. The manual should include a document describing the approach and the areas of responsibility for the internal auditors and have well documented procedures for how internal auditors are trained. To achieve a certification according to ISO 9001:2008, the clinic needs to be audited externally by a certification body. Many organizations believe that having an audit and not finding any nonconformity is proof of outstanding performance. However,

the other possibility is that it could be due to an inadequate audit procedure. If an audit is properly conducted, even in organizations with outstanding performance, areas for improvement will be found; therefore, people should put in a lot of effort to find the right certification body to undertake the audits. Some questions that might help to identify a good certification body are:

- Are they accredited to certify medical institutions?
- Have they previously certified medical or IVF clinics and how many?
- Do they have medical or IVF experts on their audit team?
- How much time do they allocate to the audit?

While to some it may seem obvious, it is important to mention, especially with respect to the factors above, that the cheapest certifying body is not necessarily the best.

Together with the audits, the management review is important for improvement of the system and for the long-term correction of errors and incidents that might occur. According to ISO 9007:2000 5.6, the management of the clinic with executive responsibility shall periodically conduct a review of the quality system and testing activities. The quality manual shall include a written agenda for these reviews, which should fulfill the demands in the standard.

INCIDENTS AND COMPLAINTS

All clinics should have a policy and procedure for the resolution of incidents and complaints received from patients, clients, and/or other parties. The routines of how these are filed and how corrective actions are taken should be documented in a clinic's quality manual. When applying a quality system it is important not to hide these incidents and complaints, but to use them as resources to improve the system. The management reviews should ensure that the incidents and complaints lead to long-term corrections and improvements in the quality of work.

STAFF MANAGEMENT

High-quality treatment can only be realized with qualified staff. Therefore, recruitment, training, and motivation of highly qualified people are the most important tasks for the management team of an organization. To make sure that a sufficient number of qualified people are working within the respective areas of the institution, a staff requirement plan should be developed. This can be organized in different ways:

1. Allocating people according to their abilities
2. Allocating people according to different responsibility levels
3. Allocating people according to the type of work that has to be done

Medical facilities need to define staffing levels for the different departments in the organization. This is a key role for management since staffing influences the quality of the service and also the cost–effectiveness/profitability of the organization. The number of employees should be carefully determined for particular departments according to their tasks and the range of services provided. Proactive staff planning where everyone understands his or her role allows for quality service to be delivered. The development of work descriptions is crucial for this system. They must be created for every position, and must clearly state the qualifications and attributes required for the employee. In addition to this formal information, the work description should also contain information about the employee's personal attributes. For various posts, different qualities are important:

1. Social competence
2. Organizing abilities
3. Communication abilities, etc.

The staff requirement plan must be set up so that it is possible to react effectively to unexpected situations. Furthermore, it must consider staff absenteeism caused by holidays, illness, and further education. A minimal presence of employees must be determined for certain areas, irrespective of the actual workload. For the development of a staff requirement plan for an IVF center, the medical as well as the non-medical areas have to be defined and considered. The question of how many people are needed to do the job properly can be answered on the basis of calculating the "influence magnitudes." The type of services offered strongly influences the number of people required. Thus, the staff requirements are different in a center in which predominantly conservative treatments and intrauterine inseminations are performed, compared with a center in which predominantly IVF and cryopreservation cycles are performed.

Training of employees

One of the most important principles for the management of a medical institution is: "give your employees the chance to be the best." This means that if you expect your employees to do their work at the highest quality level possible, you should give them proper training. In principle, there are two different types of educational events:

1. Internal events of further education
2. External events of further education (i.e., conventions, conferences, workshops, etc.)

The advantage of internal events of further education is that they can be offered on a regular basis and are usually "low-budget projects," whereas external events need more organizational and financial input. However, when carefully planned, external educational events sometimes have a higher motivational aspect. So the management team should take care to offer a balanced program of internal and external educational events. To make it possible to use the clinics' resources adequately, educational and training requirements for the organizational needs should be evaluated on an ongoing basis since unexpected events (e.g., loss of a key employee) can occur. For example, at the beginning of each year, the employee should decide which educational events he or she would like to visit or take part in.

This helps the management to introduce new educational opportunities, and also allows them to perform advanced planning of the specialization. It is striking to see that, in most ART centers, detailed and prospective plans have been developed for the training of medical doctors, but far less attention has been paid to the training of nurses, technicians, and so on. However, a well-trained nurse can significantly reduce the workload for the doctor and tremendously increase the patient's trust in the institution while also improving the referring doctor's satisfaction.

Therefore, besides training activities for the doctors, adequate educational events for nurses, technicians, and so on should be considered.

Interactions between management and employees

Success in reproductive medicine clearly depends on an optimal interaction between different professional groups; in other words, success can be achieved only if doctors communicate and work together with staff in the laboratory, nurses, receptionists, and so on. The same is true for the interactions between management and employees. Communication and collaboration between different professional groups of the same hierarchic rank is called "horizontal" communication, whereas communication and collaboration between professional groups of different hierarchic ranks is called "vertical" communication. One of the most important instruments for optimizing vertical communication is a staff interview. These staff interviews should occur periodically where the employee and their direct superiors discuss their collaboration and identify areas for improvement. The interview should take place in a structured way and a protocol should be written and signed by both sides, so that the content of the interview is assigned some kind of formal character. However, details of the interview can never be communicated with others without mutual consent. For the employee, the goals/opportunities of the interview are:

1. To become familiar with the goals of the department
2. To realize weaknesses and strengths
3. To be able to discuss own experiences of/opinions on the management style
4. To discuss further strategies for professional development
5. To participate in planning goals/strategies for the future

For the superior, the goals/opportunities of the interview are:

1. To discuss the co-worker's performance
2. To focus the activities of the employee on future goals of the institution
3. To increase mutual understanding in the event of problems
4. To increase the employee's responsibility
5. To get feedback on his/her management skills

For the above-mentioned reasons, the staff interview is one of the most important and powerful tools in staff development, and should be widely used in the process of continuous improvement.

The EU Tissue and Cells Directive

The increase in use, donation, and storage of human tissue has led to the creation of directives from the European Council. In March 2004, the European parliament issued a revised version of the directive on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells. When these directives were issued, there was a need to adapt the requirements to the actual setting of an IVF laboratory. However, in the meantime, these directives have been implemented by many IVF centers around the world, and a position paper has been issued by the ESHRE outlining how these directives should be applied. Independent of this position, authorities in many countries interpret the directive differently, which makes it difficult to share experiences between centers in different countries. Furthermore, auditing processes need to be adapted from country to country.

The central part of the EU directive is very clear concerning the demand for a quality system. Therefore, the directive states, "Tissue establishments shall take all necessary measures to ensure that the quality system includes at least the following documentation: standard operating procedures, guidelines, training and reference manuals." Certainly, by achieving ISO accreditation, this demand will be fulfilled, together with several other demands of the directive.

CONCLUSIONS

No internationally accepted standards exist for quality in the IVF laboratory and the IVF center as a whole. To ensure high quality and continual improvement, it is recommended that all IVF centers striving for excellence should consider a QM system. Furthermore, legal guidelines and the EU Tissue and Cells Directive clearly demand a QM system for medical institutions. A QM system allows the organization to gain control of its documents and procedures and to monitor the clinical and nonclinical outcomes. Furthermore, the issues of staff recruitment and staff development can be addressed systematically and the overall outcome will be improved. The ISO standards offer the medical facility access to an internationally endorsed and proven QM system. ART practitioners in particular have the unique opportunity of setting the standard in medicine for QM principles.

SUGGESTED READING

Alper MM, Brinsden PR, Fischer R, Wikland M. Is your IVF programme good? *Hum Reprod* 2002; 17: 8–10.

Alper MM. Experience with ISO quality control in assisted reproductive technology. *Fertil Steril* 2013, 100: 1503–8.

American Society for Reproductive Medicine. Revised guidelines for human embryology and andrology laboratories. *Fertil Steril* 2008, 90: s45–59.

Bloor G. Organisational culture, organisational learning and total quality management: A literature review and synthesis. *Aust Health Rev* 1999; 22: 162–79.

Bron MS, Salmon JW. Infertility services and managed care. *Am J Manag Care* 1998; 4: 715–20.

Brown RW. Errors in medicine. *J Qual Clin Pract* 1997; 17: 21–5.

Carson BE, Alper MM, Keck C. *Quality Management Systems for Assisted Reproductive Technology*. London, U.K.: Taylor and Francis, 2004.
Clancy C. AHRQ: Coordinating a quantity of quality. *Healthplan* 2003; 44: 42–6.
Collings J. An international survey of the health economics of IVF and ICSI. *Hum Reprod Update* 2002; 8: 265–77.
Colton D. The design of evaluations for continuous quality improvement. *Eval Health Prof* 1997; 20: 265–85.
Darr K. Risk management and quality improvement: Together at last—Part 2. *Hosp Top* 1999; 77: 29–35.
Garceau L, Henderson J, Davis LJ et al. Economic implications of assisted reproductive techniques: A systematic review. *Hum Reprod* 2002; 17: 3090–109.
Geraedts HP, Montenarie R, Van Rijk PP. The benefits of total quality management. *Comput Med Imaging Graph* 2001; 25: 217–20.
Glattacker M, Jackel WH. Evaluation of quality assurance—Current data and consequences for research. *Gesundheitswesen* 2007; 69: 277–83.
Gondringer NS. Benchmarking: Friend or foe. *AANAJ* 1997; 65: 335–6.
Greenberg L. Accreditation strengthens the disease management bridge over the quality chasm. *Dis Manag* 2003; 6: 3–8.
ISO/DIS 15189:2012. *Medical Laboratories—Particular Requirements for Quality and Competence*. Geneva, Switzerland: International Standardization Organization, 2012.
Matson PL. Internal quality control and external quality assurance in the IVF laboratory. *Hum Reprod* 1998; 13(Suppl 4): 156–65.
Minkman M, Ahaus K, Huijsman R. Performance improvement based on integrated quality management models: What evidence do we have? A systematic literature review. *Int J Qual Health Care* 2007; 19: 90–104.
Sackett DL, Rosenberg WMC, Gray JAM et al. Evidence based medicine: What it is and what it isn't. *Br Med J* 1996; 312: 71–6.
Sandle LN. The management of external quality assurance. *J Clin Pathol* 2005; 58: 141–4.
Sciacovelli L, Secchiero S, Zardo L et al. Risk management in laboratory medicine: Quality assurance programs and professional competence. *Clin Chem Lab Med* 2007; 45: 756–65.
Shaw CD. External quality mechanisms for health care: Summary of the ExPeRT project on visitatie, accreditations, EFQM and ISO assessment in European Union countries. External peer review techniques. European Foundation for Quality Management. International Organization for Standardization. *Int J Qual Health Care* 2000; 12: 169–75.
Varkey P, Reller MK, Resar RK. Basics of quality improvement in health care. *Mayo Clin Proc* 2007; 82: 735–9.
Vogelsang J. Quantitative research versus quality assurance, quality improvement total quality management and continuous quality improvement. *J Perianesth Nurs* 1999; 14: 78–81.
Warnes GM, Norman RJ. Quality management systems in ART: Are they really needed? An Australian clinic's experience. *Best Pract Res Clin Obstet Gynaecol* 2007; 21: 41–55.
Yasin MM, Meacham KA, Alavi J. The status of TQM in healthcare. *Health Mark Q* 1998; 15: 61–84.

RELEVANT INTERNET ADDRESSES

http://www.agrbm.de
http://www.asrm.com
http://www.eshre.com
http://www.ferti.net
http://www.iso.ch
http://www.isoeasy.org
http://www.ivf.net/ace
http://www.praxion.com

REFERENCES

1. Pinter E, Vitt KD. *Umfassendes Qualitätsmanagement für das Krankenhaus—Perspektiven und Beispiele*. Frankfurt, Germany: pmi-Verlag, 1996.
2. Viethen G. *Qualität im Krankenhaus. Grundbegriffe und Modelle des Qualitätsmanagements*. Stuttgart, Germany: Schattauer-Verlag, 1995.
3. ISO 9001:2008. *Quality Management Systems—Requirements*. Geneva, Switzerland: International Standardization Organization, 2008.
4. ISO/IEC 17025:2005. *General Requirements for the Competence of Testing and Calibration Laboratories*. Geneva, Switzerland: International Standardization Organization, 2005.
5. ISO/IEC Guide 25:2005. *General Requirements for the Competence of Testing and Calibration Laboratories*. Geneva, Switzerland: International Standardization Organization.
6. EN 45001:2017. *General Criteria for the Operation of Testing Laboratories*. www.iso.org.
7. American Fertility Society. Revised Guidelines for Human Embryology and Andrology Laboratories. *Fertil Steril* 2008; 90(3):s45–59.
8. Gianaroli L, Plachot M, Van Kooij R et al. Committee of the special interest group on embryology. ESHRE guidelines for good practice in IVF laboratories. *Hum Reprod* 2000; 15: 2241–6.
9. College of American Pathologists. Available from: http://cap.org
10. Association of Clinical Embryologists UK. Accreditation standards and guidelines for IVF laboratories, 2001. Available from: www.embryologists.org.uk
11. Directive 2004/23/EC of the European Parliament and of the Council of 31 March, 2004. On setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. *Official Journal of the European Union* 2004; L102, 48–58. Available from: www.eumonitor.nl
12. Huismam W. Quality system in the medical laboratory: The role of a quality manual. *Ann Biol Clin (Paris)* 1994; 52: 457–61.

Lifestyle, periconception, and fertility

34

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INTRODUCTION

Reproductive health critically impacts a couple's well-being and functional capacity throughout their lives. The reproductive system, with its controlling hormones and cyclical changes, governs physiological events at puberty, across the menstrual cycle, during pregnancy, and in parturition, lactation, and menopause. The majority of women experience some form of reproductive disorder over the course of life, and many chronic and severe reproductive disorders remain without preventive strategies, clear diagnostics, or successful treatment. Even "normal" pregnancy can reveal or precipitate underlying chronic metabolic disease. The direct cost of maternal and neonatal conditions is substantial (1).

Importantly, the reproductive health of a woman and of her partner is also the single greatest determinant of the health and well-being of their children. Definition of the periconceptional period in humans depicts the five stages of reproductive development: gametogenesis, fertilization, implantation, embryogenesis, and placentation (2). We now understand that the influence of parents begins before conception in that a compromised egg or sperm from either parent can alter the trajectory of development even if the embryo and intrauterine environment are optimal (3,4). A less than optimal environment *in utero* predisposes an individual to diseases in adulthood including obesity, heart disease, diabetes, and stroke, to an extent comparable in magnitude to genetic predisposition and lifestyle factors such as obesity and smoking (5). Understanding early life events and how they contribute to health or resilience to disease is a fundamental component of intergenerational health, whereby the health of one generation affects that of the next.

Fundamental knowledge gaps that currently exist are:

1. What environmental and genetic factors determine the optimal function of sperm and eggs?
2. What are the critical biological events and pathways in the periconception period that promote or constrain developmental competence in the oocyte and embryo, affecting health and functional capacity in later life?
3. How do environmental conditions, genes, and maternal reproductive disorders influence developmental competence in the oocyte and embryo and optimal growth in the fetus?
4. How do we best translate fundamental knowledge gains in order to better predict and diagnose reproductive disorders, improve periconception health, and maximize pregnancy outcomes?
5. What is the role of male factors in determining health in the sperm, embryo, and fetus?

GOALS OF PERICONCEPTION HEALTH

Our goal should be to make important basic discoveries and to capitalize on these to prevent disease and disability and build resilience in our community through clinical and public health interventions targeting early stages in life. This is best achieved by a cross-disciplinary approach spanning basic biomedical science, epidemiology, and translational research. Integration of cell and molecular biology, physiology, immunology, and new technologies (genomics and sensing) with clinical and epidemiological studies promise the best approach to developing new paradigms for appropriate healthcare. Periconception care is more than just improving fertility—it is also about optimal outcomes for children born as a result of both natural conception and after assisted reproduction technology (ART) use.

SOCIETAL IMPORTANCE

The global community recognizes the critical value of reproductive health and its necessity for health and resilience in our children. International commitment to reproductive health was declared at the 1994 International Conference on Population and Development in Cairo (6), reaffirmed at the 1995 Fourth World Conference on Women (7), and reinforced in 2000, when the UN Millennium Declaration specified the 5th Millennium Development Goal to "improve maternal health," with a focus on sexual and reproductive health (8). The Special Programme of Research, Development and Research Training in Human Reproduction (HRP), today a United Nations (UN) Programme, co-sponsored by the United Nations Development Programme (UNDP), the United Nations Population Fund (UNFPA), the World Bank and the World Health Organization (WHO), is the main instrument within the UN system for research in human reproduction, bringing together policy-makers, scientists, healthcare providers, clinicians, consumers, and community representatives to identify and address priorities for research to improve sexual and reproductive health (9). The year 2012 marked its 40th anniversary (10). While the quality of reproductive health in first-world countries is clearly higher than in developing countries, major opportunities for health gains exist there for women and future generations, particularly in economically disadvantaged or rural communities.

A GROWING UNDERSTANDING OF PERICONCEPTION CARE

Exposure to teratogens and nutrient deficiency were linked to congenital defects during the last century and these concepts dominated maternal–fetal research. In the twenty-first century, the greatest health gains stand to be made from research addressing more cryptic but pervasive

ill-health outcomes with long latencies that are functional rather than structural, which emerge through interactions between the individual and the environment and which have effects that endure across generations.

There are multiple points of vulnerability throughout the pre-birth and post-birth phases of life that are prone to the positive or negative impacts of internal and external influences. We and others have shown that the very earliest stages of embryogenesis are most susceptible. At this time the organism is rapidly developing and must exhibit great plasticity to best survive the number and scale of critical transitions from zygote to fetus (11).

The earliest determinant of life potential is the oocyte, the developmental competence of which is influenced by the local hormonal, growth factor, and cellular environment of the ovarian follicle in which it grows (12,13). After fertilization, developmental plasticity is desirable so that the early embryo can respond to the demands and opportunities of the outside world by adaptation, rather than by adhering to a standard fixed phenotype that may be inappropriate to the changing external environment. Plasticity can be exerted at the cellular level by adjustment of cell numbers and fates, and at the molecular level by changes in gene expression pathways or the more permanent effects of epigenetics (14–16). Together these processes exert modifications through which the periconception environment can modulate the phenotype to "best suit" the prevailing or predicted post-birth environment. Cytokines and growth factors secreted by maternal tract cells, as well as metabolic substrates and other physiochemical agents, are implicated as signals through which the embryo senses its local environment (17). The balance of pro-survival and pro-apoptotic cytokines can influence embryo survival and program epigenetic changes in response to environmental cues (18). Remarkably these cytokines are affected not only by the woman's environment and her health, but also by her partner's. The male seminal fluid delivers signaling molecules that interact with female tissues to alter gene expression and impact the molecular composition of the oviduct and uterine fluids at conception (19). This seminal fluid priming can influence endometrial receptivity for implantation, the progression of pregnancy, and the health of offspring after birth (19).

The reason why the periconception phase of early development is so vulnerable may reflect the importance of this phase as an opportunity for evolutionary selection and adaptation to be exerted. From an evolutionary perspective, imposing constraints and selection pressures upon the conceptus is necessary to avoid unfavorable investment of reproductive resources and to maximize offspring health. The mammalian female has limited opportunities for pregnancy during her reproductive lifespan and each pregnancy costs resources and poses a risk to her own health. The majority of early embryos fail to survive and only ~60% of embryos that implant persist beyond the second week. Decreased implantation rates result from the absence or suppression of molecules that are essential for endometrial receptivity, the mechanisms of which are diverse and include abnormal cytokine and hormonal signaling as well as epigenetic alterations (20,21). There are evolutionary advantages associated with active female-controlled processes for discerning the suitability of male gametes and embryos (22). The female immune response is "aware" of fetal transplantation antigens and is competent to discriminate the reproductive fitness and compatibility of the male partner and the integrity and developmental competence of the conceptus tissue (23,24). Since the immune response is modulated by the individual's infectious, inflammatory, stress, nutritional, and metabolic status, immune influence on progression or disruption of pregnancy may be further influenced by environmental stressors and resource availability. Emerging evidence suggests that the immune system can integrate these signals to exert executive quality control in order to either accommodate or reject the conceptus. "Immune-mediated quality control" facilitates optimal female reproductive investment and explains the evolutionary advantage of engaging the immune system in the events of reproduction (18,25).

With plasticity and maternal selection come the risk of poor outcomes—when embryo sensing of the external environment fails to properly indicate and match the reality, where compromises made to favor immediate survival are suboptimal for longevity of life after birth, or when maternal quality control systems are inappropriately executed or otherwise faulty. In broad terms it seems that extreme adaptation causes loss of functional capacity and resistance to future stressors, while maintenance of capacity in early intrauterine life improves the likelihood of subsequent health and resilience in adulthood (26). If capacity is lost in early embryonic and fetal development, the possibility of dysfunction in later life becomes higher (Figure 34.1).

The permanent effects of exerting early plasticity are often not readily observable until later in fetal or postnatal

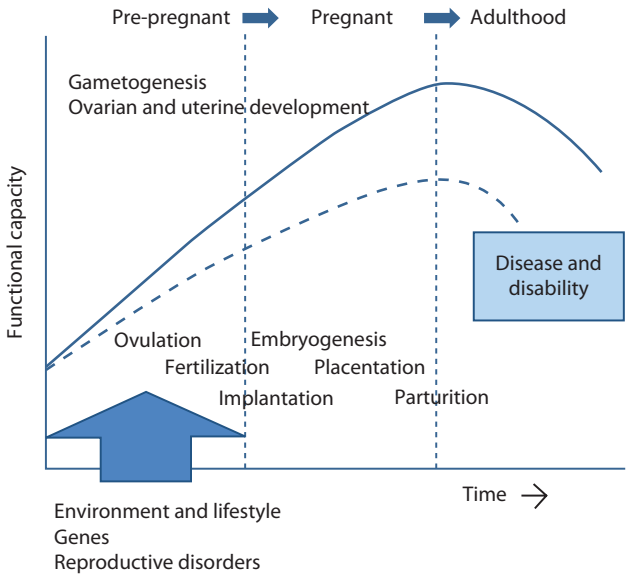


Figure 34.1 Adaptation to adverse influences in early life causes loss of functional capacity after birth.

life. Changes in cell numbers and lineage allocation or in gene or protein expression in blastocysts due to perturbation in the local physiochemical or cytokine environment (27–29) cause differences in placental structure and nutrient transport function, which are key limiting factors in fetal growth (30,31). Disturbance to epigenetic regulation of both imprinted and non-imprinted genes, caused by various environmental factors, can lead to abnormal placental development and function with possible consequences for maternal morbidity, fetal development, and disease onset in later life (32). This occurs because, in adults, susceptibility or resilience to stressors and insults that precipitate disease are affected by the cellular composition of tissues, particularly the numbers of stem and pluripotent cells and the epigenetic programming of gene regulation laid down at this time (33).

Experimental perturbations at various stages of pregnancy implicate the first days of life as the most susceptible period for later fetal and postnatal growth impairment (34). Altered embryo development or insufficient maternal support of the conceptus at implantation can lead to later miscarriage, or "shallow" placental development resulting in pre-eclampsia, fetal growth restriction, and/or preterm delivery (35,36). In turn, these conditions affect growth after birth and impart a "thrifty" phenotype that leads to metabolic disorder and onset of chronic disease. Thus, maternal stress in the periconception period due to nutritional, metabolic, immunological, infectious, pharmacological, or psychosocial perturbations can exert subtle but permanent alterations in the life-course trajectory of the offspring (Figure 34.2).

Epidemiological evidence in humans is consistent with the animal data showing that environment before birth

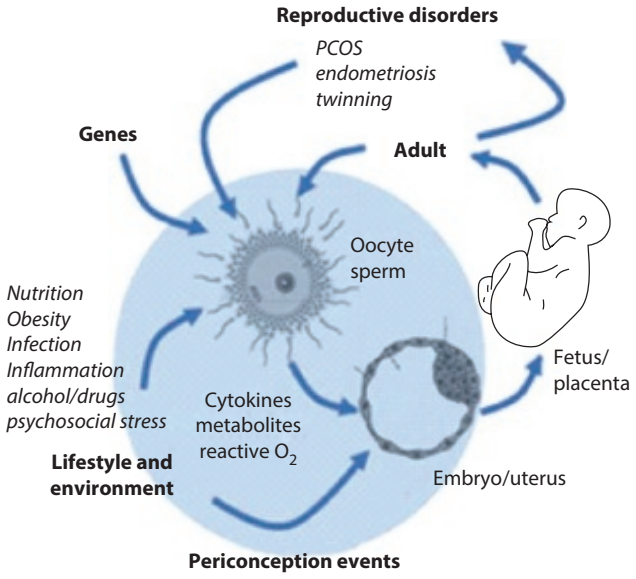


Figure 34.2 Periconception events are influenced by genes, a range of lifestyle/environmental factors, and maternal factors including reproductive disorders, impacting fetal development and adult outcomes.

sets in train either good health or disease in later life, and that early pregnancy is the most vulnerable period (28,37,38). Vulnerability to pathologies of pregnancy that precipitate poor perinatal outcomes is further influenced by maternal, paternal, and fetal genotype (39). Low birth weight for gestational age predisposes to later incidence of cardiovascular disease, impaired glucose tolerance, hypertension, and hyperlipidemia, particularly when there is postnatal catch-up growth due to over-nutrition (40–44). The association between perinatal parameters and adult health is evident even after adjusting for lifestyle factors, occupation, income, diet, and socioeconomic status.

Maternal reproductive disorders such as polycystic ovary syndrome (PCOS), obesity, endometriosis, and ovulation disorders influence periconception events, alter endometrial receptivity and quality control sensing, and impart stress on the gametes and embryo (Figure 34.2) (45,46). These reproductive disorders share inflammatory pathways, hormonal aberrations, decidual senescence and vascular abnormalities that may impair pregnancy success through common mechanisms (47). Chronic sexually transmitted infection is another key factor that influences the maternal environment. Either in combination or alone, these disorders result in an increased risk of preterm birth, fetal growth restriction, placental pathologies, and hypertensive disorders. Systemic hormonal aberrations and inflammatory and metabolic factors acting on the endometrium, myometrium, cervix, and placenta are all associated with an altered milieu during implantation and pregnancy, thus contributing to the genesis of obstetric complications (47).

ART, which is now the method of conception for many children around the world, also potentially inflicts substantial stress on the embryo (48). We now recognize that *in vitro* embryo culture in media that are deficient in maternal signaling factors, the gonadotropin-induced altered hormone environment imposed on the oocyte prior to conception, and the disordered endometrium in a stimulated cycle predispose to growth restriction and attendant lifelong effects on children (48–52). Clinical practice until now shows that the *in vitro* culture of human embryos does not confer major adverse effects on the offspring, but possible consequences in late childhood or adulthood are still to be explored, keeping in mind that even the first children conceived by ART are still relatively young (53). There is evidence that transgenerational programming is a key factor in PCOS and that other forms of reproductive dysfunction can be programmed *in utero* (54–56). Competition in the womb through twinning or higher-order multiple pregnancy, irrespective of ART or spontaneous occurrence, also causes fetal growth impairment and can bring about adverse life-long consequences (57).

FACTORS THAT AFFECT FERTILITY

Weight, exercise, and nutrition

The prevalence of overweight young couples in the reproductive age of life is steadily increasing (58), and there

is now abundant evidence that female weight disorders, both under- and over-weight, impair spontaneous fertility (59,60). Obesity has been linked to male fertility because of lifestyle changes, internal hormonal environment alterations, and sperm genetic factors (61). Either paternal or maternal obesity may negatively affect ART outcomes (62,63). Female obesity has been shown to be associated with poor pregnancy outcomes, including increased rates of congenital abnormalities, cesarean delivery, pre-eclampsia, gestational diabetes, fetal macrosomia, stillbirth, and post-term pregnancy (64). It has been reported that physical activity improves cardiovascular risk factors, hormonal profile, and reproductive function. These improvements include decreases in abdominal fat, blood glucose, blood lipids and insulin resistance, as well as improvements in menstrual cyclicity, ovulation, and fertility, decreases in testosterone levels and Free Androgen Index, and increases in sex hormone binding globulin (65). It is also recommended to advise overweight and/or obese women to lose weight prior to ART use (66). There is substantial evidence pointing to the adverse effects of obesity on the egg and embryo (67,68). Recent data also suggest a non-genomic transfer of metabolic disorders via sperm and, if confirmed, this implies much more attention needs to be paid to optimization of male and female health and nutrition prior to pregnancy (69,70).

Diet

There are a number of dietary factors that have an impact on reproduction.

Vitamins

While there is little conclusive evidence on the effects of vitamins on fertility, more substantive evidence, particularly on folic acid, is published on their effects on reducing congenital abnormalities (71). It is therefore advised that women take up to 500 μg of folate for a minimum of one month prior to conception and, where there is a higher risk of abnormality, 5 mg should be taken. It is recommended that women avoid the retinol form of vitamin A and foods containing this form of vitamin A while considering consuming vitamin D and achieving appropriate sunlight exposure, given the alleged deficiency of this in many populations (72–74).

Iodine

Many women seeking pregnancy are iodine deficient and iodine is often added to prenatal supplements or foods (75,76). All women who are pregnant, breastfeeding, or considering pregnancy should take an iodine supplement of 150 μg each day for a minimum of one month prior to conception (72).

Male antioxidants

Oxidative stress is frequently described in infertile males and the role of antioxidants has been debated (77). While several commercial preparations exist, attention to increased fruit and vegetables in the diet and avoidance of adverse lifestyle factors including environmental chemical exposure should be the first step (78).

Alcohol

It remains unclear as to the level of alcohol consumption allowable in the periconception period and several official bodies recommend complete abstinence (74). A large Danish national study of more than 90,000 participants concluded that even low amounts of alcohol consumption during early pregnancy increased the risk of spontaneous abortion substantially (79). The role of alcohol in fetal alcohol syndrome is well known (80). It would therefore seem prudent for the woman to avoid alcohol during the periconception period.

Caffeine

Caffeine is the most popular neurostimulant and is found in drinks and foods across all cultures. A high consumption of caffeine may be associated with impaired fecundity, although the evidence is not conclusive (74,81–83). While a safe level of caffeine has not been defined, it seems reasonable to keep this below 200–300 mg per day (less than two cups of coffee per day) (84–86).

Fish consumption

Certain types of fish that are high in mercury should be avoided, while acknowledging that a high-polyunsaturated diet as given by fish is desirable (72,73,87).

Smoking

Smoking can affect all stages of reproduction including folliculogenesis, steroidogenesis, embryo transport, endometrial receptivity, endometrial angiogenesis, uterine blood flow, and uterine myometrium (88). However, the effect of smoking on fertility is underestimated by the public (89). A meta-analysis of the literature indicates that smoking is a very significant risk factor for male and female infertility and that it negatively affects the outcomes of *in vitro* fertilization (IVF) cycles (90–92). For female smokers this can be as high an odds ratio for infertility as 1.6 (95% confidence interval: 1.34–1.91) (91). For every one cigarette per day, there is a 1% increase in relative risk of miscarriage (93). Sperm studies have shown increased oxidative stress, a lower sperm count, and abnormal sperm fertilizing capacity, with a significantly reduced chance of pregnancy in a female partner (94,95). Passive smoking is also important in increasing complications in pregnancy as well as in IVF cycles (93,96). There are many studies showing that intervention programs for smoking can be successful.

Illicit drugs

Marijuana increases female infertility (97) and significantly affects sperm function and form (98). Cocaine impairs ovarian responsiveness and alters sperm function (99,100), while heroin and methadone also have significant effects (59,101). Anabolic steroids can reduce testicular sperm production, while the role of other lifestyle drugs is still to be explored (102).

Other prescription drugs

There are many drugs that appear to affect fertility and congenital abnormalities and alter reproductive outcomes (74,103). These should be assessed during initial consultations and the patient should be recommended to seek alternatives if actively trying to become pregnant.

Stress

There is growing evidence that psychosocial stress is associated with negative reproductive outcomes, including IVF therapies (104–108). Appropriate counseling and lifestyle adjustments may ameliorate these effects. Based on the best available evidence in the literature, the European Society of Human Reproduction and Embryology (ESHRE) have recently developed guidelines for routine psychosocial care at infertility and medically assisted reproduction clinics (109).

Environmental pollutants

While there is a vast and controversial literature on this subject, some environmental agents may adversely affect outcomes of reproductive interventions (87,110–112). It would seem prudent to ask all patients for their occupational and environmental exposures to endocrine-disrupting chemicals such as bisphenol A, phthalates, insecticides, and other potentially dangerous products (50,72,73,110,112,113).

Vaccinations

There are few data on the impacts of vaccinations on fertility, but the serious consequences of becoming infected with rubella, herpes zoster, varicella zoster, and influenza indicate that immunization prior to pregnancy is appropriate (72,114).

Sexually transmitted diseases

It is increasingly evident that bacterial and viral infections of the reproductive tissues can alter immune and inflammatory parameters in such a way as to impede periconception events and reduce fertility. The recommendation is that couples (both partners) should seek advice from their clinical care provider regarding the detection and treatment of any infection of the reproductive tract, remembering that many (such as chlamydia) are widespread in the community and may not necessarily result in signs or symptoms.

Occupational factors

Evidence suggests that the circadian clock regulates each part of the reproductive axis from timing of neuronal activity in hypothalamic neurons to the day–night variation in the release of pregnancy hormones. Dysregulation of circadian rhythms, as often occurs with shift work, results in increased risk of adverse consequences at each step of the reproductive pathway (115). Other common workplace exposures such as prolonged working hours, lifting, standing, and heavy physical workload may also increase the risk of adverse obstetric and neonatal outcomes (116).

PRE-PREGNANCY PREPARATION

Given the theoretical and practical background to periconception health, the desire of infertile couples to seek specialist treatment, and the opportunity to favorably influence outcomes of fertility treatment, all clinics should have a program to assess adverse genetic and lifestyle influences on reproduction and an intervention protocol to minimize their detrimental effects. This is best achieved at the first interview with the doctor or nurse. Action can then be advised while there is time for an effective plan to be instituted by the clinic and couple (Figure 34.3). This may be as simple as taking folic acid and changing diet to optimize the periconception environment through to active weight loss programs, smoking cessation interventions, and elimination of inappropriate alcohol and drug use. There is currently no evidence assessing this approach and a recent Cochrane review was unable to identify any randomized trials on lifestyle intervention in infertile couples (117). There is more research on weight management and fertility. Several groups have described programs for weight loss in the context of a fertility clinic, with the best known being that by Clark from Adelaide (Fertility Fitness)

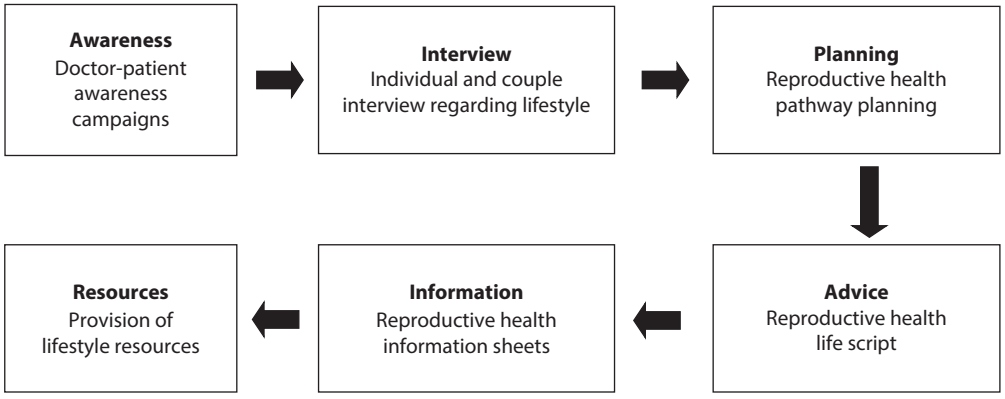


Figure 34.3 An approach to assessing and managing lifestyle in a clinical setting.

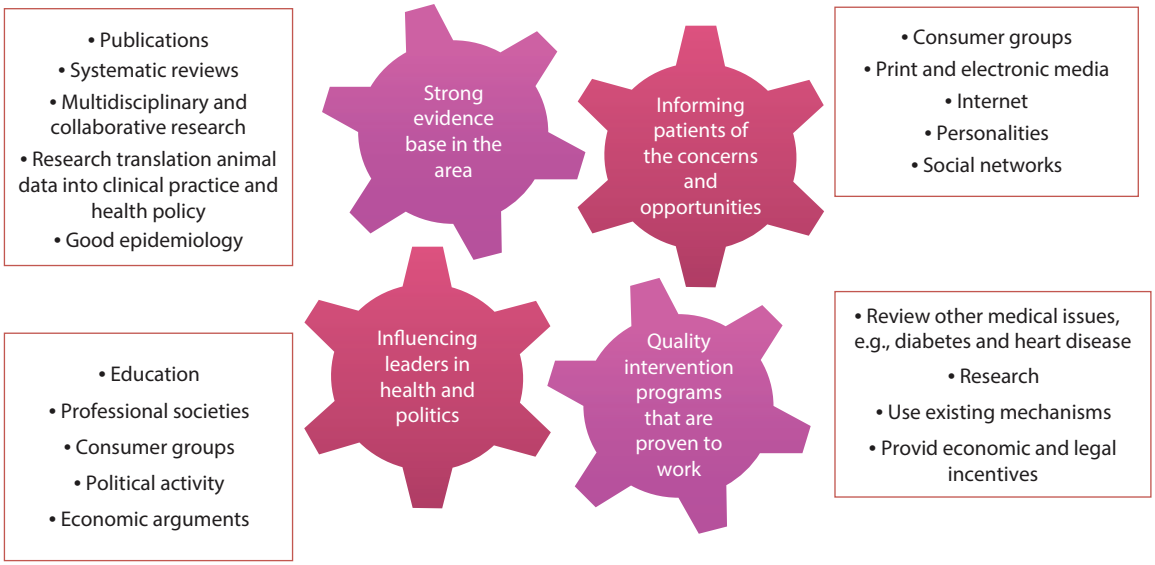


Figure 34.4 A society-wide approach to achieving lifestyle changes.

(118,119) and the FAST study (120). In this program, 5% weight loss was associated with a dramatic improvement in spontaneous and IVF pregnancy rates. Legro and colleagues have published compelling evidence for significant weight loss in a PCOS population that could be applied to other groups (121). Other popular community or expert-based facilities are available in the general community to improve lifestyle prior to pregnancy or while actively intervening.

There is a responsibility on governments to facilitate and encourage various aspects of preconception care including promoting vaccination, controlling alcohol and smoking use, providing a safe workplace, and giving general reproductive education (Figure 34.4). The clinic and individual, however, have an even greater role in safeguarding reproductive security by ensuring any pregnancy is conceived with gametes and embryos that have had the best chance to achieve their full genetic potential.

SUMMARY

In summary, there is compelling evidence that external and endogenous events in women and men impact preconception and very early pregnancy to benefit or constrain the later health of the neonate, child, and adult. Events in the pre- and peri-implantation period, spanning gametogenesis, conception, and early placental morphogenesis, have the power to impart long-term susceptibility or resilience in our children and community.

Defining the nature and actions of these external and endogenous events is now within reach. We know several of the key interlocutory signals between the oocyte and follicle, the sperm and oocyte, and the conceptus and uterus, but their full identity and interaction with environmental factors, reproductive disorders, and genetic backgrounds remain to be elucidated. Some of the most potent stressors of embryos and gametes are lifestyle factors—very young or older age, obesity, sexually transmitted infection, drugs, alcohol, diet, vitamin deficiency, and psychosocial stress. Understanding how these factors affect periconception biology will inform how public health initiatives can be targeted to modify behaviors and educate prospective parents. Similarly, the maternal reproductive disorders that impact early development are amenable to better diagnosis and clinical treatments. Defining their effects on the ovary and uterus, gametes, embryo, and placenta, and their interactions with environmental factors in the context of different genetic settings, is required to focus and prioritize clinical interventions. Despite the complexity in these interactions, we postulate that several stressors converge through a few key common inflammatory and metabolic pathways. Therefore, the prospect of identifying drug targets or interventions to minimize, reverse, or protect against adverse early environments may also be achievable.

REFERENCES

1. Australian Institute of Health and Welfare 2016. *Australia's Health 2016*. Australia's health series no. 15. Cat. no. AUS 199. Canberra: AIHW.
2. Steegers-Theunissen RP, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: The importance of one-carbon metabolism. *Hum Reprod Update* 2013; 19(6): 640–55.
3. Lane M, Robker RL, Robertson SA. Parenting from before conception. *Science* 2014; 345(6198): 756–60.
4. Norman RJ. 2015 RANZCOG Arthur Wilson Memorial Oration 'From little things, big things grow: The importance of periconception medicine'. *Aust N Z J Obstet Gynaecol* 2015; 55(6): 535–40.
5. Barker DJ, Clark PM. Fetal undernutrition and disease in later life. *Rev Reprod* 1997; 2(2): 105–12.

6. United Nations. Report of the International Conference on Population and Development (ICPD), Cairo, September 5–13, 1994. Available at: http://www.un.org/popin/icpd/conference/offeng/poa.html. Accessed Jul 10, 2017.
7. United Nations. Report of the Fourth World Conference on Women, Beijing, September 4-15, 1995. Available at: http://www.un.org/womenwatch/daw/beijing/pdf/Beijing%20full%20report%20E.pdf. Accessed Jul 10, 2017.
8. Sachs JD. *Investing in Development: A Practical Plan to Achieve the Millennium Development Goals*. Earthscan, London, 2005.
9. WHO. Research on sexual and reproductive health throughout the world. 2015. Available from: http://www.who.int/reproductivehealth/about_us/hrp/en/
10. Benagiano G, d'Arcangues C, Harris Requejo J, Schafer A, Say L, Merialdi M. The special programme of research in human reproduction: Forty years of activities to achieve reproductive health for all. *Gynecol Obstet Invest* 2012; 74(3): 190–217.
11. Thompson JG, Lane M, Robertson SA. Adaptive responses of embryos to their microenvironment and consequences for post-implantation development. In: *Early Life Origins of Health and Disease*. Owens JS, Wintour M (eds). Landes Bioscience, New York, 2005, pp. 58–69.
12. Sutton ML, Gilchrist RB, Thompson JG. Effects of *in-vivo* and *in-vitro* environments on the metabolism of the cumulus–oocyte complex and its influence on oocyte developmental capacity. *Hum Reprod Update* 2003; 9(1): 35–48.
13. Mtango NR, Potireddy S, Latham KE. Oocyte quality and maternal control of development. *Int Rev Cell Mol Biol* 2008; 268: 223–90.
14. Young LE. Imprinting of genes and the Barker hypothesis. *Twin Res* 2001; 4(5): 307–17.
15. Morgan HD, Santos F, Green K, Dean W, Reik W. Epigenetic reprogramming in mammals. *Hum Mol Genet* 2005; 14(1): R47–58.
16. Kaminsky ZA, Tang T, Wang SC et al. DNA methylation profiles in monozygotic and dizygotic twins. *Nat Genet* 2009; 41(2): 240–5.
17. Robertson SA, Chin PY, Glynn DJ, Thompson JG. Peri-conceptual cytokines—Setting the trajectory for embryo implantation, pregnancy and beyond. *Am J Reprod Immunol* 2011; 66(Suppl 1): 2–10.
18. Robertson SA, Chin PY, Schjenken JE, Thompson JG. Female tract cytokines and developmental programming in embryos. *Adv Exp Med Biol* 2015; 843: 173–213.
19. Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ, Robertson SA. Maternal tract factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *Proc Natl Acad Sci U S A* 2014; 111(6): 2200–5.
20. Robertson SA, Moldenhauer LM. Immunological determinants of implantation success. *Int J Dev Biol* 2014; 58(2–4): 205–17.
21. Cakmak H, Taylor HS. Implantation failure: Molecular mechanisms and clinical treatment. *Hum Reprod Update* 2011; 17(2): 242–53.
22. Eberhard WG. Postcopulatory sexual selection: Darwin's omission and its consequences. *Proc Natl Acad Sci U S A* 2009; 106(Suppl 1): 10025–32.
23. Robertson SA, Guerin LR, Moldenhauer LM, Hayball JD. Activating T regulatory cells for tolerance in early pregnancy—The contribution of seminal fluid. *J Reprod Immunol* 2009; 83(1–2): 109–16.
24. Trowsdale J, Betz AG. Mother's little helpers: Mechanisms of maternal-fetal tolerance. *Nat Immunol* 2006; 7(3): 241–6.
25. Robertson SA. Immune regulation of conception and embryo implantation—All about quality control? *J Reprod Immunol* 2010; 85(1): 51–7.
26. Watkins AJ, Papenbrock T, Fleming TP. The preimplantation embryo: Handle with care. *Semin Reprod Med* 2008; 26(2): 175–85.
27. Lane M, Gardner DK. Differential regulation of mouse embryo development and viability by amino acids. *J Reprod Fertil* 1997; 109(1): 153–64.
28. Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* 2000; 127(19): 4195–202.
29. Sjoblom C, Roberts CT, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. *Endocrinology* 2005; 146(5): 2142–53.
30. Tam PP. Postimplantation development of mitomycin C-treated mouse blastocysts. *Teratology* 1988; 37(3): 205–12.
31. Godfrey KM. The role of the placenta in fetal programming—A review. *Placenta* 2002; 23(Suppl A): S20–7.
32. Nelissen EC, van Montfoort AP, Dumoulin JC, Evers JL. Epigenetics and the placenta. *Hum Reprod Update* 2011; 17(3): 397–417.
33. Vaiserman AM. Epigenetic programming by early-life stress: Evidence from human populations. *Dev Dyn* 2015; 244(3): 254–65.
34. Hoet JJ, Ozanne S, Reusens B. Influences of pre- and postnatal nutritional exposures on vascular/endocrine systems in animals. *Environ Health Perspect* 2000; 108(Suppl 3): 563–8.
35. Fowden AL, Forhead AJ, Coan PM, Burton GJ. The placenta and intrauterine programming. *J Neuroendocrinol* 2008; 20(4): 439–50.
36. Maltepe E, Bakardjiev AI, Fisher SJ. The placenta: Transcriptional, epigenetic, and physiological integration during development. *J Clin Invest* 2010; 120(4): 1016–25.
37. Barker DJ, Gluckman PD, Robinson JS. Conference report: Fetal origins of adult disease—Report of the First International Study Group, Sydney, 29–30 October 1994. *Placenta* 1995; 16(3): 317–20.

38. Stein AD, Lumey LH. The relationship between maternal and offspring birth weights after maternal prenatal famine exposure: The Dutch Famine Birth Cohort Study. *Hum Biol* 2000; 72(4): 641–54.
39. van Dijk M, Mulders J, Poutsma A, Konst AA, Lachmeijer AM, Dekker GA, Blankenstein MA, Oudejans CB. Maternal segregation of the Dutch preeclampsia locus at 10q22 with a new member of the winged helix gene family. *Nat Genet* 2005; 37(5): 514–9.
40. Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ. Catch-up growth in childhood and death from coronary heart disease: Longitudinal study. *BMJ* 1999; 318(7181): 427–31.
41. Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am J Physiol Endocrinol Metab* 2000; 279(1): E83–7.
42. Ong KK, Ahmed ML, Emmett V, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: Prospective cohort study. *BMJ* 2000; 320(7240): 967–71.
43. Veening MA, Van Weissenbruch MM, Delemarre-Van De Waal HA. Glucose tolerance, insulin sensitivity, and insulin secretion in children born small for gestational age. *J Clin Endocrinol Metab* 2002; 87(10): 4657–61.
44. Parker L, Lamont DW, Unwin N, Pearce MS, Bennett SM, Dickinson HO, White M, Mathers JC, Alberti KG, Craft AW. A lifecourse study of risk for hyperinsulinaemia, dyslipidaemia and obesity (the central metabolic syndrome) at age 49-51 years. *Diabet Med* 2003; 20(5): 406–15.
45. Davies MJ, Norman RJ. Programming and reproductive functioning. *Trends Endocrinol Metab* 2002; 13(9): 386–92.
46. Lord JM, Norman R. Obesity, polycystic ovary syndrome, infertility treatment: Lifestyle modification is paramount. *BMJ* 2006; 332(7541): 609.
47. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, Petraglia F. Infertility and reproductive disorders: Impact of hormonal and inflammatory mechanisms on pregnancy outcome. *Hum Reprod Update* 2016; 22(1): 104–15.
48. Thompson JG, Kind KL, Roberts CT, Robertson SA, Robinson JS. Epigenetic risks related to assisted reproductive technologies: Short- and long-term consequences for the health of children conceived through assisted reproduction technology: More reason for caution? *Hum Reprod* 2002; 17(11): 2783–6.
49. De Rycke M, Liebaers I, Van Steirteghem A. Epigenetic risks related to assisted reproductive technologies: Risk analysis and epigenetic inheritance. *Hum Reprod* 2002; 17(10): 2487–94.
50. Basatemur E, Sutcliffe A. Follow-up of children born after ART. *Placenta* 2008; 29(Suppl B): 135–40.
51. Richani D, Sutton-McDowall ML, Frank LA, Gilchrist RB, Thompson JG. Effect of epidermal growth factor-like peptides on the metabolism of *in vitro*-matured mouse oocytes and cumulus cells. *Biol Reprod* 2014; 90(3): 49.
52. Sharkey AM, Macklon NS. The science of implantation emerges blinking into the light. *Reprod Biomed Online* 2013; 27(5): 453–60.
53. Chronopoulou E, Harper JC. IVF culture media: Past, present and future. *Hum Reprod Update* 2015; 21(1): 39–55.
54. Dumesic DA, Abbott DH, Padmanabhan V. Polycystic ovary syndrome and its developmental origins. *Rev Endocr Metab Disord* 2007; 8(2): 127–41.
55. Norman RJ, Hickey T, Moran L. Genetic/epigenetic and environmental origins of PCOS. *Early Hum Dev* 2007; 83: S43–S4.
56. Rhind SM, Rae MT, Brooks AN. Effects of nutrition and environmental factors on the fetal programming of the reproductive axis. *Reproduction* 2001; 122(2): 205–14.
57. Davies MJ. Fetal programming: The perspective of single and twin pregnancies. *Reprod Fertil Dev* 2005; 17(3): 379–86.
58. Ng M, Fleming T, Robinson M et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; 384(9945): 766–81. doi:10.1016/S0140-6736(14)60460-8
59. Ramlau-Hansen CH, Thulstrup AM, Nohr EA, Bonde JP, Sorensen TI, Olsen J. Subfecundity in overweight and obese couples. *Hum Reprod* 2007; 22(6): 1634–7.
60. Hassan MA, Killick SR Negative lifestyle is associated with a significant reduction in fecundity. *Fertil Steril* 2004; 81(2): 384–92.
61. Shukla KK, Chambial S, Dwivedi S, Misra S, Sharma P. Recent scenario of obesity and male fertility. *Andrology* 2014; 2(6): 809–18.
62. Campbell JM, Lane M, Owens JA, Bakos HW. Paternal obesity negatively affects male fertility and assisted reproduction outcomes: A systematic review and meta-analysis. *Reprod Biomed Online* 2015; 31(5): 593–604.
63. Rittenberg V, Seshadri S, Sunkara SK, Sobaleva S, Oteng-Ntim E, El-Toukhy T. Effect of body mass index on IVF treatment outcome: An updated systematic review and meta-analysis. *Reprod Biomed Online* 2011; 23(4): 421–39.
64. Mission JF, Marshall NE, Caughey AB. Obesity in pregnancy: A big problem and getting bigger. *Obstet Gynecol Surv* 2013; 68(5): 389–99.
65. Orio F, Muscogiuri G, Ascione A, Marciano F, Volpe A, La Sala G, Savastano S, Colao A, Palomba S. Effects of physical exercise on the female reproductive system. *Minerva Endocrinol* 2013; 38(3): 305–19.

66. Sim KA, Partridge SR, Sainsbury A. Does weight loss in overweight or obese women improve fertility treatment outcomes? A systematic review. *Obes Rev* 2014; 15(10): 839–50.
67. Wu LL, Norman RJ, Robker RL. The impact of obesity on oocytes: Evidence for lipotoxicity mechanisms. *Reprod Fertil Dev* 2011; 24(1): 29–34.
68. McPherson NO, Bell VG, Zander-Fox DL, Fullston T, Wu LL, Robker RL, Lane M. When two obese parents are worse than one! Impacts on embryo and fetal development. *Am J Physiol Endocrinol Metab* 2015; 309(6): E568–81.
69. Fullston T, Ohlsson Teague EM, Palmer NO, DeBlasio MJ, Mitchell M, Corbett M, Print CG, Owens JA, Lane M. Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. *FASEB J* 2013; 27(10): 4226–43.
70. Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ. Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. *Nature* 2010; 467(7318): 963–6.
71. Prevention of neural tube defects: Results of the medical research council vitamin study. MRC Vitamin Study Research Group. *Lancet* 1991; 338(8760): 131–7.
72. The Royal Australian College of General Practitioners. *Guidelines for Preventive Activities in General Practice*. 9th Edition. East Melbourne, Victoria: RACGP, 2016.
73. SA Maternal & Neonatal Clinical Network. *Vitamin and Mineral Supplementation in Pregnancy, G.o.S.A*. Department of Health, South Australia, 2014.
74. Anderson K, Nisenblat V, Norman R. Lifestyle factors in people seeking infertility treatment—A review. *Aust N Z J Obstet Gynaecol* 2010; 50(1): 8–20.
75. Zimmermann M, Delange F. Iodine supplementation of pregnant women in Europe: A review and recommendations. *Eur J Clin Nutr* 2004; 58(7): 979–84.
76. Food Standards Australia and New Zealand. *Proposal P1003 Mandatory Iodine Fortification for Australia Assessment Report*. AfU, 2008. Available from: http://www.foodstandards.gov.au/code/proposals/documents/P1003%20Mandatory%20Iodine%20fortification%20Aust%20AR%20FINAL.pdf.
77. Tremellen K. Oxidative stress and male infertility—A clinical perspective. *Hum Reprod Update* 2008; 14(3): 243–58.
78. Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. *Cochrane Database Syst Rev* 2014; 12: CD007411.
79. Andersen AM, Andersen PK, Olsen J, Gronbaek M, Strandberg-Larsen K. Moderate alcohol intake during pregnancy and risk of fetal death. *Int J Epidemiol* 2012; 41(2): 405–13.
80. Mukherjee RA, Hollins S, Abou-Saleh MT, Turk J. Low level alcohol consumption and the fetus. *BMJ* 2005; 330(7488): 375–6.
81. Greenwood DC, Alwan N, Boylan S et al. Caffeine intake during pregnancy, late miscarriage and stillbirth. *Eur J Epidemiol* 2010; 25(4): 275–80.
82. Savitz DA, Chan RL, Herring AH, Howards PP, Hartmann KE. Caffeine and miscarriage risk. *Epidemiology* 2008; 19(1): 55–62.
83. Golding J. Reproduction and caffeine consumption—A literature review. *Early Hum Dev* 1995; 43(1): 1–14.
84. Higdon JV, Frei B. Coffee and health: A review of recent human research. *Crit Rev Food Sci Nutr* 2006; 46(2): 101–23.
85. Signorello LB, McLaughlin JK. Maternal caffeine consumption and spontaneous abortion: A review of the epidemiologic evidence. *Epidemiology* 2004; 15(2): 229–39.
86. Nisenblat V, Norman RJ. The effects of caffeine on reproductive outcomes in women. UpToDate, 2015. Available from: https://www.uptodate.com/contents/the-effects-of-caffeine-on-reproductive-outcomes-in-women
87. McDiarmid MA, Gardiner PM, Jack BW. The clinical content of preconception care: Environmental exposures. *Am J Obstet Gynecol* 2008; 199(6 Suppl 2): S357–61.
88. Dechanet C, Anahory T, Mathieu Daude JC, Quantin X, Reyftmann L, Hamamah S, Hedon B, Dechaud H. Effects of cigarette smoking on reproduction. *Hum Reprod Update* 2011; 17(1): 76–95.
89. Practice Committee of the American Society for Reproductive Medicine. Smoking and infertility: A committee opinion. *Fertil Steril* 2012; 98(6): 1400–6.
90. Waylen AL, Metwally M, Jones GL, Wilkinson AJ, Ledger WL. Effects of cigarette smoking upon clinical outcomes of assisted reproduction: A meta-analysis. *Hum Reprod Update* 2009; 15(1): 31–44.
91. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: A systematic review and meta-analysis. *Hum Reprod* 1998; 13(6): 1532–9.
92. Feichtinger W, Papalambrou K, Poehl M, Krischker U, Neumann K. Smoking and *in vitro* fertilization: A meta-analysis. *J Assist Reprod Genet* 1997; 14(10): 596–9.
93. Pineles BL, Park E, Samet JM. Systematic review and meta-analysis of miscarriage and maternal exposure to tobacco smoke during pregnancy. *Am J Epidemiol* 2014; 179(7): 807–23.
94. Frey KA, Navarro SM, Kotelchuck M, Lu MC. The clinical content of preconception care: Preconception care for men. *Am J Obstet Gynecol* 2008; 199(6 Suppl 2): S389–95.
95. Zitzmann M, Rolf C, Nordhoff V., Schrader G, Rickert-Fohring M, Gassner P, Behre HM, Greb RR, Kiesel L, Nieschlag E. Male smokers have a decreased

success rate for *in vitro* fertilization and intracytoplasmic sperm injection. *Fertil Steril* 2003; 79 (Suppl 3): 1550–4.
96. Neal MS, Hughes EG, Holloway AC, Foster WG. Sidestream smoking is equally as damaging as mainstream smoking on IVF outcomes. *Hum Reprod* 2005; 20(9): 2531–5.
97. Mueller BA, Daling JR, Weiss NS, Moore DE. Recreational drug use and the risk of primary infertility. *Epidemiology* 1990; 1(3): 195–200.
98. Battista N, Pasquariello N, Di Tommaso M, Maccarrone M. Interplay between endocannabinoids, steroids and cytokines in the control of human reproduction. *J Neuroendocrinol* 2008; 20(Suppl 1): 82–9.
99. Thyer AC, King TS, Moreno AC, Eddy CA, Siler-Khodr TM, Schenken RS. Cocaine impairs ovarian response to exogenous gonadotropins in nonhuman primates. *J Soc Gynecol Investig* 2001; 8(6): 358–62.
100. George VK, Li H, Teloken C, Grignon DJ, Lawrence WD, Dhabuwala CB. Effects of long-term cocaine exposure on spermatogenesis and fertility in peripubertal male rats. *J Urol* 1996; 155(1): 327–31.
101. Ragni G, De Lauretis L, Bestetti O, Sghedoni D, Gambaro V. Gonadal function in male heroin and methadone addicts. *Int J Androl* 1988; 11(2): 93–100.
102. Pasqualotto FF, Lucon AM, Sobreiro BP, Pasqualotto EB, Arap S. Effects of medical therapy, alcohol, smoking, and endocrine disruptors on male infertility. *Rev Hosp Clin Fac Med Sao Paulo* 2004; 59(6): 375–82.
103. Pandiyan N. Medical drugs impairing fertility. In: *Reproductive Health and the Environment*. Nicolopolou-Stamati P, Hens L, Howard CV (eds). Dordrecht, The Netherlands: Springer, 2007, pp. 187–205.
104. Nakamura K, Sheps S, Arck PC. Stress and reproductive failure: Past notions, present insights and future directions. *J Assist Reprod Genet* 2008; 25(2–3): 47–62.
105. Barzilai-Pesach V, Sheiner EK, Sheiner E, Potashnik G, Shoham-Vardi I. The effect of women's occupational psychologic stress on outcome of fertility treatments. *J Occup Environ Med* 2006; 48(1): 56–62.
106. Smeenk JM, Verhaak CM, Vingerhoets AJ, Sweep CG, Merkus JM, Willemsen SJ, van Minnen A, Straatman H, Braat DD. Stress and outcome success in IVF: The role of self-reports and endocrine variables. *Hum Reprod* 2005; 20(4): 991–6.
107. Klonoff-Cohen H, Natarajan L. The concerns during assisted reproductive technologies (CART) scale and pregnancy outcomes. *Fertil Steril* 2004; 81(4): 982–8.
108. Zorn B, Auger J, Velikonja V, Kolbezen M, Meden-Vrtovec H. Psychological factors in male partners of infertile couples: Relationship with semen quality and early miscarriage. *Int J Androl* 2008; 31(6): 557–64.
109. Gameiro S, Boivin J, Dancet E et al. ESHRE guideline: Routine psychosocial care in infertility and medically assisted reproduction—A guide for fertility staff. *Hum Reprod* 2015; 30(11): 2476–85.
110. Homan GF, Davies M, Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: A review. *Hum Reprod Update* 2007; 13(3): 209–23.
111. Foster WG, Neal MS, Han MS, Dominguez MM. Environmental contaminants and human infertility: Hypothesis or cause for concern? *J Toxicol Environ Health B Crit Rev* 2008; 11(3–4): 162–76.
112. Mendola P, Messer LC, Rappazzo K. Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult female. *Fertil Steril* 2008; 89(2 Suppl): e81–94.
113. Wong WY, Zielhuis GA, Thomas CM, Merkus HM, Steegers-Theunissen RP. New evidence of the influence of exogenous and endogenous factors on sperm count in man. *Eur J Obstet Gynecol Reprod Biol* 2003; 110(1): 49–54.
114. Practice Committee of American Society for Reproductive Medicine. Vaccination guidelines for female infertility patients: A committee opinion. *Fertil Steril* 2013; 99(2): 337–9.
115. Gamble KL, Resuehr D, Johnson CH. Shift work and circadian dysregulation of reproduction. *Front Endocrinol (Lausanne)* 2013; 4: 92.
116. Palmer KT, Bonzini M, Bonde JP, Multidisciplinary Guideline Development Group, Health and Work Development Unit, Royal College of Physicians, and Faculty of Occupational Medicine. Pregnancy: Occupational aspects of management: Concise guidance. *Clin Med* 2013; 13(1): 75–9.
117. Anderson K, Norman RJ, Middleton P. Preconception lifestyle advice for people with subfertility. *Cochrane Database Syst Rev* 2010; 4: CD008189.
118. Clark AM, Ledger W, Galletly C, Tomlinson L, Blaney F, Wang X, Norman RJ. Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Hum Reprod* 1995; 10(10): 2705–12.
119. Clark AM, Thornley B, Tomlinson L, Galletley C, Norman RJ. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. *Hum Reprod* 1998; 13(6): 1502–5.
120. Homan G, Litt J, Norman RJ. The FAST study: Fertility ASsessment and advice Targeting lifestyle choices and behaviours: A pilot study. *Hum Reprod* 2012; 27(8): 2396–404.
121. Legro RS, Dodson WC, Kris-Etherton PM et al. Randomized controlled trial of preconception interventions in infertile women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2015; 100(11): 4048–58.

The environment and reproduction

35

MACHE SEIBEL

I used to walk along the beach,
a favorite thing to do.
Until the plastic and the trash completely
spoiled my view.
The place I take my rod and reel to catch my
favorite dish.
Has elevated mercury, so I can't eat the fish.

From *Protect Environment* by Mache Seibel

It is fascinating that so much is written about detox diets and detox cleanses and so little effort is devoted to detoxing the environment. Considering that the primary purpose of our existence is reproduction, it is clear that much more attention should be given to the role of the environment on the reproductive process (1). Toxic and environmental hazards can affect reproduction at any point in the process. They can affect fertility, conception, pregnancy, and/or delivery. And, of course, they can affect the male and the female (2).

Added to these environmental hazards is the fact that many women are delaying childbearing, setting the stage for less fertility, compromised further by additional insult to the reproductive system by environmental factors.

There were 80,000 synthetic compounds used in the U.S.A. during the last half-century (3). There needs to be much more clarity regarding the role these compounds play on our most basic human goal—reproduction—because it is being affected. It is estimated that over 1000 new chemicals are being introduced into the world every year, yet fewer than 5% have been investigated for their effect on reproduction.

We know that women are commonly being exposed to small amounts of potentially toxic materials such as lead, mercury, and polychlorinated biphenyls (PCBs) (4). Roughly a third of women aged 16–49 years who participated in the National Health and Nutrition Examination Survey (NHANES) had levels at or above the median for two of these three chemicals and 23% had elevated levels for all three. Although this is below levels that are thought to be clinically impactful, having that much toxic material in women of childbearing age clearly needs greater study and prevention.

Fertility studies in Pennsylvania have shown decreased total fertility rates from 1901 to 1985 (5), and the overall U.S. pregnancy rate in 1996 was 9% lower than it was in 1990 (6). Women who work in jobs where they regularly have job exposure to phthalates take longer to conceive and those exposed to pesticides are associated with lower fetal weights (7). There is evidence that the quality and quantity of semen in normal men is also declining (8,9). What role do the thousands of compounds in our everyday environment play in these declining rates? What role does environmental exposure play in the spectrum of infertility patients that present to our clinics? What evidence is available for interpreting exposures, and what clinical considerations can we yield from such research?

To address these questions, we will consider the environment not as the world at large, but rather as three *microenvironments*: the follicle, the seminal fluid, and the amniotic sac. From this vantage point, the inhabitants are the egg, the sperm, and the unborn child. In this way, the impact of the environment is not a story of what the world will be like decades from now, but rather focuses on an increased awareness of the impact of the environment on our reproductive-aged patients and children today.

MECHANISMS OF ACTION

Direct damage to the cell membrane or intracellular components is only one way compounds can cause tissue injury. Some compounds alter the communication between different cells by mimicking or blocking normal pathways. Endocrine-disrupting chemicals (EDCs) are thought to affect reproduction by directly or indirectly mimicking, stimulating, antagonizing, altering, or displacing natural hormones (10). Exposure to such agents at critical stages of development can have a significant impact upon cellular, and ultimately fetal, development. Incomplete development of DNA repair mechanisms, detoxification enzymes, and the blood–brain barrier can exacerbate a chemical's effect on the developing fetus. Moreover, there is an increasing body of research suggesting that epigenetic modulation may be an underlying mechanism of action. These effects, however, may not be seen for years.

The theory of EDCs can be traced back to the publication of *Silent Spring* (1962), by Rachel Carson (11). In a serialized printing in the *New Yorker* she proposed a connection between the population changes in wildlife ecology and the increasing rates of human cancer. She associated both changes with widespread use of agricultural and manufacturing chemicals. Her work eventually prompted a government investigation that culminated in the banning of the pesticide dichlorodiphenyltrichloroethane (DDT) in 1972. It was 20 years later that Theo Colborn and colleagues advanced the EDC theory that is now widely accepted (12–15): EDCs can exert more influence over the development of affected offspring than the genes they inherit. EDCs have been shown to have deleterious effects on animal and fish reproduction (16), and both synthetic and natural EDCs have been shown to impair the development of the male reproductive tract and external genitalia. Examples include pesticides, phthalate, dioxins and phytoestrogens, including newly synthesized resveratrol analogs, which affect Leydic cells during fetal

development and in adults (17). Compelling data exist on the role of EDCs in other hormone-driven diseases, such as the rising prevalence of endometriosis in industrialized countries (18).

BIOLOGICAL PLAUSIBILITY

Xenoestrogens, alkylphenolic chemicals (bisphenol A [BPA] (19) and PCBs), phthalates, dioxins, lead, mercury, and pesticides are ubiquitous in the global environment. They are unavoidable for the majority of us and have been reported to have a myriad of effects (Table 35.1). Many of these toxicants came under increased scrutiny when animal experiments began to demonstrate biological plausibility for human harm.

Sharpe et al. (121) demonstrated that gestational and lactational exposure of rats to xenoestrogens resulted in reduced testicular size and sperm production (20). Dicofol, an estrogenic organochloride pesticide, was observed to induce a significant decrease in ovarian follicles and the number of estrous cycles in rabbits (21), while follicle destruction has been reported in rhesus monkeys exposed to PCBs (22). The list of mammalian studies linking subfertility to environmental toxicants is extensive, including studies demonstrating embryotoxicity for DDT, methoxychlor, and hexachlorocyclohexane (23). Most human exposure is through food, air, or, in the case of trihalomethanes (THMs), absorption through skin. Exposure to the aforementioned compounds has been well documented, but is only now being monitored more closely. There were few data about non-occupational exposure to potential toxicants until the Centers for Disease Control and Prevention (CDC) began testing in 1999 (116 compounds) and 2003 (148 compounds) (24). *National Geographic* published an article in 2006 about a journalist who had his blood tested for levels of environmental toxicants to see what the average American accumulates in a lifetime. The tests, which cost around $15,000, revealed 165 of 320 chemicals tested, including levels of a fire retardant used on airline seats 10 times higher than the average American, because of the many hours spent in airplanes (25). The article highlighted the fact that these chemicals are ubiquitous not only in the environment, but also in our bodies.

The correlation between animal experiments and human experience was nicely demonstrated by Swan et al. when they examined the relationship between neonatal anogenital distance (AGD), a sexually dimorphic feature considered to be a sensitive indicator of masculinization and phthalate metabolites (26). Phthalates (diesters of 1,2-benzenedicarboxylic acid) are a ubiquitous group of chemicals found in hundreds of products ranging from soft plastic vinyl toys and flooring to shampoos, soaps, and nail polish. High-molecular-weight phthalates are used in the manufacturing of flexible vinyl for flooring, wall coverings, food contact applications, and medical devices. Low-molecular-weight phthalates are used in personal care products as solvents and plasticizers for making lacquers, varnishes, and coatings used in pharmaceuticals for timed release drugs. Humans rapidly metabolize phthalate diesters (their half-lives are generally less than 24 hours), and thus do not accumulate them. Urinary biomarkers (phthalate monoesters), therefore, represent exposure in the last one to two days only. Swan et al. evaluated mother–son pairs who had been recruited for an unrelated pregnancy cohort study ($n = 85$) and found a significant inverse relationship between the level of phthalate metabolites in the mother's third-trimester urine and the son's AGD at birth. Higher prenatal phthalate metabolite levels correlated with a shorter AGD, which, in turn, was associated with incomplete testicular descent and smaller penile volume. These findings demonstrate the effect an environmental chemical can have on morphological development. Although implied, further investigation is needed to comment specifically on fertility or fecundity.

Making the jump from biological plausibility to biological truth can be a difficult task. The randomized controlled trial (RCT) is widely recognized as the gold standard in medical research, but the use of such trials poses distinct challenges when studying toxicity. RCTs would be unethical, as deliberately exposing individuals to potentially toxic chemicals is neither realistic nor to be condoned. Given these limitations, Stephen Genuis argues that clinical trials are not the only objective and credible way of establishing causality of a disease (27). He poses a simple analogy: it would be absurd to require an RCT to confirm the efficacy of parachutes to "prevent death and major trauma related to gravitational challenge" (28). In other words, not all research topics can be evaluated in identical manners. An example is the potential occupational hazard of nurses and other healthcare professionals who work on oncology floors. Therapy-related leukemia has been known to occur in patients receiving alkylating agents for cancer treatment. Another study looked at the impact of both alkylating and non-alkylating agents on the personnel who handle these agents over a six-week period as they care for cancer patients. The study found a statistical increase in abnormalities of chromosome 5 and increased abnormalities of chromosomes 7 and 11 (29). Since many of the women in the health-related workplace are of reproductive age, handling of these and other agents can pose a real threat, in particular for spontaneous abortion, which doubles before the 12th week and increases to 3.5-fold afterwards following exposure to antineoplastic, anesthetic gases, antiviral drugs, sterilizing agents (disinfectants), and X-rays (30).

There are other challenges for interpreting environmental toxicant studies. Time-lag bias is a limitation that is highlighted by our experience with *in utero* diethylstilbestrol (DES) exposure and vaginal cancer: compounds can have devastating effects in the long term that are not immediately recognizable. Variations in genetic vulnerability and phenotypic response can also mask a compound's impact. For example, studies on BPA metabolism have shown induction of hepatic cytochrome p450s in humans (31,32). Individuals have p450 isoenzyme variation and thus will respond to bisphenols with different levels of metabolic activity. Furthermore, it can be difficult

Table 35.1 Summary of common environmental toxicants

	Phthalates	Pesticides	PCBs	Dioxins	PBDEs	Bisphenols	Heavy metals
Sources of exposure	Plastic toys, shampoos, soaps, nail polish, other personal care products, medical devices, timed-release drug coatings, flooring lacquers, and varnishes	DDE, DDT, organochlorides, and "non-persistent pesticides." DDT is banned in most countries, save Mexico, China, India, and parts of Africa. It is found in fruits, vegetables, flowers, antimicrobial soaps, and air exposure in agricultural areas	Banned in the U.S.A. and most countries. PCBs have dioxin-like properties. They were used for cutting oils, as lubricants, and as electrical insulators. Sources are contaminated fish and game consumption	Result from industrial activities and fires. Found in fatty meats, fish, and dairy products	Flame retardants in mattresses, furniture, pillows, carpets, electronic devices, TVs, DVD players, and computers	Polycarbonate plastics: rigid water bottles, soda bottles, and plastic food containers	Lead: pre-1978 paint, old pipes. Mercury: old thermometers, large fish such as tuna, shark, king mackerel, and swordfish, and electrical tilefish. Pressure-treated wood can leach chromium and arsenic
Potential effects	↑ TTP ↑ AGD	↓ Fecundability ↓ Success with IVF ↓ Semen quality ↑ Spontaneous abortion ↑ Preterm birth ↑ SGA	↓ Response to ovulation induction ↓ Fecundability ↓ Lactation ↓ Sperm quality ↑ Endometriosis Altered menstrual cycle	↑ Cancer ↑ Birth defects Change in sex ratio ↑ Endometriosis	Reproductive development disruption	↑ Breast cancer Prostate changes	↓ IQ in offspring ↓ Semen quality/ quantity ↑ Spontaneous abortion ↑ TTP ↑ Preterm labor
How to decrease exposure	Unavoidable	Wash produce well, buy organic, vacuum frequently in agricultural areas	Avoid eating fish or game from areas known to be contaminated	Avoid fatty meats Avoid areas known to be contaminated	Unavoidable	Avoid hard plastic bottles and food containers, but likely unavoidable	Remove old paint and pressure-treated lumber Be cautious/limit certain fish consumption

Abbreviations: PCB, polychlorinated biphenyl; PBDE, polybrominated diphenyl ether; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; IVF, *in vitro* fertilization; TTP, time to pregnancy; AGD, anogenital distance; SGA, small for gestational age.

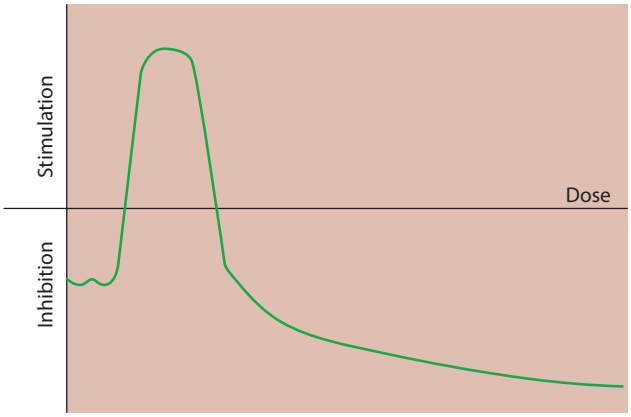


Figure 35.1 Hormetic/biphasic dose–response curve. The non-monotonic, hormetic, or "biphasic" dose–response curve describes the action of certain agents at different doses such that a very low dose of a chemical agent may trigger the opposite response to a very high dose.

to interpret dose–response curves because hormonal toxicants do not always respond according to the classic dose–response curve. Estradiol, for instance, has a negative feedback mechanism upon gonadotropin-releasing hormone (GnRH) release from the hypothalamus until it reaches a critical concentration, at which point it begins to increase the release of GnRH. This culminates in the luteinizing hormone surge that initiates ovulation. If EDCs are hormone mimickers, they probably act in the same fashion: effects may be seen at extremely low concentrations, but not at the higher concentrations used to test for chemical toxicity (33). This has been described as a non-monotonic, hormetic, or "biphasic" dose–response curve (Figure 35.1) and is described frequently in the endocrinology literature but not in the assessment of environmental agents. Lastly, environmental health research is complicated by the phenomenon of bioaccumulation. Humans are exposed to thousands of compounds over a lifetime and it is therefore difficult to sort out the relationship between a specific compound and a specific outcome (34). The CDC's *National Report on Human Exposure to Environmental Chemicals* (24) is in its third edition and has only evaluated 148 compounds through blood and urine analysis of the known 80,000 synthetic compounds in our environment. With time-lag bias, phenotypic variation, the inability to perform RCTs, unpredictable dose–response mechanisms, and the bioaccumulation of multiple compounds at once, it is apparent how difficult "proof" of causality can be in environmental toxicology.

THE SEMINAL PLASMA MICROENVIRONMENT

At levels measured in parts per trillion (ppt) and parts per billion (ppb), hormones such as insulin and estradiol are bioactive in cells and tissue. EDCs appear to be bioactive at equally low levels found in our bloodstream (33). Of great concern is the postulation that the seminal plasma acts as a chemical concentrator, increasing levels of various environmental toxicants in the fluid surrounding our next generation. Men living in agrarian areas where use of pesticides is high have higher pesticide levels in their blood and semen and lower sperm counts and motility than men living further away (35).

As mentioned previously, humans rapidly metabolize phthalate diesters, and do not accumulate them. Despite fast metabolization, the omnipresence of phthalates in our environment raises concern. A study in 2003 from Columbia University Center for Children's Environmental Health found that among 60 pregnant women tested, 100% had measurable urinary phthalate levels despite being sampled from different areas of both New York City ($n = 30$) and Krakow, Poland ($n = 30$) (36). Concurrent research from the Harvard School of Public Health found a dose–response relationship between urine levels of phthalate metabolites and a decrease in sperm motility and concentration in a cohort of 168 infertile men (37). Additional studies demonstrated similar results (34,38). A conflicting study from Sweden, however, found no change in semen quality in 234 young men recruited at the time of their medical exam for entry into the military based on urine phthalate levels (39). Russ Hauser, a Harvard researcher, brings to light multiple methodological and analytical differences between these studies in a review article that calls into question the validity of the Swedish study and comparability of the study results (38). Most notable are the study population differences: the Swedish study recruited young men, and the American studies had older infertile patients.

Although not conclusive, the data suggest phthalates play a role in decreased sperm quality and possibly fertility. Conflicting data also exist for many other potential reproductive toxicants. The THMs are a group of chemical by-products of the chlorination processes used to disinfect drinking water. They have been a source of investigation for infertility and birth defects. One THM, bromodichloromethane, has been associated with low sperm counts and increased abnormal semen morphology (40), but the majority of studies in both rats and humans have not found conclusive evidence that THMs decrease sperm quality or quantity (41). Additional investigations have questioned the relationship between THM exposure and spontaneous abortion (42,43). In 2000, an international workshop gathered to assess the impact of disinfectant by-products on reproduction and concluded that more research on methods of exposure assessment needed to be done in order to properly evaluate exposure risk (44). Since that time, a well-documented case–control study of over 2400 pregnancies in North Carolina did not find an association between THMs and spontaneous abortion (45). The study enrolled patients at seven weeks' gestation or less, sampled weekly drinking water from three distinct THM-profile regions, and specifically analyzed exposure during critical periods of fetal development.

Another chemical that causes concern is the pesticide dichlorodiphenyldichloroethylene (DDE), a persistent remnant of DDT, which, although no longer being

produced in the U.S.A., is sporadically used in Mexico (35), India, and China (46). Despite initially using pilot data, two studies were unable to demonstrate an association between sperm quality and DDE in both infertile U.S. patients (n = 12) and older Swedish fishermen (n = 195) (47,48). At this time, the evidence suggesting a risk of DDE to male fertility at casual exposure levels is still being evaluated.

PCBs, much like DDT, have been banned in the U.S.A. since the late 1970s. PCBs are synthetic, persistent, halogenated, lipophilic substances that are still ubiquitous in our environment today. They have varying hormonal functions: some act as weak estrogens and some are antiestrogenic. PCBs were used as early as 1881 in cutting or thinning oils, as lubricants, and as electrical insulators. In the 1930s they were reported to cause "chloracne" and even death from liver failure in occupational exposures (49). The Hudson River is perhaps the most famous site of PCB contamination from over 30 years of General Electric dumping PCBs until they were successfully sued to stop in 1975. We encounter PCBs primarily through the diet, but they can enter our systems by dermal contact (household dust) and inhalation. The half-life in some cases is >10 years, hence their existence today (50).

Inhibition stimulation

Dose

The connection between seminal plasma PCB levels and sperm quality was first shown in 1986 (51). It became clear in 2002 that PCBs were not the guilty molecules, but it was their active metabolites that were responsible for gamete abnormalities (52,53). Several environmental exposure studies show a consistent decrease in sperm quality in relation to seminal plasma PCB metabolite levels across different age groups: 18–21-year-olds (54), 30-year-old infertile couples, and 39- and 50-year-old fishermen (48). Russ Hauser at the Harvard School of Public Health has spent over six years studying PCBs and their effects on male factor infertility. He makes a strong case that the epidemiological data support an inverse association of PCBs with reduced semen quality, specifically reduced sperm motility. The associations found are generally consistent across studies, despite a range of PCB levels, methods of measuring PCB levels, and methods of measuring semen quality (38).

Non-persistent pesticides or "contemporary-use" pesticides are those that are currently in use for killing insects, weeds, and other pests. While non-persistent in the environment, heavy use of pest control in the developed world means that most people receive at least some exposure to low levels of these chemicals (see Table 35.2).

Several epidemiological studies on occupational exposure to contemporary-use pesticides have been reported. In one cross-sectional study, greenhouse workers (n = 122) exposed to over a dozen pesticides were stratified into low-, medium-, or high-exposure groups. The highest-exposure group showed a higher proportion of abnormal sperm and lower median sperm counts in workers with more than 10 years of experience compared to those with fewer than five years (55). The study was appropriately adjusted for sexual abstinence and other potential cofounders. Juhler et al. investigated dietary exposure to pesticides and semen quality in a cross-sectional study of organic farmers compared to traditional farmers (56). Through food frequency questionnaires and pesticide monitoring programs they found that men with a lower intake of organic food had lower proportions of normally shaped sperm using strict criteria after controlling for various confounders (2.5% vs 3.7%; p = 0.003). However, there were no differences between groups in 14 other semen parameters. Oliva et al. obtained similar results in Argentina (57), but Larsen et al. did not find significant differences in sperm quality between Danish farmers who sprayed pesticides and those who did not (58). Unfortunately, for the sake of clarity, none of these studies looked at individual pesticide exposure, only exposure in general.

This lack of specificity indicated the ever-present need for more controlled investigations that can link measurable quantities of these newer compounds to sperm quality and ultimately fertility. Several studies have specifically investigated exposure to organophosphate pesticides (59,60) and found similar results to the broad cross-sectional studies mentioned previously. Whorton et al. (61) studied workers who packaged carbaryl (a common insecticide marketed under the name Sevin since 1958) and found an increased

Table 35.2 Occupational exposures to metals, solvents, and pesticides and their effects on male reproduction and biological markers

Female	Male	Children
↓ Fertility	↓ Fertility	↓ Birth weight
↑ Early pregnancy loss	↑ Genetically abnormal sperm	↓ Size
↑ Late pregnancy loss	↓ Sperm counts	Developmental abnormalities
↑ Preterm birth	Germinal epithelium abnormalities	
Abnormalities of the reproductive systems	Abnormal hormone function	

Sources: Data from Figà-Talamanca I et al. Occupational exposures to metals, solvents, and pesticides: Recent evidence on male reproductive effects and biological markers. *Occup Med* 2001; 51(3): 174–88; Whorton MD et al. Infertility in male pesticide workers. *Lancet* 1977; 2: 1259–61; Bretveld RW et al. Pesticide exposure: The hormonal function of the female reproductive system disrupted? *Reprod Biol Endocrinol* 2006; 4: 30.

incidence of oligozoospermia (<20 million sperm/mL) compared with a reference group of chemical workers. A total of 15% of exposed workers had sperm concentrations below the reference value of 20 million sperm/mL compared with 5.5% of non-exposed controls ($p = 0.07$). Wyrobek et al. (62) reported an association between carbaryl exposure and sperm morphology soon thereafter. The distribution of abnormal sperm morphology was significantly higher for exposed workers ($p < 0.005$), and the proportion of teratospermic men (>60% abnormal) was larger in the exposed group (29%; $n = 50$) compared with controls (12%, $n = 34$; $p = 0.06$). Meeker et al. (63) found an inverse relationship between sperm concentration and motility in 272 men recruited from infertile couples and urinary levels of 1-naphthol, a metabolite of both carbaryl and naphthalene. They suggested that "an interquartile range increase in carbaryl metabolite levels in urine is associated with a 4% decrease in sperm motility", and may result in a significant increase in the number of subfertile men across the U.S. population. In summary, there are human data supporting the association between contemporary-use pesticides and decreased semen quality, but the public health implications are yet to be determined.

The estrogenic monomer BPA is used in the manufacture of polycarbonate plastic products, in resins lining metal cans, in dental sealants, and in blends with other types of plastic products. Typical products include polyvinyl chloride, medical tubing, water pipes, soda bottles, and baby bottles (Table 35.1). Over time, the ester bonds linking BPA molecules in polycarbonate and resins undergo hydrolysis, resulting in the release of free BPA into food, beverages, and the environment. This hydrolysis is accelerated by heat or contact with acidic and basic substances, such that repeated washing or contact with substances of different acidity lead to increased leaching of BPA from polycarbonate. BPA levels have been found in rivers and streams, drinking water, indoor air, and leaching out of landfills (64). Numerous monitoring studies now show almost ubiquitous human exposure to biologically active levels of this chemical (65). The Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) currently consider daily exposure of BPAs of <50 μg/kg to be safe based on megadose studies in which the lowest tested dose was 1000-fold higher. Now, over 40 studies have been published, reporting significant effects in rats and mice at doses <50 μg/kg (66). Previous "safe levels" of exposure are now under scrutiny as older studies are being recognized as limited because of assay sensitivity. Moreover, it is difficult, but not impossible, to conduct laboratory experiments and avoid contamination from polycarbonate lab plastics. Mechanistically, BPAs exert estrogenic effects through the classic nuclear estrogen receptor, by acting as selective estrogen receptor modulators, and by initiating rapid responses via estrogen receptors presumably associated with the plasma membrane (64). BPAs bind very little to sex hormone-binding protein and thus have an unconjugated free fraction of 8% that can be delivered to cells more easily than estradiol, which has a free fraction of 3.5% (67). Additionally, pregnant women have a significantly higher affinity for BPAs than nonpregnant women (68). Indeed, BPAs have been detected in fetal cord serum, maternal serum during pregnancy, and amniotic fluid (69). For unclear reasons, the level of BPAs found in the amniotic fluid of 15–18-week gestations is five-times higher than serum levels, but returns to a concentration similar to fetal and maternal serum in the third trimester (Figure 35.2). Although the metabolism of BPAs is not completely understood, these findings can be explained by the development of fetal capacity to metabolize BPAs in the late second trimester, possibly by the liver.

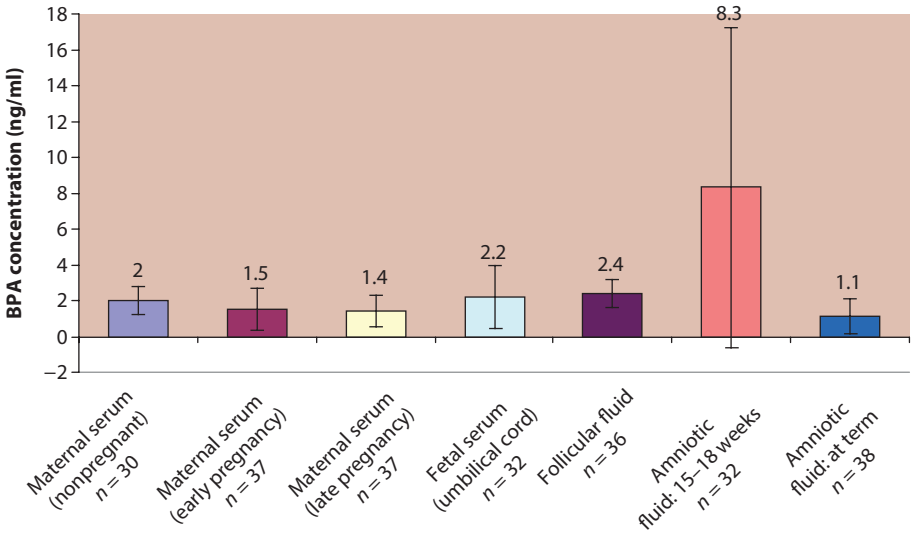


Figure 35.2 Bisphenol A (BPA) concentrations in human biological fluids. Columns represent mean BPA values, and "whiskers" give the 95% confidence intervals of the values. The amniotic fluid at 15–18 weeks was significantly elevated ($p < 0.0001$) compared with other biological fluids. (From Tsutsumi O. *J Steroid Biochem Mol Biol* 2005; 93: 325–30, with permission.)

Metabolism research, like epidemiological studies, is just beginning to be published to accompany a large body of animal research already accessible (70). Whereas BPAs are newer molecules, heavy metals have long been implicated in impairing fertility. The most frequently studied metals are lead and mercury. Physicians have recognized lead as a reproductive toxicant for well over a century. Lead salts, in fact, were once used as abortificants. The effects on reproduction were well summarized in 1944 (71): "It is generally agreed that if pregnancy does occur it is frequently characterized by miscarriage, intrauterine death of the fetus, and premature birth, but if living children are born, they are usually smaller, weaker, slower in development, and have a higher infant mortality".

Beyond broad generalizations, however, there is little evidence supporting the claim that lead affects fertility per se. Animal studies have shown altered spermatogenesis at 35 μg/dL (the CDC-cited safe level is <10 μg/dL), and a few case reports have observed similar findings in humans with levels over 40 μg/dL (72). It has been demonstrated that lead crosses into the seminal fluid, but in general, studies focused on male fertility and lead exposure are lacking. In Denmark, a prospective cohort of workers in a battery manufacturing plant had average serum lead levels of 35.9 μg/dL, but no decrease in the birth rate (odds ratio = 0.98, 95% confidence interval 0.88–1.12) (73). A study of welders in Canada demonstrated a decrease in sperm quality, but did not correlate those findings with decreased fertility (74). Likewise, there is scant evidence of increased spontaneous abortion rates or increased time to pregnancy (TTP). No doubt, more information on the impact of lead on reproduction will be forthcoming as newer techniques of investigation are developed. However, indirect evidence is provided by the fact that in the Nurses II study, which evaluated over 213,000 human years, overall infertility increased the closer one lived to a major road. The more we understand our urban environment, the more hostile it seems to be to the reproductive process (75).

THE FOLLICLE MICROENVIRONMENT

One by-product of *in vitro* fertilization (IVF) has been access to follicular fluid for studies demonstrating the presence of toxicants (76–79). The pesticides DDE, mirex, hexachloroethane, and 1,2,4-trichlorobenzene, along with PCBs, BPA, and phthalates, have been implicated in infertility, but have not consistently demonstrated adverse IVF or pregnancy outcomes. Variables examined include number of oocytes retrieved, recovered, and fertilized, cleavage rates, and pregnancy rates. In a Canadian study of 21 IVF couples, higher DDE levels correlated with failed fertilization, but higher follicular PCB levels correlated with pregnancy success (76). A study of IVF patients in 1984 showed that oocyte recovery and embryo cleavage rates were inversely related to chlorinated hydrocarbon concentrations (79), although a subsequent study showed a positive relationship (78). Most remarkable, however, is the fact that in all of these studies, pesticides are present in follicular fluid at the time of resumption of meiosis when chromosome susceptibility is at its highest. For the most part, follicular toxicant concentrations are lower than serum levels (68,76). Knowing the relationship between serum and follicle concentrations has allowed speculation on the fertility outcomes of non-IVF patients based on serum levels. Law et al. (80) pulled frozen third-trimester blood samples from 380 planned pregnancies recruited for the 1959–1965 Collaborate Perinatal Project and compared serum levels of PCBs and DDE with TTP and fecundability. Dose–response curves with proportional hazards suggested that as PCB and DDE levels increase, the probability of pregnancy decreases. Since DDE and PCBs are lipophilic, the serum levels obtained in this study were adjusted for maternal lipid volume (an appropriate adjustment not done in most published reports). Once adjusted, the increased TTP attributed to DDE disappeared and the PCB effect became considerably weaker, leaving no significant difference in TTP or fecundability based on either substance's concentration (80). These results echo the findings of a cohort of Swedish fishermen from which multiple papers have been published showing no relationship between fish consumption (including persistent organochlorine and PCB exposure) and TTP, miscarriage rate, stillbirths, or subfertility (81). In the end, there is little evidence to support the association between DDE, PCBs, and subfertility. Once again, however, the presence of such toxic substances bathing the preovulatory oocyte is worrisome given the protective barrier the reproductive organs pose to the passage of most substances. More studies will be required in order to understand whether there is any adverse impact of these substances on oocyte DNA. However, a more recent study has shown that PCBs can reduce the number of antral follicles and increase follicular atresia with an end result of earlier menopause (82).

Just as paternal lead and mercury exposure is widely thought to impair fertility, so is maternal exposure. Lead has been shown to destroy oocytes and to lead to follicular atresia in rodents and primates (83), but has only been measured in follicular fluid in two published human studies available on MEDLINE (84,85), while mercury measurement has not been reported at all. Various rodent and nonhuman primate studies have demonstrated suppression of menarche, decreased circulating progesterone levels, and less frequent menstrual cycles with lead exposure (72). Nevertheless, examination of human epidemiological data is less conclusive. First, the mechanism of lead's toxicity is not well understood: it is unclear whether there is a direct toxic effect on the ovary, the effect is mediated through a central neuroendocrine dysfunction, or both. Second, older studies demonstrate an association between high-dose occupational exposure and spontaneous fetal loss (86), but a later study was unable to find an association between the two when 304 women living near a lead smelter in Yugoslavia were compared with 335 women from a nearby town with low serum lead levels (87).

As mentioned, mercury levels have not been reported in follicular fluid, but mercury's effect on the developing

fetus *in utero* has been subject to a great deal of scrutiny. Maternal tobacco use would seem to be one of the simplest areas to modify regarding toxin accumulation in the ovarian follicular fluid. Objective measurements of tobacco compounds and their metabolites in follicular fluid correlate with subjective measures of lower ovarian, gamete, and embryo quality in smokers and in those exposed to passive smoke (88). A range of chemical toxins, such as nicotine, carbon monoxide, and follicular fluid cadmium concentration, is also reported to be higher in smokers than in nonsmokers (89).

THE AMNIOTIC SAC MICROENVIRONMENT

Over a decade ago, the endocrinologist Howard Bern of UC Berkeley in California coined the phrase the "fragile fetus" while explaining the vulnerability of the developing fetus *in utero* to insult and exposure. This phrase has proven true when it comes to lead and mercury exposure.

For years it was assumed that the developing fetus was protected by the placenta. However, a study in 2005 by the Environmental Working Group tested the blood of 10 randomly selected infants and the results showed that there were 200 toxic elements in the cord blood of the babies. These included mercury, industrial chemicals, pollutants, and pesticides (90). Mercury is a common atmospheric element that is released from the earth's crust. Inorganic mercury gets converted to soluble forms that are deposited into soil and water by precipitation. Sources such as coal mining "rain down" mercury across the earth, depositing it on both land and sea. These soluble mercury forms are then methylated via microbes or non-enzymatic processes, and readily taken up by proteins. Methyl mercury then rapidly accumulates in the food chain through predatory fish. Humans, at the top of the food chain, acquire mercury through food consumption as well. The greatest concern with mercury is not related to fertility—there is little evidence correlating mercury poisoning and infertility—but to the developmental effects of *in utero* exposure. In theory, the fetus is particularly vulnerable to mercury (91). In adults, methyl mercury can be converted to inorganic mercury by intestinal flora and 90% is eventually removed from our system through the feces. The fetus, with neither gut flora, nor a fully functional liver, nor the ability to defecate, is virtually guaranteed to rapidly accumulate this heavy metal. We must also consider the process of urination: *in utero* urine is cycled from the amniotic fluid into the developing fetus's nose and mouth, and back into the amniotic fluid, unlike in adults, where urination is an essential mechanism for clearing toxicants from the body. Couple higher toxicant levels with deficient excretion mechanisms and there is the potential for alarming toxicant accumulation. Moreover, because levels of circulating binding proteins are lower in the fetus, there is a higher concentration of circulating unbound toxicants. Lastly, the blood–brain barrier is more permeable during development and thus the developing brain proportionally receives greater exposure to toxicants than the adult brain. In theory, this can increase the vulnerability of the fetus to neurotoxins such as mercury. We have known for decades that lead and mercury pass through the placenta, into the amniotic fluid, and directly to the baby in concentrations near those of the maternal blood serum. Umbilical cord blood levels of lead are usually only 10%–20% lower than maternal serum levels (92), but mercury (methyl mercury) levels are generally higher than maternal serum levels (72). Mercury has been the most publicized environmental toxicant in relation to reproductive health, which explains the FDA's recommended limitations on fish consumption in pregnancy. Current FDA recommendations are for women of childbearing age to avoid fish that are likely to contain high levels of methyl mercury (>1 μg/g), including swordfish, shark, tilefish, and king mackerel. Fortunately, many U.S. sport fish have levels lower than this (Figure 35.3). In Massachusetts, though, there are no bodies of water that have safe levels of mercury for fish consumption by women of childbearing age. A statement to this effect was issued by the Massachusetts Department of Public Health in 2004. This was an extension on an advisory from 1994 to 2001 (93), a previously issued statewide fish consumption advisory that cautioned pregnant women to avoid eating fish from all freshwater bodies due to concerns about mercury contamination, and now includes women of childbearing age who may become pregnant, nursing mothers, and children under 12 years of age. Epidemiological studies show that an increase of only 1 ppm of mercury lowers the average cognitive score of a child (94), while high-dose exposure can lead to neonatal central nervous system damage and even death. Minamata disease—mercury poisoning caused by dumping in Japan's Bay of Minamata—is an example of the potential effects of high-dose exposure (95). More recently, a 2014 update from the FDA recommended women and children follow three safety tips for eating fish and shellfish:

1. Do not eat shark, swordfish, king mackerel, and tilefish because of high mercury levels.
2. Eat up to 12 oz (two average meals) weekly of fish and shellfish low in mercury such as shrimp, canned light tuna, salmon, pollock, and catfish. Albacore ("white") tuna has more mercury than canned light tuna.
3. Check local advisories about the safety of fish caught by family and friends.

However, fish are an important source of omega-3 fatty acids, which are essential for fetal neurodevelopment, and there is some evidence that higher fish consumption is correlative with greater cognitive development. A cohort study of 8947 women in England found that women who consumed over 340 g (12 oz) of fish per week during pregnancy had children with significantly greater outcomes on 14 of 23 neurodevelopmental measures, including various fine-motor, communication, and social development milestones and verbal IQ at eight years of age (96).

Unfortunately, recommendations about limiting fish consumption in pregnancy may overestimate the risk of chronic low-dose mercury intake and underestimate the benefit of omega-3 fatty acids. The challenge is to find the correct balance.

Two important prospective observational studies—the Seychelles Child Development Study (97) (n = 779)

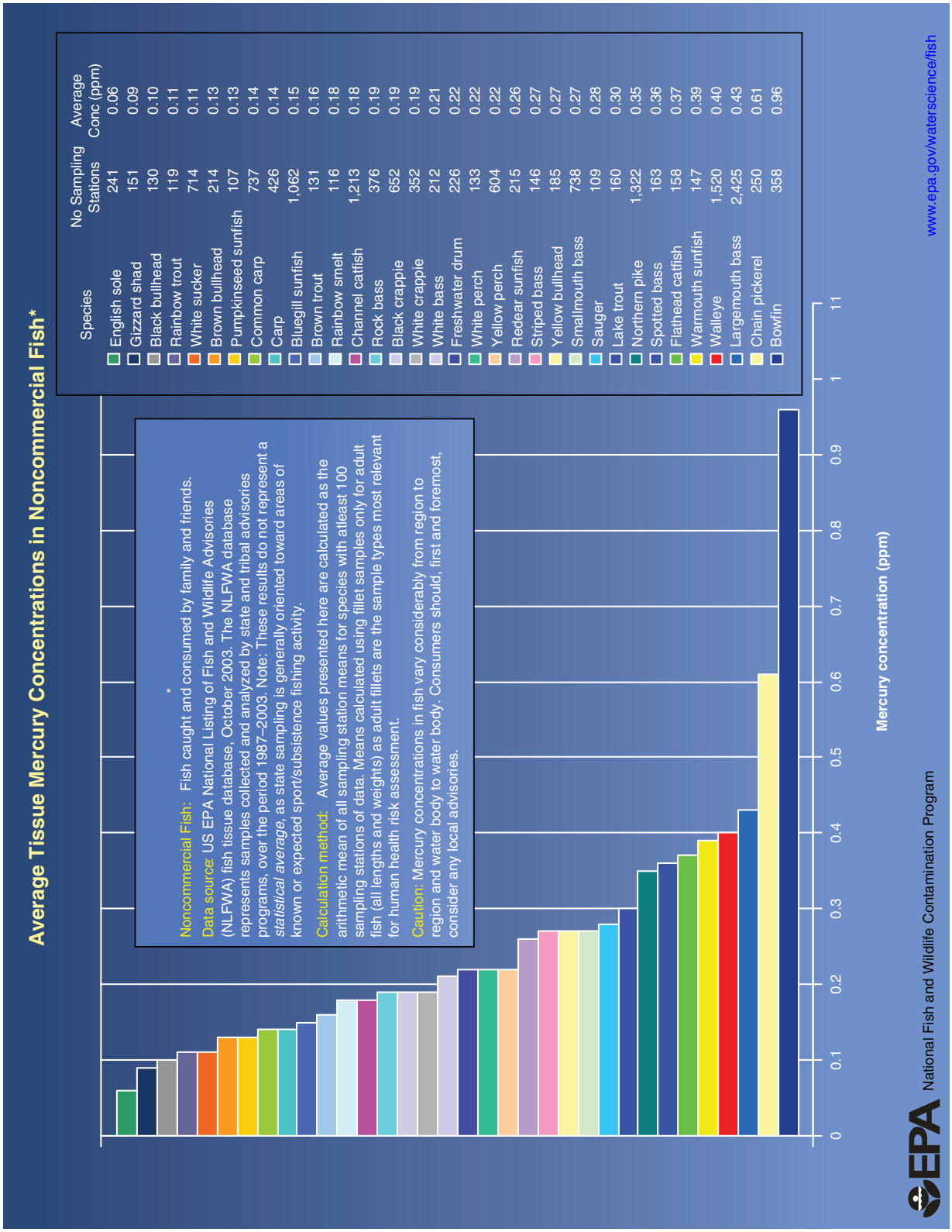


Figure 35.3 Average tissue mercury concentrations in noncommercial fish. (From www.epa.gov/waterscience/fish, accessed 2012).

and the Faroe Islands Cohort Study (98) (n = 878)—followed fish consumption and cognitive function for 9 and 14 years, respectively, and reported conflicting results. The Seychelles study has not shown an association between *in utero* methyl mercury levels and neurocognitive function, while the Faroe Islands Cohort Study has consistently shown a negative correlation after correcting for postnatal exposure. In response to these two studies, the World Health Organization (WHO) published a new maternal serum cutoff level of 5.6 μg/dL and a tolerable weekly mercury intake of 1.6 μg/kg to "protect the developing fetus and embryo, the most sensitive subgroup of the population" (95). A second way of evaluating fetal mercury exposure is by cord blood mercury levels. The U.S. National Research Council has raised concern for cord blood levels >58 ppm (5.8 μg/L) (99). Large fish-consuming populations have been shown to have mercury level averages that are substantially above this threshold. In Taiwan, 65 pregnant women filled out a questionnaire in the third trimester and gave blood samples, placenta tissue, and cord blood after delivery. A total of 89% of the maternal blood mercury concentrations exceeded the U.S. National Research Council recommended value. Levels were highest in women who ate fish more than three times a week while pregnant (100). In Hawaii, a study of 308 newborns showed a mean cord blood concentration of 4.8 μg/L, with 28% above the recommended safety value (101). While it is clear that fish consumption is correlated with both maternal and fetal mercury levels, the developmental significance is still being evaluated.

Other amniotic sac microenvironment toxicants have been implicated in affecting fertility. Guo et al. studied one of the only prospective cohorts of high-dose PCB exposure when contaminated cooking oil was used in Taiwan in 1979 (102). In 1998, they contacted children exposed to PCBs *in utero* and performed sperm analysis. They found abnormal sperm motility and morphology and decreased ability to penetrate hamster eggs. Fertility rates have yet to be reported.

The translation of subfertility across generations is one of the most interesting and concerning concepts to arise out of reproductive environmental health research. Belgian investigators failed to show a difference in serum concentration of the pesticide by-product DDE in fertile men (n = 73) and infertile men (n = 82), but a sub-analysis of the blood of mothers of the patients demonstrated higher serum pesticide levels in mothers of subfertile men (n = 19) than in those of fertile men (n = 23) (103).

While these results can only offer hypotheses on the role of exposure to pesticides *in utero* and fertility, they do provide reason for caution toward potential toxicants and increase the need to verify their potential risks.

THE GLOBAL ENVIRONMENT

The global environment is the most difficult to deconstruct. We do know that, in 2001, more than 1.2 billion pounds of active ingredients were used in the U.S.A. according to the American College of Obstetricians and Gynecologists (104). In truth, the collection of studies presented here probably reflects alteration in the functionality of semen or ovarian follicles. There is, however, the potential for environmental agents to affect the systems that support pregnancy. For example, environmental estrogens may change the hormonal balance that allows sufficient endometrial growth, affects angiogenesis necessary to support a developing placenta, or causes/worsens endometriosis and tubal patency. These details are sure to be elucidated in the near future. Nevertheless, regional differences in fertility rates highlight the potential effect of the global environment on fertility (105).

A study that falls into this category of the "global environment" was published in 1999 by Khattak et al. Their prospective case–control trial on the effect of occupational maternal exposure to organic solvents involved pregnant women exposed to solvents and matched by age, gravity, smoking, and alcohol usage to comparable pregnant women exposed to a recognized non-teratogenic agent. In addition to an increased incidence of miscarriage (54/117 [46.2%] vs. 24/125 [19.2%]; $p < 0.001$), the women in the exposure group were 13-times more likely to have children with major cardiovascular or central nervous system malformations (106). The authors concluded that occupational exposure to organic solvents in pregnancy is associated with increased risk of major fetal malformations. Where this effect takes place is not clear: it could be either the gametes, the amniotic microenvironment, or both. Animal research has demonstrated that fetal alterations may continue to impact future generations through persistent epigenetic changes. Genome methylation or histone acylation can alter gene expression without modifying the DNA sequence and can transmit from generation to generation with a higher penetrance than DNA mutations themselves (107). Anway et al. illustrated this concept when they observed that rats exposed to vinclozolin (an antiandrogenic compound that is used as a fungicide in wine vineyards) or methoxychlor (an estrogenic compound that has replaced DDT as a pesticide) resulted in increased male infertility in F1 generation rats and persisted in over 90% of all male rats through the next three generations (108). These kinds of results in humans have only been hinted at by the association of elevated maternal serum levels of DDE with grown children's fertility rates (100), as well as the transgenerational effect of DES and vaginal cancer (109).

Even more interesting (and complicating) are reports that environmental agents that are thought to be toxic may actually enhance reproduction. Higher follicular fluid levels of PCBs have been associated with better IVF outcomes (76), higher DDE levels have been associated with reduced TTP (110), and *in vitro* work has shown that DDE stimulates the aromatase enzyme system of granulosa cells in synergy with follicle-stimulating hormone (111) and thus may speed follicle maturation. Additionally, in Denmark, 192 IVF couples with paternal exposure to pesticides, fungicides, and herbicides had a 21% spontaneous abortion rate compared with 28% in the reference population of

2925 couples (112). How these findings will ultimately be interpreted is not presently clear.

CLINICAL CONSIDERATIONS

It is relatively easy to accept research indicating the presence of environmental toxicants in the semen, the oocyte, and the amniotic fluid. Translation of the epidemiological and observational research to the bedside is challenging. Clinically, how do we counsel our patients? We agree that providers should be cautious, not alarming, given that the literature is suggestive, not conclusive, of links between EDCs and decreased reproductive performance (Table 35.3) (113). One way to incorporate knowledge about environmental exposures is to include questions during history taking about exposures to solvents, pesticides, or heavy metals. Ask patients where they live and where they have lived, where they work, what they eat, how much fish they consume, and what exposures their parents may have had to plastics, heavy metals, pesticides, and industrial solvents. Several formal exposure evaluations have been published for this purpose (114,115). Encourage patients, particularly those thinking of starting a family, to eat organic. A summary of the 12 fruits and vegetables that contain the most pesticides, known as the "Dirty Dozen", is vailable from the Environmental Working Group (EWG) (116). If at all possible, these foods should be selected from an organic source. If organic options are either not available or are too expensive, the "Clean Fifteen" list contains the fruits and vegetables that contain the least pesticides (Table 35.4) and make excellent substitutions.

The knowledge gained from history-taking can be used to advise patients according to the precautionary principle (117). Educate patients on potential toxic exposures, and let them make efforts to avoid them. The data gathered can also become the basis for observational research opportunities to understand better which substances are most deleterious, and at what levels.

The role of toxin decontamination is neither well studied nor reported, but certainly is not new. Hippocrates wrote of solariums, religious groups recommend fasting, Aborigines wrote of sweat lodges and hot baths, Egyptians applied body wraps, and some Scandinavian cultures utilized saunas and steam baths (27). None of these decontamination methods have been studied and reported upon in the scientific literature, despite the many such services advertised in every community in the world. In the end, the scientific communities at large remain unimpressed with the impact of EDCs. The National Academy of Science report on "Hormonally Active Agents in the Environment" (118) was unable to come to a consensus opinion on the topic; the WHO state-of-the-science assessment in 2002 (119) concluded that organochlorines (PCBs and DDE) do affect pregnancy in wildlife, but was uncertain about the effect on humans. Congress mandated the EPA, through the advisory of the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), to expand its current mandate to test all food-use pesticides and drinking water contaminants for hormonal activity. This is to include evaluation of the 80,000-plus registered chemicals under the Toxic Substances Control Act of 1998—a daunting task that requires prioritization. The EPA report on the first 200 of these chemicals was updated in November 2015 (120).

Table 35.3 The fertility/fecundity impact of chemical exposure during adulthood

Substance	Potential effect on females	Potential effect on males
Bisphenol A	Oocyte chromosomal abnormalities, recurrent abortions	Poor semen quality
Chlorinated hydrocarbons	Menstrual abnormalities, reduced fertility, endometriosis, fetal loss	Poor semen quality and hormonal changes
Disinfection by-products	Fetal loss, irregular menses	—
Ethylene oxide	Fetal loss	Poor semen quality and miscarriage in partner
Glycol ethers (paints, thinners, printing inks)	Fetal loss, reduced fertility	Decreased semen quality
Heavy metals (Pb, Hg, Cd)	Fetal loss, reduced fertility, irregular menses	Abnormal sperm, reduced fertility
Pesticides	Irregular menses, reduced fertility, fetal loss	Poor semen quality, miscarriage in female partner
Phthalates (plastic additives)	Fetal loss, irregular menses, lower fertility	Decreased semen quality
Solvents (benzene, toluene xylene, and others)	Fetal loss, irregular menses, lower fertility	Reduced fertility, decreased semen quality
Cigarette smoke	Reduced fertility, miscarriage, early menopause	Reduced fertility, decreased semen quality

Source: Modified from "Challenged conceptions: Environmental chemicals and fertility"; https://www.epa.gov/fish-tech, accessed 2017, p. 5.

Table 35.4 Summary of the "Dirty Dozen" and "Clean Fifteen" lists of fruits and vegetables containing the most and least pesticides, respectively

Dirty Dozen	Buy these organic	Clean Fifteen	Lowest in pesticide
1	Apples	1	Onions
2	Celery	2	Sweet corn
3	Strawberries	3	Pineapples
4	Peaches	4	Avocado
5	Spinach	5	Asparagus
6	Nectarines—imported	6	Sweet peas
7	Grapes—imported	7	Mangoes
8	Sweet bell peppers	8	Eggplant
9	Potatoes	9	Cantaloupe—domestic
10	Blueberries—domestic	10	Kiwi
11	Lettuce	11	Cabbage
12	Kale/collard greens	12	Watermelon
		13	Sweet potatoes
		14	Grapefruit
		15	Mushrooms

Source: From EWG's Shopper's Guide to Pesticides. https://www.ewg.org/foodnews/#.WYvBQoqQxE4.

There has been much progress and expansion in investigating the role that different compounds play in our reproductive health. There is clearly much more work needed, however, to draw any definitive conclusions. We must ask ourselves: why are we allowing an "innocent until proven guilty" approach for chemical agents dispersed into our environment, when we insist on the opposite approach for all pharmaceuticals (27)? Why do we need an act of Congress to verify that consuming pesticides and toxic chemicals are bad for us and for our potential to reproduce? If toxins take years to eventually be cleared from the soil and water, why would or should we have them lingering in our bodies? It is time for advocacy not only for the well-being of our future children, but also for the continuance of them.

Some simple habits that can be explained to patients to limit their toxicity include:

1. Read labels: if you cannot pronounce it, do not buy it. There is an extensive list on the EWG's website.
2. Go organic. Although it costs more, so does eating pesticides and other harmful substances. The less distance one's food travels, the less exposure to chemicals it likely has.
3. Avoid chemicals. Cosmetics and water are common harbingers of toxins, but so are canned goods, scented perfumes, air fresheners, and household cleaners. You can create your own cleaners with lemon juice and vinegar and use essential oils as air fresheners.
4. Drink filtered water from bottles that do not have BPA. Metal containers and glass bottles are far safer than plastic ones.
5. Do not microwave in plastics or unmarked containers. If you do microwave in plastic, it must say "microwave safe". This includes leftover Chinese food or other take-out plastic containers.

REFERENCES

1. Sutton P, Woodruff TJ, Perron J et al. Toxic environmental chemicals: The role of reproductive health professionals in preventing harmful exposures. *Am J Obstet Gynecol* 2012; 207: 164.
2. http://www.uptodate.com/contents/occupational-and-environmental-risks-to-reproduction-in-females
3. Trubo R. Endocrine-disrupting chemicals probed as potential pathways to illness. *JAMA* 2005; 294: 291–3.
4. Thompson MR, Boekelheide K. Multiple environmental chemical exposures to lead, mercury and polychlorinated biphenyls among childbearing-aged women (NHANES 1999–2004): Body burden and risk factors. *Environ Res* 2013; 121: 23.
5. Nonaka K, Miura T, Peter K. Recent fertility decline in Dariusleut Hutterites: An extension of Eaton and Mayer's Hutterite fertility study. *Hum Biol* 1994; 66: 411–20.
6. Ventura SJ, Mosher WD, Curtin SC, Abma JC, Henshaw S. Trends in pregnancies and pregnancy rates by outcome: Estimates for the United States, 1976–96. *Vital Health Stat* 2000; 21: 1–47.
7. Burdorf A, Brand T, Jaddoe VW et al. The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: The Generation R Study. *Occup Environ Med* 2011; 68: 197.
8. Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med* 1995; 332: 281–5.
9. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992; 305: 609–13.
10. Brevini TA, Zanetto SB, Cillo F. Effects of endocrine disruptors on developmental and reproductive functions. *Curr Drug Targets Immune Endocr Metabol Disord* 2005; 5: 1–10.
11. Carson R, Darling L, Darling L. *Silent Spring*. Cambridge, MA: Houghton Mifflin, Riverside Press, 1962.
12. Colborn T. Nontraditional evaluation of risk from fish contaminants. In: *Proceedings of a Symposium on Issues in Seafood Safety*. Ahmed F (ed.). Washington, DC: National Academy of Sciences, Institute of Medicine, Food, and Nutrition Board, 1991.

13. Colborn T, Vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 1993; 101: 378–84.
14. Colborn T. Pesticides—How research has succeeded and failed to translate science into policy: Endocrinological effects on wildlife. *Environ Health Perspect* 1995; 103(Suppl 6): 81–5.
15. Colborn T, Dumanoski D, Myers JP. *Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival? A Scientific Detective Story*. New York, NY: Dutton, 1996.
16. Damstra T. Potential effects of certain persistent organic pollutants and endocrine disrupting chemicals on the health of children. *J Clin Toxicol* 2002; 40: 457–65.
17. Svechnikov K, Izzo G, Landreh L et al. Endocrine disruptors and Leydig cell function. *J Biomed Biotechnol* 2010; 2010.
18. Heilier JF, Nackers F, Verougstraete V et al. Increased dioxin-like compounds in the serum of women with peritoneal endometriosis and deep endometriotic (adenomyotic) nodules. *Fertil Steril* 2005; 84: 305–12.
19. NIEHS: Bisphenol A (BPA) Questions and Answers about Bisphenol A. http://www.niehs.nih.gov/health/top-ics/agents/sya-bpa/index.cfm
20. Sharpe et al. Disturbances of sperm maturation and minipuberty: Is there a connection? *Biomed Res Int* 2014; 2014: 912746.
21. Jadarmkunti UC, Kaliwal BB. Effect of dicofol formulation on estrous cycle and follicular dynamics in albino rats. *J Basic Clin Physiol Pharmacol* 1999; 10: 305–14.
22. Muller WF, Hobson W, Fuller GB et al. Endocrine effects of chlorinated hydrocarbons in rhesus monkeys. *Ecotoxicol Environ Saf* 1978; 2: 161–72.
23. Alm H, Tiemann U, Torner H. Influence of organochlorine pesticides on development of mouse embryos *in vitro*. *Reprod Toxicol* 1996; 10: 321–6.
24. CDC. *Third National Report on Human Exposure to Environmental Chemicals*. Atlanta, GA: CDC, 2005.
25. Duncan D. The pollution within. *National Geographic* 2006; 210: 116–43.
26. Swan SH, Main KM, Liu F et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect* 2005; 113: 1056–61.
27. Genuis SJ. Health issues and the environment—An emerging paradigm for providers of obstetrical and gynaecological health care. *Hum Reprod* 2006; 21: 2201–8.
28. Smith GC, Pell JP. Parachute use to prevent death and major trauma related to gravitational challenge: Systematic review of randomised controlled trials. *BMJ* 2003; 327: 1459–61.
29. McDiarmid MA, Rogers B, Oliver MS. Chromosomal effects of non-alkylating drug exposure in oncology personnel. *Environ Mol Mutagen* 2014; 55: 369.
30. Lawson CC, Rocheleau CM, Whelan EA, et al. Occupational exposures among nurses and risk of spontaneous abortion. *Am J Obstet Gynecol* 2012; 206: 327.e1.
31. Niwa T, Tsutsui M, Kishimoto K et al. Inhibition of drug-metabolizing enzyme activity in human hepatic cytochrome P450s by bisphenol A. *Biol Pharm Bull* 2000; 23: 498–501.
32. Niwa T, Fujimoto M, Kishimoto K et al. Metabolism and interaction of bisphenol A in human hepatic cytochrome P450 and steroidogenic CYP17. *Biol Pharm Bull* 2001; 24: 1064–7.
33. Welshons WV, Thayer KA, Judy BM et al. Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* 2003; 111: 994–1006.
34. Hauser R, Williams P, Altshul L, Calafat AM. Evidence of interaction between polychlorinated biphenyls and phthalates in relation to human sperm motility. *Environ Health Perspect* 2005; 113: 425–30.
35. Younglai EV, Holloway AC, Foster WG. Environmental and occupational factors affecting fertility and IVF success. *Hum Reprod Update* 2005; 11: 43–57.
36. Adibi JJ, Perera FP, Jedrychowski W et al. Prenatal exposures to phthalates among women in New York City and Krakow, Poland. *Environ Health Perspect* 2003; 111: 1719–22.
37. Duty SM, Silva MJ, Barr DB et al. Phthalate exposure and human semen parameters. *Epidemiology* 2003; 14: 269–77.
38. Hauser R. The environment and male fertility: Recent research on emerging chemicals and semen quality. *Semin Reprod Med* 2006; 24: 156–67.
39. Jonsson BA, Richthoff J, Rylander L, Giwercman A, Hagmar L. Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology* 2005; 16: 487–93.
40. Fenster L, Waller K, Windham G et al. Trihalomethane levels in home tapwater and semen quality. *Epidemiology* 2003; 14: 650–8.
41. Shaw GM, Ranatunga D, Quach T et al. Trihalomethane exposures from municipal water supplies and selected congenital malformations. *Epidemiology* 2003; 14: 191–9.
42. Waller K, Swan SH, DeLorenze G, Hopkins B. Trihalomethanes in drinking water and spontaneous abortion. *Epidemiology* 1998; 9: 134–40.
43. Waller K, Swan SH, Windham GC, Fenster L. Influence of exposure assessment methods on risk estimates in an epidemiologic study of total trihalomethane exposure and spontaneous abortion. *J Expo Anal Environ Epidemiol* 2001; 11: 522–31.
44. Arbuckle TE, Hrudey SE, Krasner SW et al. Assessing exposure in epidemiologic studies to disinfection byproducts in drinking water: Report from an international workshop. *Environ Health Perspect* 2002; 110(Suppl 1): 53–60.

45. Savitz DA, Singer PC, Herring AH et al. Exposure to drinking water disinfection by-products and pregnancy loss. *Am J Epidemiol* 2006; 164: 1043–51.
46. Garcia AM. Pesticide exposure and women's health. *Am J Ind Med* 2003; 44: 584–94.
47. Hauser R, Chen Z, Pothier L, Ryan L, Altshul L. The relationship between human semen parameters and environmental exposure to polychlorinated biphenyls and p,p'-DDE. *Environ Health Perspect* 2003; 111: 1505–11.
48. Rignell-Hydbom A, Rylander L, Giwercman A et al. Exposure to CB-153 and p,p'-DDE and male reproductive function. *Hum Reprod* 2004; 19: 2066–75.
49. Schroeder M. Did Westinghouse keep mum on PCBs? *Business Week* August 12, 1991: 68–70.
50. Phillips DL, Smith AB, Burse VW et al. Half-life of polychlorinated biphenyls in occupationally exposed workers. *Arch Environ Health* 1989; 44: 351–4.
51. Bush B, Bennett AH, Snow JT. Polychlorobiphenyl congeners, p,p'-DDE, and sperm function in humans. *Arch Environ Contam Toxicol* 1986; 15: 333–41.
52. Dallinga JW, Moonen EJ, Dumoulin JC et al. Decreased human semen quality and organochlorine compounds in blood. *Hum Reprod* 2002; 17: 1973–9.
53. https://www.dhs.wisconsin.gov/environmental/pcb-fish.htm
54. Richthoff J, Rylander L, Jonsson BA et al. Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. *Environ Health Perspect* 2003; 111: 409–13.
55. Abell A, Ernst E, Bonde JP. Semen quality and sexual hormones in greenhouse workers. *Scand J Work Environ Health* 2000; 26: 492–500.
56. Juhler RK, Larsen SB, Meyer O, Jensen ND, Spanò M, Giwercman A, Bonde JP. Human semen quality in relation to dietary pesticide exposure and organic diet. *Arch Environ Contam Toxicol* 1999; 37(3): 415–23.
57. Oliva A, Spira A, Multigner L. Contribution of environmental factors to the risk of male infertility. *Hum Reprod* 2001; 16: 1768–76.
58. Larsen SB, Joffe M, Bonde JP. Time to pregnancy and exposure to pesticides in Danish farmers. ASCLEPIOS Study Group. *Occup Environ Med* 1998; 55: 278–83.
59. Padungtod C, Savitz DA, Overstreet JW et al. Occupational pesticide exposure and semen quality among Chinese workers. *J Occup Environ Med* 2000; 42: 982–92.
60. Kamijima M, Hibi H, Gotoh M et al. A survey of semen indices in insecticide sprayers. *J Occup Health* 2004; 46: 109–18.
61. Whorton MD, Milby TH, Stubbs HA, Avashia BH, Hull EQ. Testicular function among carbaryl exposed exployees. *J Toxicol Environ Health* 1979; 5: 929–41.
62. Wyrobek AJ, Watchmaker G, Gordon L et al. Sperm shape abnormalities in carbaryl-exposed employees. *Environ Health Perspect* 1981; 40: 255–65.
63. Meeker JD, Ryan L, Barr DB et al. The relationship of urinary metabolites of carbaryl/naphthalene and chlorpyrifos with human semen quality. *Environ Health Perspect* 2004; 112: 1665–70.
64. Welshons WV, Nagel SC, Vom Saal FS. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. *Endocrinology* 2006; 147: S56–69.
65. Calafat AM, Kuklenyik Z, Reidy JA et al. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environ Health Perspect* 2005; 113: 391–5.
66. Vom Saal FS, Welshons WV. Large effects from small exposures. II. The importance of positive controls in low-dose research on Bisphenol A. *Environ Res* 2006; 100: 50–76.
67. Nagel SC, Vom Saal FS, Welshons WV. The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: Physiology of delivery modifies estrogenic activity. *Proc Soc Exp Biol Med* 1998; 217: 300–9.
68. Ikezuki Y, Tsutsumi O, Takai Y, Kamei Y, Taketani Y. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. *Hum Reprod* 2002; 17: 2839–41.
69. Schonfelder G, Wittfoht W, Hopp H et al. Parent bisphenol A accumulation in the human maternal–fetal–placental unit. *Environ Health Perspect* 2002; 110: A703–7.
70. Tsutsumi O. Assessment of human contamination of estrogenic endocrine-disrupting chemicals and their risk for human reproduction. *J Steroid Biochem Mol Biol* 2005; 93: 325–30.
71. Cantarow A, Trumper M. *Lead Poisoning*. Baltimore, MD: Williams & Wilkins, 1944.
72. Miller R, Bellinger D. Metals. In: *Occupational and Environmental Reproductive Hazards: A Guide for Clinicians*. Paul M (ed.). Baltimore, MD: Williams & Wilkins, 1993.
73. Bonde JP, Kolstad H. Fertility of Danish battery workers exposed to lead. *Int J Epidemiol* 1997; 26: 1281–8.
74. Bigelow PL, Jarrell J, Young MR, Keefe TJ, Love EJ. Association of semen quality and occupational factors: Comparison of case–control analysis and analysis of continuous variables. *Fertil Steril* 1998; 69: 11–8.
75. Mahalingaiah S, Hart JE, Laden F et al. Adult air pollution exposure and risk of infertility in the Nurses' Health Study II. *Hum Reprod* January 2, 2016 (online), doi:10.1093/humrep/dev330.
76. Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing *in vitro* fertilization. *Arch Environ Contam Toxicol* 2002; 43: 121–6.

77. Foster WG, Jarrell JF, Younglai EV et al. An overview of some reproductive toxicology studies conducted at Health Canada. *Toxicol Ind Health* 1996; 12: 447–59.
78. Jarrell J, Villeneuve D, Franklin C et al. Contamination of human ovarian follicular fluid and serum by chlorinated organic compounds in three Canadian cities. *CMAJ* 1993; 148: 1321–7.
79. Trapp M, Baukloh V, Bohnet HG, Heeschen W. Pollutants in human follicular fluid. *Fertil Steril* 1984; 42: 146–8.
80. Law DC, Klebanoff MA, Brock JW, Dunson DB, Longnecker MP. Maternal serum levels of polychlorinated biphenyls and 1,1-dichloro-2,2-bis (p-chlorophenyl) ethylene (DDE) and time to pregnancy. *Am J Epidemiol* 2005; 162: 523–32.
81. Axmon A, Rylander L, Stromberg U et al. Polychlorinated biphenyls in serum and time to pregnancy. *Environ Res* 2004; 96: 186–95.
82. Shuzhen L, Chunna Y, Dong C. Aroclor 1234 impairs the development of ovarian follicles by inducing the apoptosis of granulosa cells. *Toxicol Res* 2015, 4: 302–310.
83. Vermande-Van Eck GJ, Meigs JW. Changes in the ovary of the rhesus monkey after chronic lead intoxication. *Fertil Steril* 1960; 11: 223–34.
84. Silberstein T, Saphier O, Paz-Tal O et al. Lead concentrates in ovarian follicle compromises pregnancy. *J Trace Elem Med Biol* 2006; 20: 205–7.
85. Paksy K, Gati I, Naray M, Rajczy K. Lead accumulation in human ovarian follicular fluid, and *in vitro* effect of lead on progesterone production by cultured human ovarian granulosa cells. *J Toxicol Environ Health* 2001; 62: 359–66.
86. Rom WN. Effects of lead on the female and reproduction: A review. *Mt Sinai J Med* 1976; 43: 542–52.
87. Murphy MJ, Graziano JH, Popovac D et al. Past pregnancy outcomes among women living in the vicinity of a lead smelter in Kosovo, Yugoslavia. *Am J Public Health* 1990; 80: 33–5.
88. Cooper AR, Moley KH. Maternal tobacco use and its preimplantation effects on fertility: More reasons to stop smoking. *Semin Reprod Med* 2008; 26: 204–12.
89. Hannoun A, Nassar AH, Usta M, Musa AA. Effect of female nargile smoking on *in vitro* fertilization outcome. *Eur J Obstet Gynecol Reprod Biol* 2010; 150: 171–4.
90. http://www.ewg.org/reports/bodyburden2/execsumm.php
91. Makri A, Goveia M, Balbus J, Parkin R. Children's susceptibility to chemicals: A review by developmental stage. *J Toxicol Environ Health* 2004; 7: 417–35.
92. Angell NF, Lavery JP. The relationship of blood lead levels to obstetric outcome. *Am J Obstet Gynecol* 1982; 142: 40–6.
93. Condon S. A public health response to mercury in fish. 2007. Available from: https://www.necir.org/2015/07/19/high-mercury-in-mass-fish/
94. Axelrad DA, Bellinger DC, Ryan LM, Woodruff TJ. Dose–response relationship of prenatal mercury exposure and IQ: An integrative analysis of epidemiologic data. *Environ Health Perspect* 2007; 115: 609–15.
95. FAO/WHO. *Evaluation of certain food additives and contaminants. Sixty-Seventh Report of the Joint FAO/WHO Expert Committee on Food Additives*. Rome, Italy: WHO Technical Report Series, 940, 2006.
96. Hibbeln JR, Davis JM, Steer C et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): An observational cohort study. *Lancet* 2007; 369: 578–85.
97. Davidson PW, Myers GJ, Weiss B, Shamlaye CF, Cox C. Prenatal methyl mercury exposure from fish consumption and child development: A review of evidence and perspectives from the Seychelles child development study. *Neurotoxicology* 2006; 27: 1106–9.
98. Debes F, Budtz-Jorgensen E, Weihe P, White RF, Grandjean P. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. *Neurotoxicol Teratol* 2006; 28: 536–47.
99. NRC. *National Research Council: Toxicological Effects of Methylmercury*. Washington, DC: National Academy Press, 2000.
100. Hsu CS, Liu PL, Chien LC, Chou SY, Han BC. Mercury concentration and fish consumption in Taiwanese pregnant women. *BJOG* 2007; 114: 81–5.
101. Sato RL, Li GG, Shaha S. Antepartum seafood consumption and mercury levels in newborn cord blood. *Am J Obstet Gynecol* 2006; 194: 1683–8.
102. Guo YL, Hsu PC, Hsu CC, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. *Lancet* 2000; 356: 1240–1.
103. Charlier CJ, Foidart JM. Comparative study of dichlorodiphenyldichloroethylene in blood and semen of two young male populations: Lack of relationship to infertility, but evidence of high exposure of the mothers. *Reprod Toxicol* 2005; 20: 215–20.
104. Exposure to Toxic Environmental Agents. Committee on Health Care for Underserved Women, American Society for Reproductive Medicine Practice Committee, The University of California, San Francisco Program on Reproductive Health and the Environment. 2013.
105. Carpenter DO, Shen Y, Nguyen T, Le L, Lininger LL. Incidence of endocrine disease among residents of New York areas of concern. *Environ Health Perspect* 2001; 109(Suppl 6): 845–51.
106. Khattak S, K-Moghtader G, McMartin K et al. Pregnancy outcome following gestational exposure to organic solvents: A prospective controlled study. *JAMA* 1999; 281: 1106–9.
107. Nakao M. Epigenetics: Interaction of DNA methylation and chromatin. *Gene* 2001; 278: 25–31.
108. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 2005; 308: 1466–9.

109. Crews D, McLachlan JA. Epigenetics, evolution, endocrine disruption, health, and disease. *Endocrinology* 2006; 147: S4–10.
110. Cohn BA, Cirillo PM, Wolff MS et al. DDT and DDE exposure in mothers and time to pregnancy in daughters. *Lancet* 2003; 361: 2205–6.
111. Younglai EV, Holloway AC, Lim GE, Foster WG. Synergistic effects between FSH and 1,1-dichloro-2,2-bis(P-chlorophenyl)ethylene (P,P'-DDE) on human granulosa cell aromatase activity. *Hum Reprod* 2004; 19: 1089–93.
112. Hjollund NH, Bonde JP, Ernst E et al. Pesticide exposure in male farmers and survival of *in vitro* fertilized pregnancies. *Hum Reprod* 2004; 19: 1331–7.
113. Giudice LC. Infertility and the environment: The medical context. *Semin Reprod Med* 2006; 24: 129–33.
114. Miller CS, Prihoda TJ. The environmental exposure and sensitivity inventory (EESI): A standardized approach for measuring chemical intolerances for research and clinical applications. *Toxicol Ind Health* 1999; 15: 370–85.
115. Rea WJ. *Chemical Sensitivity: Tools of Diagnosis and Methods of Treatment*. Volume 4. Boca Raton, FL: Lewis Publishers, 1992.
116. http://www.ewg.org/foodnews/summary/
117. Cranor CF. Toward understanding aspects of the precautionary principle. *J Med Philos* 2004; 29: 259–79.
118. NRC. *National Research Council: Hormonally Active Agents in the Environment*. Washington, DC: National Academy Press, 1999.
119. IPCS. International programme on chemical safety. In: *Global Assessment of the State-of-the-Science of Endocrine Disruptors*. Damstra T, Barlow S, Bergman A, Kavlock R, van der Kraak G (eds). Geneva, Switzerland: World Health Organization, 2002.
120. http://www.epa.gov/laws-regulations/summary-toxic-substances-control-act
121. Sharpe et al. Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. *Environ Health Perspect*. 1995 Dec; 103(12): 1136–43.

Indications for *in vitro* fertilization treatment

36

From diagnosis to prognosis

IDO BEN-AMI, ARIEH RAZIEL, YARIV GIDONI, BART C.J.M. FAUSER, and RAPHAEL RON-EL

INTRODUCTION

Since the birth of the first *in vitro* fertilization (IVF) baby almost 40 years ago, dramatic developments have occurred in IVF. IVF was initially designed to overcome the problem of tubal infertility, but is now widely held to represent the treatment of choice for unexplained infertility, male factor infertility, endometriosis, and ovarian dysfunction resistant to ovulation induction (1,2). The introduction of intracytoplasmic sperm injection (ICSI) has rendered severe forms of male infertility amenable to treatment and further widened the scope of IVF. High-profile publicity given to the latest achievements with IVF has led to its perception as a panacea for all those having difficulty in conceiving a pregnancy. This has been reflected in the rapid expansion of both the indications for IVF and the current annual number of IVF cycles worldwide (3). The degree to which IVF merits this growth in application remains unclear, however, since prospective randomized trials comparing the effectiveness of IVF with simpler fertility treatments remain scarce.

In recent years, increasing attention has been given to the balance between benefits, burdens, and risks of IVF treatment, and the concept of achieving pregnancy at all costs has been increasingly rejected (4). The level of provision of IVF treatment varies greatly from country to country, and few provide access to IVF treatment to all those who may benefit (5). The challenge is therefore twofold: firstly to identify those couples for whom the potential benefits of IVF treatment merit the associated risks and costs; and secondly to improve the risk/benefit balance in favor of the latter. In recent years, progress has been made on both counts. New studies focusing on IVF outcomes have further clarified those factors that determine outcome and offer the prospect of individualizing ovarian stimulation protocols and embryo transfer policies. The concept of considering indications for IVF has become more sophisticated than simply identifying a cause for infertility that might be amenable to IVF.

CONVENTIONAL APPROACH: DIAGNOSIS AS THE INDICATION FOR IVF

The original indication for IVF, tubal disease, remains an important medical indication for IVF, but in terms of numbers of patients treated, other indications have become more important. National guidelines for IVF continue to focus primarily on underlying diagnoses when determining indications for IVF (Table 36.1). Over the years, a consensus has grown as to what constitute the primary medical indications (Figure 36.1). This is reflected in the similar frequencies of indications revealed by independent databases. Variations between databases may simply reflect differences in definition or population. Patients with low-grade endometriosis may, for instance, be considered as having either a tubal or an idiopathic indication. Depending on inclusion and exclusion criteria, infertility is categorized as idiopathic in 10% to more than 30% of cases.

The extent to which the underlying pathology itself can impact on the chance of success has been the subject of considerable study. Initial reports indicated certain causes of infertility to be associated with a lower chance of success than others. However, large published studies on the effect of the cause of female infertility have shown no significant effect on outcome of IVF (Table 36.2, Figure 36.2) (2,6). Instead, pregnancy chances were again determined by female age, duration of infertility, and previous pregnancy (2). In recent years, the impacts of certain underlying causes of infertility on IVF outcome have become clearer.

Endometriosis

Early reports from major IVF centers indicated that IVF success rates in women were not adversely affected by endometriosis (7,8). These were followed by a number of studies that reported a significant decrease in the fertilization rate *in vitro* in women with endometriosis (9,10). Endometriosis may cause infertility by distorting adnexal anatomy, interfering with oocyte capture, impairing oocyte development, early embryogenesis, or endometrial receptivity (11). The rate of endometriosis-associated assisted reproduction technology (ART) cycles has decreased over time. As compared with male factor infertility, endometriosis is associated with increased cancellation and decreased hyperstimulation risks. Despite reduced oocyte yield and higher medication dose, the differences in pregnancy and live birth rates may be of limited clinical significance, suggesting comparable pregnancy outcomes per transfer (12).

Data, mostly uncontrolled, indicate that surgery at any stage of endometriosis enhances the chances of natural

Table 36.1 *In vitro* fertilization indications as recommended by the Dutch Society of Obstetrics and Gynaecology

1. Tubal pathology
   - If tubal surgery is not a realistic option, IVF is the method of choice.
   - In case of impaired tubal function but no occlusion is present, or following tubal surgery, IVF is the method of choice after an infertility duration of two years or longer. Depending on the female age, IVF can be done after a shorter duration of infertility.
2. Unexplained infertility (idiopathic)[a]
   - In case of idiopathic infertility, IVF is indicated if the duration is three years or longer. If the woman is older than 36 years, IVF may be considered earlier.
3. Male infertility
   - TMC <1 million: first treatment of choice is ICSI.
   - TMC >1 and <10 million: IVF can be performed if infertility duration is two years or longer.[a]
   - TMC >10 million: treat as unexplained infertility.
4. Endometriosis
   - In case of mild or moderate endometriosis, treat as unexplained infertility.
   - In case of severe endometriosis, policy is to treat as tubal pathology
5. Cervical factor/immunological infertility[a]
   - After an infertility duration of two years, IVF is indicated. This may be considered sooner if the woman is over 36 years of age.
6. Hormonal disturbances[a]
   - Anovulatory cycle abnormalities are indications for IVF if 12 cycles of treatment with ovulation induction have been unsuccessful.

[a] In these situations, intrauterine insemination treatment merits consideration before proceeding to IVF.
Abbreviations: ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; TMC, total motile sperm count.

conception (13). The risk of compromised ovarian function following surgery due to excision of excessive tissue or damage to hilar vessels sparked a rule of no surgery before IVF. Women with endometrioma undergoing IVF/ICSI have similar reproductive outcomes compared with those without the disease, although their cycle cancellation rate is significantly higher. Surgical treatment of endometrioma does not alter the outcome of IVF/ICSI treatment compared with no surgical intervention. Considering that the reduced ovarian reserve may be attributed to the presence of endometrioma per se, and the potential detrimental impact from surgical intervention, individualization of care for women with endometrioma prior to IVF/ICSI is recommended (14). Indeed, during the last decade, there has been a spread of conservative management, with increasing agreement that endometriomas with a mean diameter less than 4 cm should not be systematically removed before IVF trial (15,16). This recommendation is clearly stated in both

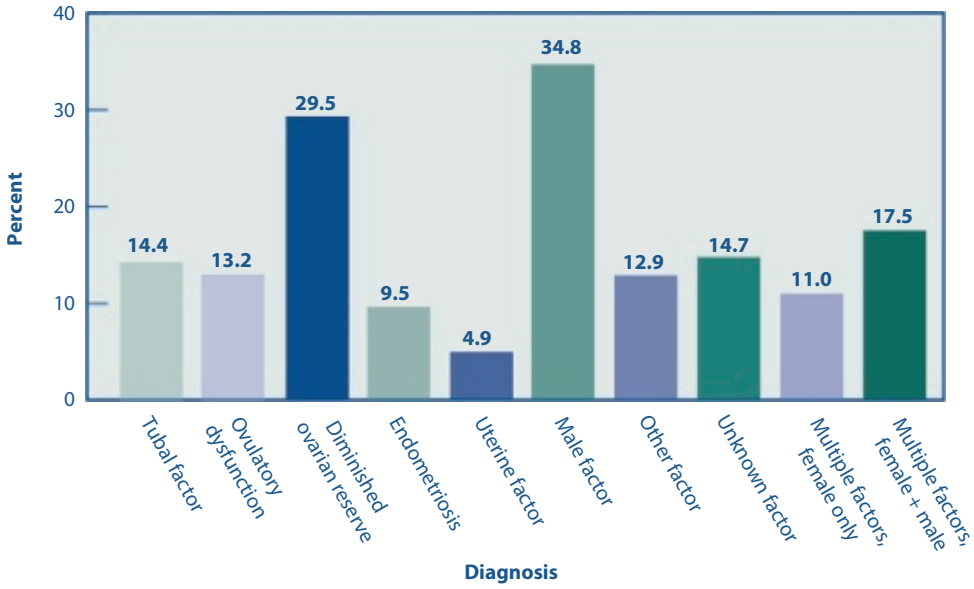


Figure 36.1 Percentages of assisted reproduction technology cycles using fresh non-donor eggs or embryos by infertility diagnosis (CDC 2013): total percentages are greater than 100 because more than one diagnosis can be reported for each cycle.

Table 36.2 Impact of cause of infertility on livebirth rate from *in vitro* fertilization

Cause of infertility	Number of cycles	Live birth rate (%) (95% confidence interval)		
		Per treatment cycle	Per egg collection	Per embryo transfer
Tubal disease	19,096	13.6 (13.0–14.0)	15.0 (14.5–15.6)	16.5 (15.9–17.1)
Endometriosis	4117	14.2 (13.2–15.3)	15.9 (14.7–17.0)	17.9 (16.6–19.3)
Unexplained	12,340	13.4 (12.9–14.1)	15.2 (14.6–15.9)	19.7 (18.8–20.5)
Cervical	4232	14.2 (13.2–15.3)	16.2 (15.1–17.4)	18.8 (17.5–20.2)

Source: Adapted from Templeton A et al. *Lancet* 1996; 348: 1402–6.

the American Society of Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) currently available guidelines for the management of endometriosis (17,18). Medical treatment (e.g., three to six months of gonadotropin-releasing hormone [GnRH] analogs) improves the outcome of IVF. When age, ovarian reserve, and male and tubal status permit, surgery should be considered immediately so that time is dedicated to attempts to conceive naturally. In other cases, the preference is for administration of GnRH analogs before IVF, and no surgery beforehand (19).

Tubal dysfunction

No randomized controlled studies have been performed comparing tubal surgery and IVF in patients with tubal damage or dysfunction. The decision to carry out IVF rather than tubal surgery therefore has a large subjective element, and tends to be based on a clinical assessment of the severity of tubal damage, the age of the patient, and the availability of specialized surgical services and IVF.

The impact of tubal dysfunction on IVF outcome is similarly controversial (20,21). Although tubal disease in general is not associated with poor outcome from IVF, there is a substantial body of evidence that distal tubal disease associated with hydrosalpinx may affect the chances of success from IVF treatment. Several retrospective studies have indicated that hydrosalpinges negatively influence the chance of success with IVF by decreasing implantation rates or toxic effects on the embryo or endometrium (22–24). In a meta-analysis evaluating differences in pregnancy rates after IVF in tubal infertility with and without hydrosalpinx, pregnancy rates of 31.2% were observed in the absence of hydrosalpinx and 19.7% in the presence of hydrosalpinx (odds ratio [OR] = 0.64, 95% confidence interval [CI] = 0.56–0.74) (25). A recent systematic review including five randomized trials observed that the odds of achieving an ongoing pregnancy were twice as great after laparoscopic salpingectomy for hydrosalpinges before IVF (OR = 2.14, 95% CI = 1.23–3.73) (26). In a randomized study, proximal tubal occlusion was shown to be as effective as salpingectomy at improving implantation rates when compared to no intervention (27). Any discussion of the potential risks and benefits should also highlight the potential effect of delaying IVF

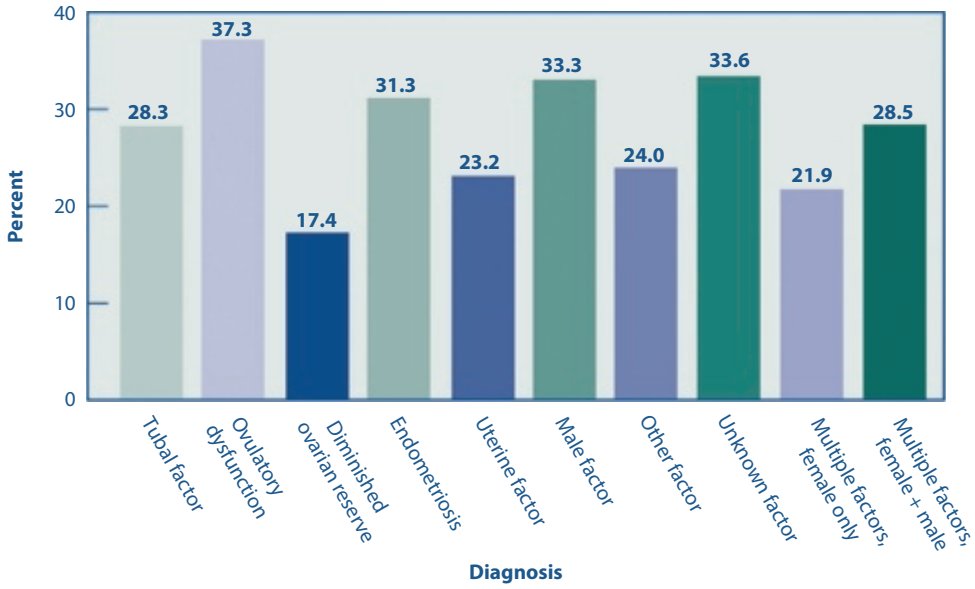


Figure 36.2 Percentages of assisted reproduction technology cycles using fresh non-donor eggs or embryos that resulted in live births by infertility diagnosis (CDC 2013).

treatment, especially in older patients where other factors may play the determining role.

Anovulation

Chronic anovulation is a common cause of infertility. Most anovulatory women have irregular menstrual cycles and normal serum follicle-stimulating hormone (FSH) concentrations (World Health Organization [WHO] group 2) (28,29). Depending on the criteria used, polycystic ovary syndrome (PCOS) is diagnosed in approximately 60%–70% of these women (30,31). Cumulative singleton live birth rates of up to 71% in two years can be achieved in this group of patients with classical induction of ovulation by applying clomiphene citrate (CC) as first-line treatment and exogenous gonadotropins as second-line treatment (32). Alternative treatment options such as IVF should therefore be avoided as first-line therapy in these patients, except for subgroups with a poor prognosis. Those women who may benefit from IVF as first-line therapy can be identified by older age, longer duration of infertility, and a higher insulin:glucose ratio (32). When classical ovulation induction fails, IVF is a feasible therapeutic option (33). Although PCOS patients are typically characterized by producing an increased number of oocytes, they are often of poor quality, leading to lower fertilization, cleavage, and implantation rates, and a higher miscarriage rate (34). Despite reduced overall fertilization, IVF pregnancy rates in PCOS patients appeared to be comparable to normo-ovulatory women (35–37). In a meta-analysis of IVF outcomes in women diagnosed with PCOS on the basis of the Rotterdam criteria (38), it was shown that the cycle cancellation rate is significantly increased in patients with PCOS (12.8% vs. 4.1%; OR = 0.5; 95% CI = 0.2–1.0). Duration of stimulation is significantly longer in patients with PCOS (1.2 days; 95% CI = 0.9–1.5), even when the daily dose of FSH is similar to that of women without PCOS. Although PCOS subjects produced more oocytes, a lower fertilization rate was observed (33).

In a study in which IVF outcomes were compared between a carefully defined group of women with WHO 2 anovulatory infertility and a matched control group of women with tubal infertility (39), obese women suffering from WHO 2 anovulatory infertility were at an increased risk of having their IVF cycle cancelled due to insufficient response. However, once oocyte retrieval was achieved, live birth rates were comparable with controls.

A retrospective cohort study that aimed to determine whether the diagnosis of PCOS independently predicts increased rates of pregnancy complications relative to control subjects after fresh IVF with or without ICSI found higher risks of gestational diabetes, hypertensive disorders of pregnancy, and large for gestational age >90th percentile (40).

Male factor infertility

Poor semen quality is the single cause of infertility in approximately 20% of infertile couples, and is an important contributing factor in another 20%–40% of them (41). Fortunately, high female fecundity can often compensate for the presence of low sperm concentrations (42). In those couples presenting with male factor infertility, intrauterine insemination (IUI) with washed and prepared sperm can be an effective treatment (43). The additional value of ovarian stimulation to IUI in this context remains a topic of debate (44,45). Ovarian stimulation with CC does not appear to increase the efficacy of IUI (46,47), but when the female partner is over 35 years of age, the addition of gonadotropin ovarian stimulation does appear to increase pregnancy rates, but at the expense of a higher incidence of multiple pregnancy (44).

Best results with IUI are achieved when the total motile sperm count in the insemination specimen exceeds a threshold of approximately 10 million and 14% or more of sperm have normal morphology (strict criteria; WHO III standard) (48,49). Higher counts do not further increase the likelihood for success and IUI is seldom successful if fewer than 1 million total motile sperm are present (49).

The results of IVF in the treatment of male factor infertility are determined primarily by the age of the woman (50), the degree of sperm motility, and sperm morphology (51–53). Many studies have reported a strong correlation between impaired semen parameters and fertilization capacity in IVF, and when severe male factor infertility is present, total fertilization failure (TFF) may occur. In many centers, a post-wash total motile sperm count of less than 500,000 is considered to indicate ICSI treatment (54), while others apply a cutoff value of 1 million (Table 36.3). These values remain largely arbitrary, since few reliable data are available that enable the prediction of the chance of TFF in a given couple (53).

Although ICSI has transformed the fertility prognosis for couples with severe male factor infertility (including those where TFF occurs during IVF), the appropriate indications for ICSI remain controversial (55). While in some countries ICSI tends to be restricted to treating severe oligoasthenospermy and TFF, other European and U.S. centers apply a more liberal policy to the use of ICSI, primarily reflecting differences in national or local funding policy. However, absolute indications for ICSI are agreed to include the use of microsurgical (epididymal or testicular) aspirated spermatozoa (Table 36.3). While many clinics have a lower clinical threshold for applying ICSI, and some apply it to all cases of IVF, this approach is not supported

Table 36.3 Indications for intracytoplasmic sperm injection

- TMC <1 million
- <4% normal morphology and TMC <5 million
- No or poor fertilization in the first IVF cycle when TMC <10 million
- No or poor fertilization in two IVF cycle when TMC >10 million
- Epididymal or testicular spermatozoa

Abbreviations: IVF, *in vitro* fertilization; TMC, total motile sperm count.

by well-designed prospective studies. In one study comparing IVF to ICSI in couples with tubal infertility but with normozoospermic semen, no differences in fertilization rates were observed (56). There is some evidence that ICSI may have detrimental effects, leading to poorer embryo development compared to IVF (57,58). In a multicenter randomized study comparing ICSI to IVF in the treatment of unexplained infertility, no benefit of ICSI was demonstrated (59). However, ICSI may yield higher fertilization rates for oocytes matured *in vitro* (60) and cryopreserved oocytes (61), which often exhibit a hardened zona (62). Among fresh IVF cycles in the U.S.A., ICSI use increased from 36.4% in 1996 to 76.2% in 2012, with the largest relative increase among cycles without male factor infertility. Compared with conventional IVF, ICSI use was not associated with improved post-fertilization reproductive outcomes, irrespective of male factor infertility diagnosis (63).

Unexplained infertility

The incidence of unexplained infertility ranges between 10% and as high as 30% among infertile populations, depending on diagnostic criteria (64). Spontaneous pregnancy chances in these untreated couples vary from 30% to 70% within 2 years (65). In the absence of a specific medical cause, a specific treatment for unexplained infertility is lacking (66). These couples are exposed to several empirical treatments, such as CC (67), controlled ovarian hyperstimulation combined with IUI (COH/IUI), and/or IVF with or without ICSI (66). In general, IUI has been shown to result in pregnancy rates varying between 2% and 4% per cycle. However, when combined with vigorous ovarian stimulation, complication rates (especially high-order multiple pregnancies) are unacceptably high (68).

Steures et al. demonstrated in a randomized controlled trial (RCT) that in couples with a spontaneous pregnancy prognosis between 30% and 40%, six months of COH/IUI led to the same percentage of pregnancies as six months of expectant management (69). Whether treatment in couples with a poorer prognosis is superior to expectant management is, unfortunately, not yet sufficiently investigated in RCTs. Likewise, in a randomized comparison of 250 couples between a single IVF cycle and six months of expectant management, no difference in pregnancy rates was observed when bilateral tubal occlusion was excluded (70). For the group of patients with more subtle abnormalities (such as endometriosis, minor tubal disease, oligospermia or unexplained infertility) proper management should focus on prognosis rather than diagnosis. The prognosis of a given couple for spontaneous pregnancy should be weighed against pregnancy chances after more invasive treatment strategies such as IUI (with or without ovarian stimulation) or IVF.

A recent randomized controlled study compared the time to pregnancy and healthcare costs (i.e., costs related to treatment, pregnancy, and newborn care), as well as the efficacy and adverse events, of two infertility treatment strategies for couples with unexplained infertility who were candidates for ovulation induction with IUI as their initial treatment (71). Compared with conventional infertility treatment and when the woman was younger than 40 years, an accelerated approach to IVF that started with CC/IUI, but eliminated gonadotropin/IUI, resulted in a shorter time to pregnancy, with fewer treatment cycles, and at a suggested cost savings (71).

In conclusion, a true cause for the infertility cannot be found in many couples presenting with fertility problems. Therefore, causal therapy is only possible in a small proportion of patients. For the remaining couples, a pragmatic prognosis-oriented approach should be applied. Most importantly, chances for spontaneous pregnancy should be assessed for each given couple. Evidence is accumulating that female age is by far the most crucial factor in determining chances for pregnancy, either spontaneously or after fertility therapy. This becomes even more predominant over the years, since women in the Western world tend to delay their wish to conceive. Increasing attention is now focusing on the identification of prognostic factors capable of determining the chance of spontaneous conception and of successful outcome to infertility treatment in individual couples. When considering treatment options for couples with unexplained infertility, it is prudent to consider simple treatment before complex treatment and to balance what is known about effectiveness against the cost and adverse effects of different treatments.

OTHER INDICATIONS FOR IVF AND ASSOCIATED TECHNOLOGIES

Fertility preservation

Women with malignancy or other illnesses that require treatments that have a negative effect on future fertility (i.e., chemotherapy and radiation therapy) may be candidates for urgent IVF and cryopreservation of embryos before the initiation of the treatment, if time and health allow (72). Oocyte cryopreservation is a viable option for women having no male partner (73) who ask for fertility preservation either because of severe malady, an emerging premature ovarian failure (74), healthy aging women, and those who anticipate delayed childbearing (73,74).

Gestational surrogacy

For women with no functional uterus, either due to developmental anomaly (e.g., Mayer–Rokitansky–Kuster–Hauser syndrome), advanced disease (multiple myomas or severe intrauterine adhesions), or previous hysterectomy, and for women suffering from severe medical conditions that preclude pregnancy, gestational surrogacy offers the opportunity to have their own genetic offspring (75).

Preimplantation genetic diagnosis and screening

Preimplantation genetic diagnosis (PGD) has been developed for patients at high risk of transmitting a genetic abnormality to their children, which includes all monogenic defects (autosomal recessive, autosomal dominant, and X-linked disorders) (76). More recently, DNA amplification-based PGD applications have broadened and

include sibling-donor selection through human leukocyte antigen (HLA) matching (77), and the analysis of familial chromosomal rearrangements (78). Preimplantation genetic screening (PGS) applies the same technology in couples having no known chromosomal or genetic abnormalities in efforts to identify and exclude aneuploid embryos. The beneficial effect of PGS was expected to be greatest in women of advanced maternal age, since aneuploidies in clinically recognized pregnancies occur more frequently when a woman passes 35 years of age (79), and it is in these women that pregnancy chances decline sharply both in normal conception and after IVF (80). In addition to women of advanced maternal age, PGS has been offered to women with a history of recurrent miscarriage, women with a history of repeated implantation failure (i.e., several failed IVF cycles), and women with a partner with low sperm quality (severe male factor), mainly since high percentages of aneuploidies have been found in the embryos of these women. However, systematic review and meta-analysis of RCTs on PGS using fluorescence *in situ* hybridization (FISH) technology after cleavage-embryo biopsy found no evidence of a beneficial effect of PGS on live birth rates after IVF, and even deleterious effects on IVF outcomes (81). The reasons for the latter results might be attributed to the FISH technology itself, or to the stage of the embryo biopsy, which may have adverse effects on embryo development (82). A new genetic technique known as comprehensive chromosome screening (CCS), which analyzes the full chromosome complement, has been used recently in PGS cycles (83–85). This technique has been utilized on different stages of embryo biopsies, including polar body, cleavage-stage, and blastocyst-stage embryos (84,86). Furthermore, CCS can be achieved with the use of different genetic methods, including metaphase/array comparative genomic hybridization, single-nucleotide polymorphism microarray, quantitative polymerase chain reaction, and, most recently, next-generation sequencing (87–91). Recently, it was found that elective single-embryo transfer coupled with enhanced embryo selection using PGS in women older than 35 years of age reduced multiple pregnancy rates while maintaining the cumulative success rate of the IVF program (92). Although early data show promising clinical results, whether PGS–CCS improves embryo selection in IVF remains unclear and a matter of debate.

From diagnosis to prognosis

Infertility is defined as the inability of a couple to conceive within one year of regular intercourse. These infertile couples can be separated into two groups: those who are unable to conceive without therapy (i.e., absolute infertility) and those with reduced fertility chances who still have a considerable chance to conceive spontaneously with time. Disease states underlying the inability to conceive spontaneously include anovulation, complete tubal occlusion, and azoospermia. Hence, an underlying cause for the infertility can be diagnosed conclusively in these conditions. A regular fertility workup—including tests to evaluate ovulation, sperm analysis, and tests for tubal patency—can easily identify these problems.

In couples with decreased fertility, conditions such as endometriosis, oligozoospermia, or luteal phase insufficiency may be found, but it remains uncertain to what extent they contribute to the reduced fertility. Hence, in a large number of couples attending a physician for fertility problems, a clear diagnosis explaining their decreased or absent fertility cannot be found (also referred to as unexplained subfertility). Indeed, success rates per cycle of a given treatment should be weighed against costs, side effects, and inconvenience for the patient, and the chances of complications for mother and child. Risks for finance-driven overtreatment remain substantial.

Many endogenous factors play a role in determining how an individual woman will respond to IVF treatment. However, any individual approach to infertility treatment must begin with an assessment of a given couple's chance of conceiving spontaneously. The chance of achieving a spontaneous pregnancy is frequently underestimated by couples and their physicians (93). The increasing tendency to delay childbearing for career, social, or other reasons is putting physicians under greater pressure to intervene when spontaneous conception does not occur quickly. Time is increasingly an issue for couples seeking to conceive. Yet patience can pay dividends for many who are now subject to premature and unnecessary intervention. Most couples seeking help will present with subfertility rather than absolute infertility. On the basis of a modest range of investigations and certain individual characteristics, the chances of an individual couple conceiving spontaneously over a given period of time can be calculated.

Several studies developed prediction models for calculating individual chances of spontaneous conception in subfertile couples (42,94,95). On the basis of the results of a number of fertility investigations and patient parameters such as age and duration of infertility, the chance of conception over a given timeframe can be calculated. For instance, after three years of failure to conceive, the residual likelihood of spontaneous pregnancy in untreated couples with unexplained infertility falls to 40% and after five years to 20% (93,96).

When considering the appropriate moment for therapeutic intervention for couples with unexplained infertility, prognostic models may aid the clinician. However, caution is required when applying a prediction model developed elsewhere to one's own patient population. Before a prediction model can be introduced into everyday clinical practice, prospective external validation is required. Furthermore, knowledge of the development cohort is important when selecting a model for application in one's own setting. Few prediction models have been subject to validation in a different population to that in which the model was developed (97). For example, the discriminative ability and reliability of the Eimers model for predicting spontaneous pregnancy among subfertile women was measured in an independent Canadian data set (98). The model that was developed in the Dutch

population in 1994 was found to have moderate predictive power in the Canadian population, in which the birth rate was generally lower. With adjustment for the average live birth rate, the Eimers model gave reliable spontaneous pregnancy predictions. In a prospective evaluation of the performance of the Eimers model in a tertiary care center, the expected and observed incidence of spontaneous pregnancy in the different risk groups correlated well (99). More recently, the Hunault synthesis model was shown in a prospective study to accurately predict spontaneous pregnancy in subfertile couples (100).

In those with a poor chance of conceiving spontaneously, or with other fertility treatments, consideration of a number of factors will aid in assessing the likely outcome of IVF. While duration of infertility has been shown to be associated with the chance of spontaneous pregnancy (101), its impact on the chance of success with IVF treatment has been less clear (102). In a large retrospective analysis of factors affecting outcomes in IVF, there was a significant decrease in age-adjusted live birth rates with increasing duration of infertility (2). Previous pregnancy had a significantly positive impact on the chance of success with IVF, with the effect being stronger for pregnancies resulting in a live birth. This positive association with previous live birth was even stronger if it had followed IVF pregnancy. The same authors calculated a previous live birth to be associated with a live birth rate per IVF treatment cycle of 23.2% compared with 12.5% when no previous pregnancy had occurred. This association with previous pregnancy and successful outcome has since been confirmed by other studies (7,103). In a recent review and meta-analysis that aimed to identify the most relevant predictors of success in IVF, it was found that female age, duration of subfertility, basal FSH, and number of oocytes, all reflecting ovarian function, were predictors of pregnancy after IVF (104).

Ovarian aging

The most prominent determining factor for IVF outcome is the individual variability in ovarian response to stimulation. Rather than exhibiting the desired response, women can present with either a hypo-response or a hyper-response to stimulation. While hyper-response to gonadotropin stimulation can usually be prevented by modification of the stimulation regimen, a poor response to ovarian stimulation is highly resistant to therapeutic intervention (105). Strategies for stimulating "low responders" include varying the dose or day of the cycle for initiating stimulation with gonadotropins. Studies undertaken so far have been unable to demonstrate a beneficial effect of gonadotropin dose increase in patients who exhibit a poor response to standard-dose regimens (105,106). Alternative approaches include early cessation or micro-dose GnRH agonist protocols, and the adjunctive use of aromatase inhibitors, growth hormone, GnRH antagonists (107), and dehydroepiandrosterone (108). Initial small studies focusing on surrogate outcomes such as number of cancelled cycles rather than ongoing pregnancy may produce encouraging results. However, at present, no therapeutic intervention has been shown in large randomized studies to offer a solution to poor response to ovarian stimulation in IVF. It might indeed be argued that therapeutic interventions aimed at increasing the chance of meeting criteria for oocyte pickup are unethical unless ongoing pregnancy rates can also be shown to improve.

Poor response to ovarian stimulation for IVF is clearly associated with chronological aging. Maternal age is the most important factor in determining the likelihood of success with IVF. Age-related declines in response to stimulation with gonadotropins and a reduction in the number of oocytes (109), oocyte quality (110), fertilization rates (111,112), and ultimately embryos (113,114) have been well documented. Many studies point to 40 years of age as bring a significant cutoff point for effectiveness of IVF (115–118). This age-related effect on pregnancy rates is similar to that reported in donor sperm programs (119) and chances for spontaneous pregnancy. A multiple regression analysis of factors influencing IVF outcomes revealed a predicted live birth rate of 17% per cycle at 30 years of age, falling to just 7% at 40 years of age and 2% at 45 years of age (2). Although age is an important predictor of IVF outcome (120), chronological age is poorly correlated with ovarian aging. The associations between cycle cancellation and poor success rates and poor ovarian response due to diminished ovarian reserve are well established (121,122). Major individual variability exists in follicle pool depletion within the normal range of menopausal age, as complete follicle pool exhaustion may occur at between 40 and 60 years. The quantity and quality of the primordial follicle pool diminishes with age, reducing ovarian reserve (123). This results in a decline in both therapy-induced and spontaneous pregnancies (124). However, while some women above 40 years of age will show a good response to ovarian stimulation, and subsequently conceive with IVF, other women under 40 years of age may fail to respond as a result of accelerated ovarian aging (125). The concept of poor response as a feature of chronological and ovarian aging has been further supported by studies linking poor response to ovarian stimulation to subsequent early menopause (126–128). Indeed, the response of a woman to ovarian stimulation for IVF can be considered as an extended challenge of ovarian function. In recent years, attention has been given to the identification of sensitive and specific markers of ovarian aging that may enable prediction of poor or good response to ovarian stimulation. This would open the way to improved counseling and patient selection for IVF.

The value of FSH and other prognostic markers in predicting ovarian response to stimulation in IVF treatment is dealt with further in Chapter 38.

Clearly related to the ovarian response to stimulation, the number of embryos available for transfer appears to be a crucial factor in determining the chance of success with IVF (126), and this is of equal importance in older women (129). Two studies reported on the number of embryos transferred and IVF success (130,131). One study

categorized this number into more than two and two or fewer embryos transferred. Women for whom more than two embryos were transferred had significantly higher pregnancy chances (130). The second study showed higher, though not statistically significant, chances of pregnancy when transferring more embryos (131). These data suggest uterine senescence to be less important than embryo quality in determining IVF outcome in older women. Further support for this comes from the observed success of oocyte donation programs in women over the age of 40 years (132).

Lifestyle and concurrent medical conditions

There is now a substantial amount of evidence showing that environmental and lifestyle factors influence the success rates of ART (133–135), and it is therefore important that serious attempts are made to provide adequate preconceptional screening counseling and interventions in order to optimize health prior to starting IVF. The importance of full medical assessment prior to IVF treatment is increasing as the average age of patients continues to rise. A greater proportion of infertility patients may now also present with concurrent medical conditions that may impact on the safety and management of the IVF treatment as well as pregnancy. The appropriate management of the medically complicated patient presenting for IVF can be complex and often requires an interdisciplinary approach.

The most important lifestyle factor impacting on fertility outcomes is tobacco smoking. Cigarette smoke contains several thousand components (e.g., nicotine, polycyclic aromatic hydrocarbons, and cadmium) with diverse effects. Each stage of reproductive function—folliculogenesis, steroidogenesis, embryo transport, endometrial receptivity, endometrial angiogenesis, uterine blood flow, and uterine myometrium—is a target for cigarette smoke components (136). Reports have appeared linking smoking to damage of the meiotic spindle in oocytes, increasing the risk of chromosomal errors (137). In men who smoke, all parameters of sperm quality are reduced (134). Smoking in men and passive smoking in women have been associated with a longer time to achieve a pregnancy (134). The effects of cigarette smoke are dose dependent and are influenced by the presence of other toxic substances and hormonal status. Individual sensitivity, dose, time, and type of exposure also play roles in the impacts of smoke constituents on human fertility (136). Furthermore, smoking during pregnancy has long been known to increase the risk of a number of adverse obstetric and fetal outcomes such as miscarriage, placenta previa, preterm birth, and low birth weight (134). The effects of smoking on live birth rate among women who undergo IVF are similar in magnitude to the effect of an increase in female age of more than 10 years (133). As a result, smokers require twice as many IVF cycles to become pregnant as non-smokers (133). The ASRM practice committee paper published on smoking and infertility has highlighted the considerable contribution of smoking to infertility and treatment outcomes and the need for a more proactive approach to stopping smoking prior to fertility treatment (138).

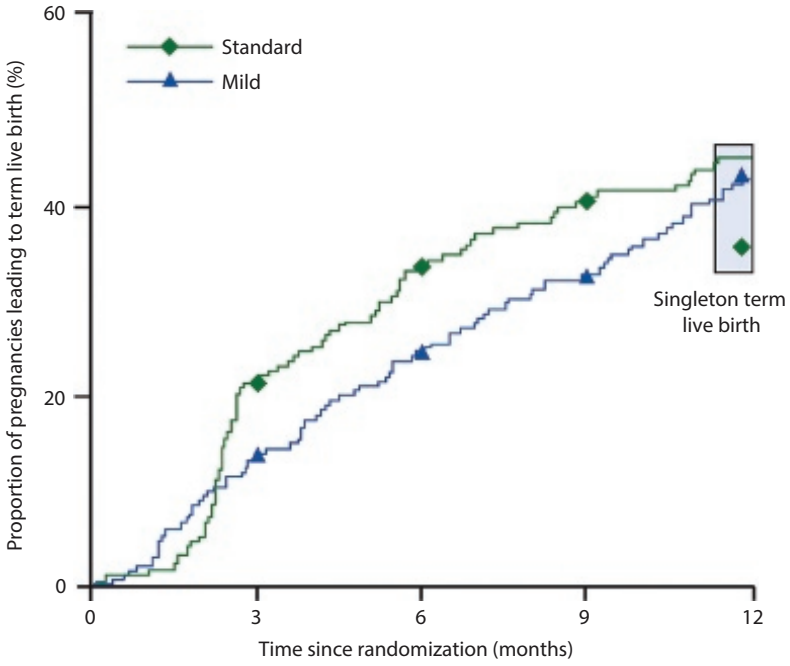
Epidemiological evidence clearly shows that being overweight contributes to menstrual disorders, infertility, miscarriage, poor pregnancy outcomes, impaired fetal well-being, and diabetes mellitus (139). Compared with women with a body mass index (BMI) of 25 kg/m^2 or less, women with a BMI $\geq$25 kg/m^2 have a lower chance of pregnancy following IVF (OR = 0.71, 95% CI = 0.62–0.81), require higher doses of gonadotrophins (weighed mean differences = 210.08, 95% CI = 149.12–271.05), and have an increased miscarriage rate (OR = 1.33, 95% CI = 1.06–1.68) (140). In men, a BMI <20 or >25 kg/m^2 is associated with reduced sperm quality (134). A number of studies have shown that weight loss can improve fecundity in overweight women, and many centers include weight loss programs as part of their fertility treatment. However, few data are available regarding the impact of type of diet on IVF outcomes.

Recent studies have highlighted the importance of certain nutritional factors for healthy gamete development and hence embryo quality. Folic acid supplementation was shown to alter the vitamin microenvironment of the oocyte (141), while seminal plasma cobalamin levels were demonstrated to effect sperm concentration (142). Concerns that folate supplementation may increase twinning rates in IVF are better addressed by practicing single-embryo transfer, rather than withholding folate supplementation (143). Women with higher vitamin D levels in their serum and follicular fluid were significantly more likely to achieve clinical pregnancy following IVF (144).

It is becoming clear that preconceptional care aimed at optimizing medical, lifestyle, and nutritional factors should be an integral part of fertility therapies, and in our center, all IVF patients attend a preconceptional clinic before commencing treatment.

Defining success in IVF

The approach of maximizing pregnancy rates per cycle has led to very complex and costly ovarian stimulation protocols with considerable risk of side effects and complications. In fact, many couples do not consider a second IVF attempt, even if they can afford one, because of the stress associated with the first treatment cycle (145). Research on less complex, more patient-friendly stimulation protocols, along with transfer of a reduced number (preferably one) of embryos, will only prosper in an environment in which singleton healthy birth is regarded as the most appropriate endpoint of infertility treatment. This primary outcome should be judged in the context of the risk of adverse effects, complications, and costs per treatment (which might include multiple cycles) or during a given treatment period. In a recent randomized study, the cumulative live singleton birth rate achieved at one year after commencing treatment was measured after two treatment strategies (145). The "conventional" strategy consisted of three cycles in which the conventional "long protocol" was applied and two embryos per cycle were transferred. The mild strategy



Number of patients

Standard	199	152	123	106	97
Mild	205	174	149	130	109

Figure 36.3 Proportions of pregnancies leading to cumulative term live birth within 12 months after starting *in vitro* fertilization. Mild: mild ovarian stimulation with gonadotropin-releasing hormone antagonist and single-embryo transfer. Standard: standard ovarian stimulation with gonadotropin-releasing hormone antagonist and dual-embryo transfer. The shaded area represents the singleton live birth rate after 12 months. (From Sharma V et al. *Fertil Steril* 2002; 78: 40–6, with permission.)

comprised four cycles in which a mild stimulation protocol was combined with the transfer of just one embryo. After one year of treatment, cumulative singleton rates were equivalent, but those treated with the mild strategy had incurred lower costs, far fewer multiple pregnancies, and lower dropout rates (Figure 36.3) (145). If IVF outcomes are expressed in terms of live singleton birth rates per period of treatment, milder regimens with fewer risks and complications will be more readily adopted into clinical practice, improving the prognosis of a complication-free, successful IVF treatment (146).

The future

As our knowledge of the factors influencing outcome following fertility therapies increases, treatment will become more individualized, maximizing cost–effectiveness and minimizing inconvenience and risk for the patient. Prognostic models based on individual factors are likely to predominate over population cost–effectiveness considerations when deciding, for instance, who receives IUI rather than IVF for the treatment of unexplained infertility. In addition, the developments of mild stimulation IVF and the prospect of improving implantation rates by optimizing embryo culture conditions and the provision of PGS will demand continuing reassessment of the cost–benefit issues. This degree of individualization requires the development and application of sophisticated, accurate, and prospectively validated prediction models. An individual approach to IVF may impact on one of the major problems still facing IVF: that of multiple pregnancy.

A major limit on the indications for IVF is the process of ovarian aging. Apart from donation, there appears to be little sign of a therapeutic intervention that is capable of circumventing this phenomenon. While the ongoing tendency to delay childbirth will increase the need for assisted conception services, the negative impact of aging on IVF outcome is likely to increase.

In the future, IVF will be increasingly applied for indications other than infertility. The growing applications for PGD are producing a new range of indications for IVF. IVF is becoming a tool simply to enable PGD, and thus prevention of hereditary disorders in normally fertile couples at risk of having children with serious medical conditions. In addition, IVF allows the creation of "designer babies" capable of donating HLA-matching tissue to treat a sick sibling. While these indications for IVF remain under close scrutiny by national regulators of IVF, they are likely to become established in the near future. The theoretical possibilities for medical therapies based on the *in vitro* culture and selective differentiation of embryonic stem cells are likely to be translated into therapeutic

reality before long. The treatment of infertility may very soon be but a minor indication for IVF.

SUMMARY

At the time of its introduction into clinical practice, the principal indication for IVF was tubal infertility. Since then the indications have multiplied, and IVF now has a central place in the treatment of female and male factor infertility, as well as the infertile couple with no clear underlying cause. The underlying indication for treatment has a limited impact on the probability of success. More important determining factors are patient age and duration of infertility. With increasing knowledge of the factors that influence a given couple's chance of conceiving either spontaneously or following fertility treatment, the emphasis is shifting from diagnosis to prognosis. The most important variable with respect to IVF is the response of the patient to ovarian stimulation. In recent years, the link between poor response to ovarian stimulation and ovarian aging has become clear, but effective remedial therapies remain elusive. Certain lifestyle factors such as smoking and obesity have also been shown to impact negatively on fertility and IVF outcomes. These factors are amenable to intervention, and due attention should be given by both clinicians and their patients to optimizing preconceptional conditions for a successful treatment and pregnancy.

REFERENCES

1. Steptoe PC, Edwards RG, Purdy JM. Clinical aspects of pregnancies established with cleaving embryos grown *in vitro*. *Br J Obstet Gynaecol* 1980; 87: 757–68.
2. Templeton A, Morris JK, Parslow W. Factors that affect outcome of *in-vitro* fertilisation treatment. *Lancet* 1996; 348: 1402–6.
3. Adamson GD, de Mouzon J, Lancaster P, Nygren KG, Sullivan E, Zegers-Hochschild F. World collaborative report on *in vitro* fertilization, 2000. *Fertil Steril* 2006; 85: 1586–622.
4. Fauser BC, Devroey P, Yen SS, Gosden R, Crowley WF, Jr., Baird DT, Bouchard P. Minimal ovarian stimulation for IVF: Appraisal of potential benefits and drawbacks. *Hum Reprod* 1999; 14: 2681–6.
5. Nachtigall RD. International disparities in access to infertility services. *Fertil Steril* 2006; 85: 871–5.
6. Hull MG, Eddowes HA, Fahy U, Abuzeid MI, Mills MS, Cahill DJ, Fleming CF, Wardle PG, Ford WC, McDermott A. Expectations of assisted conception for infertility. *BMJ* 1992; 304: 1465–9.
7. Mahadevan MM, Trounson AO, Leeton JF. The relationship of tubal blockage, infertility of unknown cause, suspected male infertility, and endometriosis to success of *in vitro* fertilization and embryo transfer. *Fertil Steril* 1983; 40: 755–62.
8. Jones HW, Jr., Acosta AA, Andrews MC et al. Three years of *in vitro* fertilization at Norfolk. *Fertil Steril* 1984; 42: 826–34.
9. Wardle PG, Mitchell JD, McLaughlin EA, Ray BD, McDermott A, Hull MG. Endometriosis and ovulatory disorder: Reduced fertilisation *in vitro* compared with tubal and unexplained infertility. *Lancet* 1985; 2: 236–9.
10. Simon C, Gutierrez A, Vidal A, de los Santos MJ, Tarin JJ, Remohi J, Pellicer A. Outcome of patients with endometriosis in assisted reproduction: Results from *in-vitro* fertilization and oocyte donation. *Hum Reprod* 1994; 9: 725–9.
11. Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. *Fertil Steril* 2008; 90: 247–57.
12. Kawwass JF, Crawford S, Session DR, Kissin DM, Jamieson DJ, National ART Surveillance System Group. Endometriosis and assisted reproductive technology: United States trends and outcomes 2000–2011. *Fertil Steril* 2015; 103: 1537–43.
13. Vercellini P, Somigliana E, Vigano P, Abbiati A, Barbara G, Crosignani PG. Endometriosis: Current therapies and new pharmacological developments. *Drugs* 2009; 69: 649–75.
14. Hamdan M, Dunselman G, Li TC, Cheong Y. The impact of endometrioma on IVF/ICSI outcomes: A systematic review and meta-analysis. *Hum Reprod Update* 2015; 21: 809–25.
15. Garcia-Velasco JA, Mahutte NG, Corona J, Zuniga V, Giles J, Arici A, Pellicer A. Removal of endometriomas before *in vitro* fertilization does not improve fertility outcomes: A matched, case–control study. *Fertil Steril* 2004; 81: 1194–7.
16. Garcia-Velasco JA, Somigliana E. Management of endometriomas in women requiring IVF: To touch or not to touch. *Hum Reprod* 2009; 24: 496–501.
17. Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility: A committee opinion. *Fertil Steril* 2012; 98: 591–8.
18. Dunselman GA, Vermeulen N, Becker C et al. European Society of Human Reproduction and Embryology. ESHRE guideline: Management of women with endometriosis. *Hum Reprod* 2014; 29: 400–12.
19. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: Pathophysiology and management. *Lancet* 2010; 376: 730–8.
20. Check JH, Lurie D, Callan C, Baker A, Benfer K. Comparison of the cumulative probability of pregnancy after *in vitro* fertilization–embryo transfer by infertility factor and age. *Fertil Steril* 1994; 61: 257–61.
21. Dor J, Seidman DS, Ben-Shlomo I, Levran D, Ben-Rafael Z, Mashiach S. Cumulative pregnancy rate following *in-vitro* fertilization: The significance of age and infertility aetiology. *Hum Reprod* 1996; 11: 425–8.

22. Barmat LI, Rauch E, Spandorfer S, Kowalik A, Sills ES, Schattman G, Liu HC, Rosenwaks Z. The effect of hydrosalpinges on IVF–ET outcome. *J Assist Reprod Genet* 1999; 16: 350–4.
23. Cohen MA, Lindheim SR, Sauer MV. Hydrosalpinges adversely affect implantation in donor oocyte cycles. *Hum Reprod* 1999; 14: 1087–9.
24. de Wit W, Gowrising CJ, Kuik DJ, Lens JW, Schats R. Only hydrosalpinges visible on ultrasound are associated with reduced implantation and pregnancy rates after *in-vitro* fertilization. *Hum Reprod* 1998; 13: 1696–701.
25. Aboulghar MA, Mansour RT, Serour GI. Controversies in the modern management of hydrosalpinx. *Hum Reprod Update* 1998; 4: 882–90.
26. Johnson N, van Voorst S, Sowter MC, Strandell A, Mol BW. Surgical treatment for tubal disease in women due to undergo *in vitro* fertilisation. *Cochrane Database Syst Rev* 2010; 1: CD002125.
27. Kontoravdis A, Makrakis E, Pantos K, Botsis D, Deligeoroglou E, Creatsas G. Proximal tubal occlusion and salpingectomy result in similar improvement in *in vitro* fertilization outcome in patients with hydrosalpinx. *Fertil Steril* 2006; 86: 1642–9.
28. Anovulatory infertility. The ESHRE Capri Workshop Group. *Hum Reprod* 1995; 10: 1549–53.
29. Rowe PJ, World Health Organization. *WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple*. Cambridge, U.K.; New York, NY: Published on behalf of the World Health Organization by Cambridge University Press, 1993.
30. van Santbrink EJ, Hop WC, Fauser BC. Classification of normogonadotropic infertility: Polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome. *Fertil Steril* 1997; 67: 452–8.
31. Laven JS, Imani B, Eijkemans MJ, Fauser BC. New approach to polycystic ovary syndrome and other forms of anovulatory infertility. *Obstet Gynecol Surv* 2002; 57: 755–67.
32. Eijkemans MJ, Imani B, Mulders AG, Habbema JD, Fauser BC. High singleton live birth rate following classical ovulation induction in normogonadotrophic anovulatory infertility (WHO 2). *Hum Reprod* 2003; 18: 2357–62.
33. Heijnen EM, Eijkemans MJ, Hughes EG, Laven JS, Macklon NS, Fauser BC. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. *Hum Reprod Update* 2006; 12: 13–21.
34. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: Impact on oocyte maturation and embryo developmental competence. *Hum Reprod Update* 2011; 17: 17–33.
35. Dor J, Shulman A, Levran D, Ben-Rafael Z, Rudak E, Mashiach S. The treatment of patients with polycystic ovarian syndrome by *in-vitro* fertilization and embryo transfer: A comparison of results with those of patients with tubal infertility. *Hum Reprod* 1990; 5: 816–8.
36. Urman B, Fluker MR, Yuen BH, Fleige-Zahradka BG, Zouves CG, Moon YS. The outcome of *in vitro* fertilization and embryo transfer in women with polycystic ovary syndrome failing to conceive after ovulation induction with exogenous gonadotropins. *Fertil Steril* 1992; 57: 1269–73.
37. Homburg R, Berkowitz D, Levy T, Feldberg D, Ashkenazi J, Ben-Rafael Z. *In vitro* fertilization and embryo transfer for the treatment of infertility associated with polycystic ovary syndrome. *Fertil Steril* 1993; 60: 858–63.
38. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004; 81: 19–25.
39. Mulders AG, Laven JS, Imani B, Eijkemans MJ, Fauser BC. IVF outcome in anovulatory infertility (WHO group 2)—including polycystic ovary syndrome—following previous unsuccessful ovulation induction. *Reprod Biomed Online* 2003; 7: 50–8.
40. Sterling L, Liu J, Okun N, Sakhuja A, Sierra S, Greenblatt E. Pregnancy outcomes in women with polycystic ovary syndrome undergoing *in vitro* fertilization. *Fertil Steril* 2016; 105: 791–7.e2.
41. Schlegel PN, Girardi SK. Clinical review 87: *In vitro* fertilization for male factor infertility. *J Clin Endocrinol Metab* 1997; 82: 709–16.
42. Collins JA, Burrows EA, Wilan AR. The prognosis for live birth among untreated infertile couples. *Fertil Steril* 1995; 64: 22–8.
43. Cohlen BJ, Vandekerckhove P, te Velde ER, Habbema JD. Timed intercourse versus intra-uterine insemination with or without ovarian hyperstimulation for subfertility in men. *Cochrane Database Syst Rev*, 2000; 2: CD000360.
44. Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. *Lancet* 2005; 365: 1807–16.
45. Cohlen B, Cantineau A, D'Hooghe T, te Velde E. Multiple pregnancy after assisted reproduction. *Lancet* 2005; 366: 452–3, author reply 453–4.
46. Martinez AR, Bernardus RE, Voorhorst FJ, Vermeiden JP, Schoemaker J. Intrauterine insemination does and clomiphene citrate does not improve fecundity in couples with infertility due to male or idiopathic factors: A prospective, randomized, controlled study. *Fertil Steril* 1990; 53: 847–53.
47. Arici A, Byrd W, Bradshaw K, Kutteh WH, Marshburn P, Carr BR. Evaluation of clomiphene citrate and human chorionic gonadotropin treatment: A prospective, randomized, crossover study during intrauterine insemination cycles. *Fertil Steril* 1994; 61: 314–8.

48. Van Voorhis BJ, Barnett M, Sparks AE, Syrop CH, Rosenthal G, Dawson J. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and *in vitro* fertilization. *Fertil Steril* 2001; 75: 661–8.
49. van der Westerlaken LA, Naaktgeboren N, Helmerhorst FM. Evaluation of pregnancy rates after intrauterine insemination according to indication, age, and sperm parameters. *J Assist Reprod Genet* 1998; 15: 359–64.
50. Forti G, Krausz C. Clinical review 100: Evaluation and treatment of the infertile couple. *J Clin Endocrinol Metab* 1998; 83: 4177–88.
51. Van Uem JF, Acosta AA, Swanson RJ, Mayer J, Ackerman S, Burkman LJ, Veeck L, McDowell JS, Bernardus RE, Jones HW, Jr. Male factor evaluation in *in vitro* fertilization: Norfolk experience. *Fertil Steril* 1985; 44: 375–83.
52. Donnelly ET, Lewis SE, McNally JA, Thompson W. *In vitro* fertilization and pregnancy rates: The influence of sperm motility and morphology on IVF outcome. *Fertil Steril* 1998; 70: 305–14.
53. Repping S, van Weert JM, Mol BW, de Vries JW, van der Veen F. Use of the total motile sperm count to predict total fertilization failure in *in vitro* fertilization. *Fertil Steril* 2002; 78: 22–8.
54. Devroey P, Vandervorst M, Nagy P, Van Steirteghem A. Do we treat the male or his gamete? *Hum Reprod* 1998; 13(Suppl 1): 178–85.
55. Devroey P, Van Steirteghem A. A review of ten years experience of ICSI. *Hum Reprod Update* 2004; 10: 19–28.
56. Staessen C, Camus M, Clasen K, De Vos A, Van Steirteghem A. Conventional *in-vitro* fertilization versus intracytoplasmic sperm injection in sibling oocytes from couples with tubal infertility and normozoospermic semen. *Hum Reprod* 1999; 14: 2474–9.
57. Ola B, Afnan M, Sharif K, Papaioannou S, Hammadieh N, Barratt CL. Should ICSI be the treatment of choice for all cases of *in-vitro* conception? Considerations of fertilization and embryo development, cost effectiveness and safety. *Hum Reprod* 2001; 16: 2485–90.
58. Verpoest W, Tournaye H. ICSI: Hype or hazard? *Hum Fertil (Camb)* 2006; 9: 81–92.
59. Bhattacharya S, Hamilton MP, Shaaban M et al. Conventional *in-vitro* fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: A randomised controlled trial. *Lancet* 2001; 357: 2075–9.
60. Barnes FL, Crombie A, Gardner DK, Kausche A, Lacham-Kaplan O, Suikkari AM, Tiglias J, Wood C, Trounson AO. Blastocyst development and birth after *in-vitro* maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. *Hum Reprod* 1995; 10: 3243–7.
61. Kuleshova L, Gianaroli L, Magli C, Ferraretti A, Trounson A. Birth following vitrification of a small number of human oocytes: Case report. *Hum Reprod* 1999; 14: 3077–9.
62. Zhang X, Rutledge J, Armstrong DT. Studies on zona hardening in rat oocytes that are matured *in vitro* in a serum-free medium. *Mol Reprod Dev* 1991; 28: 292–6.
63. Boulet SL, Mehta A, Kissin DM, Warner L, Kawwass JF, Jamieson DJ. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. *JAMA* 2015; 313: 255–63.
64. Aboulghar MA, Mansour RT, Serour GI, Al-Inany HG. Diagnosis and management of unexplained infertility: An update. *Arch Gynecol Obstet* 2003; 267: 177–88.
65. Smeenk JM, Braat DD, Stolwijk AM, Kremer JA. Pregnancy is predictable: A large-scale prospective external validation of the prediction of spontaneous pregnancy in subfertile couples. *Hum Reprod* 2007; 22: 2344–5; author reply 2345–6.
66. Brandes M, Hamilton CJ, van der Steen JO, de Bruin JP, Bots RS, Nelen WL, Kremer JA. Unexplained infertility: Overall ongoing pregnancy rate and mode of conception. *Hum Reprod* 2011; 26: 360–8.
67. Hughes E, Brown J, Collins JJ, Vanderkerchove P. Clomiphene citrate for unexplained subfertility in women. *Cochrane Database Syst Rev*, 2010; 1: CD000057.
68. Guzick DS, Carson SA, Coutifaris C et al. Efficacy of superovulation and intrauterine insemination in the treatment of infertility. National Cooperative Reproductive Medicine Network. *N Engl J Med* 1999; 340: 177–83.
69. Steures P, van der Steeg JW, Hompes PG, Habbema JD, Eijkemans MJ, Broekmans FJ, Verhoeve HR, Bossuyt PM, van der Veen F, Mol BW. Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: A randomised clinical trial. *Lancet* 2006; 368: 216–21.
70. Soliman S, Daya S, Collins J, Jarrell J. A randomized trial of *in vitro* fertilization versus conventional treatment for infertility. *Fertil Steril* 1993; 59: 1239–44.
71. Reindollar RH, Regan MM, Neumann PJ, Levine BS, Thornton KL, Alper MM, Goldman MB. A randomized clinical trial to evaluate optimal treatment for unexplained infertility: The Fast Track and Standard Treatment (FASTT) trial. *Fertil Steril* 2010; 94: 888–99.
72. Oktay K, Rodriguez-Wallberg K, Schover L. Preservation of fertility in patients with cancer. *N Engl J Med* 2009; 360: 2681; author reply 2682–3.
73. Grifo JA, Noyes N. Delivery rate using cryopreserved oocytes is comparable to conventional *in vitro* fertilization using fresh oocytes: Potential fertility preservation for female cancer patients. *Fertil Steril* 2010; 93: 391–6.
74. Oktay K, Rodriguez-Wallberg KA, Sahin G. Fertility preservation by ovarian stimulation and oocyte

cryopreservation in a 14-year-old adolescent with Turner syndrome mosaicism and impending premature ovarian failure. *Fertil Steril* 2010; 94: 753.e15–9.
75. Brinsden PR. Gestational surrogacy. *Hum Reprod Update* 2003; 9: 483–91.
76. Harton GL, De Rycke M, Fiorentino F, Moutou C, SenGupta S, Traeger-Synodinos J, Harper JC. ESHRE PGD consortium best practice guidelines for amplification-based PGD. *Hum Reprod* 2011; 26: 33–40.
77. Van de Velde H, De Rycke M, De Man C, De Hauwere K, Fiorentino F, Kahraman S, Pennings G, Verpoest W, Devroey P, Liebaers I. The experience of two European preimplantation genetic diagnosis centres on human leukocyte antigen typing. *Hum Reprod* 2009; 24: 732–40.
78. Fiorentino F, Kokkali G, Biricik A, Stavrou D, Ismailoglu B, De Palma R, Arizzi L, Harton G, Sessa M, Pantos K. Polymerase chain reaction-based detection of chromosomal imbalances on embryos: The evolution of preimplantation genetic diagnosis for chromosomal translocations. *Fertil Steril* 2010; 94: 2001–11, 2011.e1–6.
79. Hassold T, Hunt P. To err (meiotically) is human: The genesis of human aneuploidy. *Nat Rev Genet* 2001; 2: 280–91.
80. Lintsen AM, Eijkemans MJ, Hunault CC, Bouwmans CA, Hakkaart L, Habbema JD, Braat DD. Predicting ongoing pregnancy chances after IVF and ICSI: A national prospective study. *Hum Reprod* 2007; 22: 2455–62.
81. Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: A systematic review and meta-analysis of RCTs. *Hum Reprod Update* 2011; 17: 454–66.
82. Scott RT, Jr., Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: A randomized and paired clinical trial. *Fertil Steril* 2013; 100: 624–30.
83. Gutierrez-Mateo C, Colls P, Sanchez-Garcia J, Escudero T, Prates R, Ketterson K, Wells D, Munne S. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil Steril* 2011; 95: 953–8.
84. Schoolcraft WB, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. *Fertil Steril* 2010; 94: 1700–6.
85. Harper JC, Harton G. The use of arrays in preimplantation genetic diagnosis and screening. *Fertil Steril* 2010; 94: 1173–7.
86. Capalbo A, Bono S, Spizzichino L, Biricik A, Baldi M, Colamaria S, Ubaldi FM, Rienzi L, Fiorentino F. Sequential comprehensive chromosome analysis on polar bodies, blastomeres and trophoblast: Insights into female meiotic errors and chromosomal segregation in the preimplantation window of embryo development. *Hum Reprod* 2013; 28: 509–18.
87. Sher G, Keskintepe L, Keskintepe M, Maassarani G, Tortoriello D, Brody S. Genetic analysis of human embryos by metaphase comparative genomic hybridization (mCGH) improves efficiency of IVF by increasing embryo implantation rate and reducing multiple pregnancies and spontaneous miscarriages. *Fertil Steril* 2009; 92: 1886–94.
88. Fiorentino F, Spizzichino L, Bono S, Biricik A, Kokkali G, Rienzi L, Ubaldi FM, Iammarrone E, Gordon A, Pantos K. PGD for reciprocal and Robertsonian translocations using array comparative genomic hybridization. *Hum Reprod* 2011; 26: 1925–35.
89. Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT, Jr. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. *Fertil Steril* 2011; 96: 638–40.
90. Treff NR, Scott RT, Jr. Four-hour quantitative real-time polymerase chain reaction-based comprehensive chromosome screening and accumulating evidence of accuracy, safety, predictive value, and clinical efficacy. *Fertil Steril* 2013; 99: 1049–53.
91. Wells D. Next-generation sequencing: The dawn of a new era for preimplantation genetic diagnostics. *Fertil Steril* 2014; 101: 1250–1.
92. Ubaldi FM, Capalbo A, Colamaria S et al. Reduction of multiple pregnancies in the advanced maternal age population after implementation of an elective single embryo transfer policy coupled with enhanced embryo selection: Pre- and post-intervention study. *Hum Reprod* 2015; 30: 2097–106.
93. Evers JL. Female subfertility. *Lancet* 2002; 360: 151–9.
94. Eimers JM, te Velde ER, Gerritse R, Vogelzang ET, Looman CW, Habbema JD. The prediction of the chance to conceive in subfertile couples. *Fertil Steril* 1994; 61: 44–52.
95. Snick HK, Snick TS, Evers JL, Collins JA. The spontaneous pregnancy prognosis in untreated subfertile couples: The Walcheren primary care study. *Hum Reprod* 1997; 12: 1582–8.
96. Collins JA, Rowe TC. Age of the female partner is a prognostic factor in prolonged unexplained infertility: A multicenter study. *Fertil Steril* 1989; 52: 15–20.
97. Stolwijk AM, Straatman H, Zielhuis GA, Jansen CA, Braat DD, van Dop PA, Verbeek AL. External validation of prognostic models for ongoing pregnancy after *in-vitro* fertilization. *Hum Reprod* 1998; 13: 3542–9.
98. Hunault CC, Eijkemans MJ, te Velde ER, Collins JA, Habbema JD. Validation of a model predicting spontaneous pregnancy among subfertile untreated couples. *Fertil Steril* 2002; 78: 500–6.
99. Collins JA, Milner RA, Rowe TC. The effect of treatment on pregnancy among couples with unexplained infertility. *Int J Fertil* 1991; 36: 140–1, 145–52.

100. van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Hompes PG, Broekmans FJ, van Dessel HJ, Bossuyt PM, van der Veen F, Mol BW. Pregnancy is predictable: A large-scale prospective external validation of the prediction of spontaneous pregnancy in subfertile couples. *Hum Reprod* 2007; 22: 536–42.
101. Salat-Baroux J, Alvarez S, Antoine JM, Cornet D, Tibi C, Plachot M, Mandelbaum J. Results of IVF in the treatment of polycystic ovary disease. *Hum Reprod* 1988; 3: 331–5.
102. Goverde AJ, McDonnell J, Vermeiden JP, Schats R, Rutten FF, Schoemaker J. Intrauterine insemination or *in-vitro* fertilisation in idiopathic subfertility and male subfertility: A randomised trial and cost-effectiveness analysis. *Lancet* 2000; 355: 13–8.
103. Croucher CA, Lass A, Margara R, Winston RM. Predictive value of the results of a first *in-vitro* fertilization cycle on the outcome of subsequent cycles. *Hum Reprod* 1998; 13: 403–8.
104. van Loendersloot LL, van Wely M, Limpens J, Bossuyt PM, Repping S, van der Veen F. Predictive factors in *in vitro* fertilization (IVF): A systematic review and meta-analysis. *Hum Reprod Update* 2010; 16: 577–89.
105. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: A systematic review. *Hum Reprod Update* 2003; 9: 61–76.
106. van Hooff MH, Alberda AT, Huisman GJ, Zeilmaker GH, Leerentveld RA. Doubling the human menopausal gonadotrophin dose in the course of an *in-vitro* fertilization treatment cycle in low responders: A randomized study. *Hum Reprod* 1993; 8: 369–73.
107. Karande VC. Managing and predicting low response to standard *in vitro* fertilization therapy: A review of the options. *Treat Endocrinol* 2003; 2: 257–72.
108. Wiser A, Gonen O, Ghetler Y, Shavit T, Berkovitz A, Shulman A. Addition of dehydroepiandrosterone (DHEA) for poor-responder patients before and during IVF treatment improves the pregnancy rate: A randomized prospective study. *Hum Reprod* 2010; 25: 2496–500.
109. Piette C, de Mouzon J, Bachelot A, Spira A. *In-vitro* fertilization: Influence of women's age on pregnancy rates. *Hum Reprod* 1990; 5: 56–9.
110. Tucker MJ, Morton PC, Wright G, Ingargiola PE, Jones AE, Sweitzer CL. Factors affecting success with intracytoplasmic sperm injection. *Reprod Fertil Dev* 1995; 7: 229–36.
111. Ashkenazi J, Orvieto R, Gold-Deutch R, Feldberg D, Dicker D, Voliovitch I, Ben-Rafael Z. The impact of woman' age and sperm parameters on fertilization rates in IVF cycles. *Eur J Obstet Gynecol Reprod Biol* 1996; 66: 155–9.
112. Yie SM, Collins JA, Daya S, Hughes E, Sagle M, Younglai EV. Polyploidy and failed fertilization in *in-vitro* fertilization are related to patient's age and gamete quality. *Hum Reprod* 1996; 11: 614–7.
113. Hull MG, Fleming CF, Hughes AO, McDermott A. The age-related decline in female fecundity: A quantitative controlled study of implanting capacity and survival of individual embryos after *in vitro* fertilization. *Fertil Steril* 1996; 65: 783–90.
114. Sharif K, Elgendy M, Lashen H, Afnan M. Age and basal follicle stimulating hormone as predictors of *in vitro* fertilisation outcome. *Br J Obstet Gynaecol* 1998; 105: 107–12.
115. Yaron Y, Botchan A, Amit A, Kogosowski A, Yovel I, Lessing JB. Endometrial receptivity: The age-related decline in pregnancy rates and the effect of ovarian function. *Fertil Steril* 1993; 60: 314–8.
116. Roest J, van Heusden AM, Mous H, Zeilmaker GH, Verhoeff A. The ovarian response as a predictor for successful *in vitro* fertilization treatment after the age of 40 years. *Fertil Steril* 1996; 66: 969–73.
117. Legro RS, Shackleford DP, Moessner JM, Gnatuk CL, Dodson WC. ART in women 40 and over. Is the cost worth it? *J Reprod Med* 1997; 42: 76–82.
118. Malizia BA, Hacker MR, Penzias AS. Cumulative live-birth rates after *in vitro* fertilization. *N Engl J Med* 2009; 360: 236–43.
119. van Noord-Zaadstra BM, Looman CW, Alsbach H, Habbema JD, te Velde ER, Karbaat J. Delaying childbearing: Effect of age on fecundity and outcome of pregnancy. *BMJ* 1991; 302: 1361–5.
120. Chuang CC, Chen CD, Chao KH, Chen SU, Ho HN, Yang YS. Age is a better predictor of pregnancy potential than basal follicle-stimulating hormone levels in women undergoing *in vitro* fertilization. *Fertil Steril* 2003; 79: 63–8.
121. Pellicer A, Lightman A, Diamond MP, Russell JB, DeCherney AH. Outcome of *in vitro* fertilization in women with low response to ovarian stimulation. *Fertil Steril* 1987; 47: 812–5.
122. Jenkins JM, Davies DW, Devonport H, Anthony FW, Gadd SC, Watson RH, Masson GM. Comparison of "poor" responders with "good" responders using a standard buserelin/human menopausal gonadotrophin regime for *in-vitro* fertilization. *Hum Reprod* 1991; 6: 918–21.
123. te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod Update* 2002; 8: 141–54.
124. Scott RT, Opsahl MS, Leonardi MR, Neall GS, Illions EH, Navot D. Life table analysis of pregnancy rates in a general infertility population relative to ovarian reserve and patient age. *Hum Reprod* 1995; 10: 1706–10.
125. Beckers NG, Macklon NS, Eijkemans MJ, Fauser BC. Women with regular menstrual cycles and a poor response to ovarian hyperstimulation for *in vitro* fertilization exhibit follicular phase characteristics suggestive of ovarian aging. *Fertil Steril* 2002; 78: 291–7.
126. de Boer EJ, den Tonkelaar I, te Velde ER, Burger CW, Klip H, van Leeuwen FE. A low number of retrieved oocytes at *in vitro* fertilization treatment is predictive of early menopause. *Fertil Steril* 2002; 77: 978–85.

127. Nikolaou D, Lavery S, Turner C, Margara R, Trew G. Is there a link between an extremely poor response to ovarian hyperstimulation and early ovarian failure?. *Hum Reprod* 2002; 17: 1106–11.
128. Lawson R, El-Toukhy T, Kassab A, Taylor A, Braude P, Parsons J, Seed P. Poor response to ovulation induction is a stronger predictor of early menopause than elevated basal FSH: A life table analysis. *Hum Reprod* 2003; 18: 527–33.
129. van Kooij RJ, Looman CW, Habbema JD, Dorland M, te Velde ER. Age-dependent decrease in embryo implantation rate after *in vitro* fertilization. *Fertil Steril* 1996; 66: 769–75.
130. Sharma V, Allgar V, Rajkhowa M. Factors influencing the cumulative conception rate and discontinuation of *in vitro* fertilization treatment for infertility. *Fertil Steril* 2002; 78: 40–6.
131. Hauzman E, Fedorcsak P, Klinga K, Papp Z, Rabe T, Strowitzki T, Urbancsek J. Use of serum inhibin A and human chorionic gonadotropin measurements to predict the outcome of *in vitro* fertilization pregnancies. *Fertil Steril* 2004; 81: 66–72.
132. Abdalla HI, Burton G, Kirkland A, Johnson MR, Leonard T, Brooks AA, Studd JW. Age, pregnancy and miscarriage: Uterine versus ovarian factors. *Hum Reprod* 1993; 8: 1512–7.
133. Lintsen AM, Pasker-de Jong PC, de Boer EJ, Burger CW, Jansen CA, Braat DD, van Leeuwen FE. Effects of subfertility cause, smoking and body weight on the success rate of IVF. *Hum Reprod* 2005; 20: 1867–75.
134. Younglai EV, Holloway AC, Foster WG. Environmental and occupational factors affecting fertility and IVF success. *Hum Reprod Update* 2005; 11: 43–57.
135. Homan GF, Davies M, Norman R. The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: A review. *Hum Reprod Update* 2007; 13: 209–23.
136. Dechanet C, Anahory T, Mathieu Daude JC, Quantin X, Reyftmann L, Hamamah S, Hedon B, Dechaud H. Effects of cigarette smoking on reproduction. *Hum Reprod Update* 2011; 17: 76–95.
137. Zenzes MT, Wang P, Casper RF. Cigarette smoking may affect meiotic maturation of human oocytes. *Hum Reprod* 1995; 10: 3213–17.
138. Practice Committee of the American Society for Reproductive Medicine. Smoking and infertility. *Fertil Steril* 2006; 86: S172–7.
139. Norman RJ, Clark AM. Obesity and reproductive disorders: A review. *Reprod Fertil Dev* 1998; 10: 55–63.
140. Maheshwari A, Stofberg L, Bhattacharya S. Effect of overweight and obesity on assisted reproductive technology—A systematic review. *Hum Reprod Update* 2007; 13: 433–44.
141. Boxmeer JC, Brouns RM, Lindemans J, Steegers EA, Martini E, Macklon NS, Steegers-Theunissen RP. Preconception folic acid treatment affects the microenvironment of the maturing oocyte in humans. *Fertil Steril* 2008; 89: 1766–70.
142. Boxmeer JC, Smit M, Weber RF, Lindemans J, Romijn JC, Eijkemans MJ, Macklon NS, Steegers-Theunissen RP. Seminal plasma cobalamin significantly correlates with sperm concentration in men undergoing IVF or ICSI procedures. *J Androl* 2007; 28: 521–7.
143. Boxmeer JC, Fauser BC, Macklon NS. Effect of B vitamins and genetics on success of *in-vitro* fertilisation. *Lancet* 2006; 368: 200.
144. Ozkan S, Jindal S, Greenseid K, Shu J, Zeitlian G, Hickmon C, Pal L. Replete vitamin D stores predict reproductive success following *in vitro* fertilization. *Fertil Steril* 2010; 94: 1314–9.
145. Heijnen EM, Eijkemans MJ, De Klerk C et al. A mild treatment strategy for *in-vitro* fertilisation: A randomised non-inferiority trial. *Lancet* 2007; 369: 743–9.
146. Heijnen EM, Macklon NS, Fauser BC. What is the most relevant standard of success in assisted reproduction? The next step to improving outcomes of IVF: Consider the whole treatment. *Hum Reprod* 2004; 19: 1936–8.

Initial investigation of the infertile couple

37

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Infertility is defined as a failure to conceive after 12 months of unprotected intercourse (1). It affects one in seven couples (2). After one year of unprotected intercourse, 85%–90% of couples will successfully conceive. Among the remaining couples, half of them will conceive during the second year. The National Institute for Health and Care Excellence (NICE) and the American Society for Reproductive Medicine (ASRM) recommend starting infertility investigations after 12 months of unprotected intercourse (3–5). This takes into account that natural conception may occur during the period of investigation. Earlier investigation is recommended after six months of trying to conceive in women over the age of 35 years (3) due to the age-related decline in fertility as well as diminishing assisted reproduction technology (ART) outcomes in this age category. Earlier assessment may also be justified when an infertility factor is known or when it is highly suspected in the female (such as oligo/amenorrhea, tubal or uterine disease, or endometriosis) or in the male (such as undescended testes) (3,4).

The purpose of our review is to provide an overview of investigation of the infertile couple.

GENERAL ASSESSMENT OF THE COUPLE

The main objective of the fertility workup is to find a cause for infertility, particularly those that are amendable to treatment. Another objective of fertility workup includes identification of infertility-associated medical conditions such as various hormonal or genetic disorders. Serious conditions including testicular cancer might also be encountered. Fertility workup should include evaluation of the prognostic value of potential ART treatment. The etiologies of infertility are: ovulatory disorders (25%), tubal damage (20%), male factors causing infertility (30%), and uterine or peritoneal disorders (10%) (2,4,6).

The main common causes of male infertility are obstruction of the genital tract, testicular failure, varicocele, and genetic and ejaculatory disorders (7). About 25% of cases of infertility remain unexplained (6). Both members of the couple need to be evaluated. In about 40% of cases disorders are found in both the male and female (2,6). Ideally, the couple should be seen together as this has been shown to increase satisfaction (8). Sufficient time should also be allotted to allow for a comprehensive medical, reproductive, and family history and to perform a physical examination (3). History-taking is a crucial part of the infertility investigation that should not be replaced by a questionnaire (9). This should include sexual history such as frequency and timing of sexual intercourse and questions regarding a possibility of sexual dysfunction.

The initial evaluation is also used to counsel patients regarding preconception care, lifestyle changes (including avoidance of smoking and toxic exposure), and to identify situations requiring specific care, such as history of genetic diseases or consanguinity. Cessation of smoking is essential not only for general health, but also to improve fertility (10,11). Women should also be offered testing for rubella status (4) and diabetes screening in case of polycystic ovarian syndrome (PCOS) or obesity. Both partners should be tested for their HIV status and hepatitis B and C serology (4). Regular intake of folic acid is advised.

Medical evaluation and specific fertility tests for females and males will be further detailed in the following sections. The initial fertility tests are summarized in Figure 37.1.

FEMALE INVESTIGATION

History

In addition to general history-taking for both partners, a gynecological history is essential. This includes menstrual history, previous pregnancy and outcome, history of sexually transmitted disease, previous methods of contraception, and fertility treatments. Pelvic surgeries potentially leading to adhesions that impair tubal and ovarian function should be noted. Inquiries should include signs of endometriosis such as dysmenorrhea, dyspareunia, or chronic pelvic pain. Recent cervical cytology (Pap test) must be made available.

The general history should focus on weight and on endocrine diseases that could interfere with gonadal function like thyroid disease, galactorrhea or hirsutism. Occupation, environmental exposure to toxins, and drug use should be noted.

Physical investigation

Physical examination should include the patient's weight and height, identification of thyroid abnormalities, breast secretion, hirsutism, and other signs of hyperandrogenism. This is followed by pelvic examination focusing on vaginal or cervical abnormalities; uterine size, position, and mobility; and cul-de-sac or adnexal masses. Pelvic ultrasound (US), if available, could be complementary to the physical evaluation.

Tests

Baseline investigations should be performed to assess ovulatory function, ovarian reserve, uterine cavity, and tubal patency.

Ovulatory function

Regular menstrual cycle, occurring at intervals of 21–35 days (12), is usually indicative of normal ovulation (13).

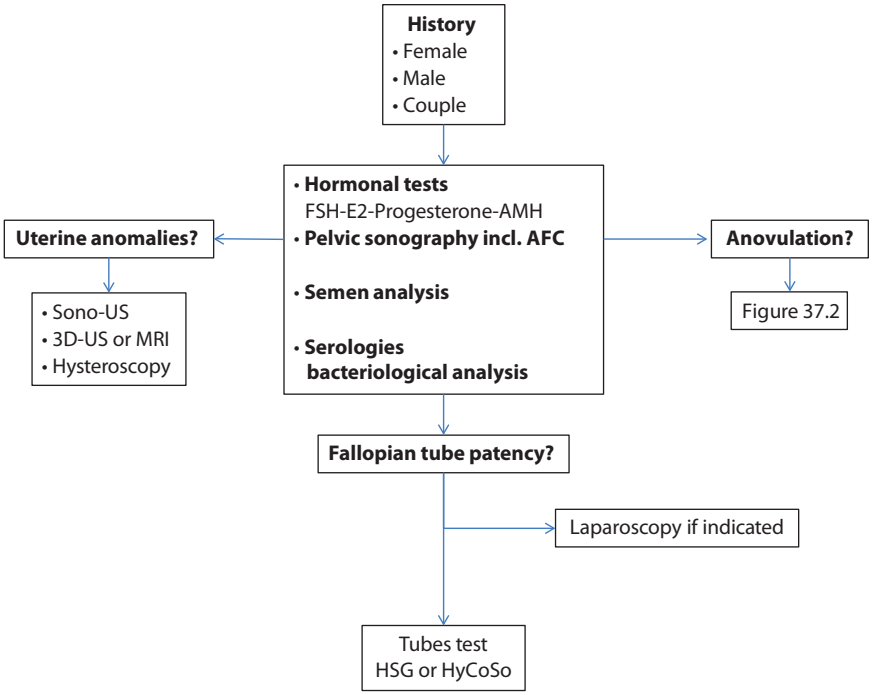

Figure 37.1 Initial infertility tests. *Abbreviations*: AFC, antral follicular count; AMH, anti-Mullerian hormone; E2, estradiol; FSH, follicle-stimulating hormone; HSG, hysterosalpingography; HyCoSy, hysterosalpingo-contrast sonography; MRI, magnetic resonance imaging; US, ultrasound.

Still, some degree of variation is normal, depending especially upon the woman's age (12). In a study of 786 cycles in 130 women, 46% of the subjects had a cycle range of seven days or more and 20% had a cycle range of 14 days or more (14).

- Ovulation was historically assessed by serial basal body temperature (BBT) measurement. Although a biphasic BBT provides presumptive evidence of ovulation, monophasic or uninterpretable BBTs are also common in ovulatory patients (15). Moreover, BBT cannot accurately predict timing of ovulation (16). This tedious test is therefore not recommended for assessing ovulation function (3,4).
- Commercially available urinary luteinizing hormone (LH) kits identify the mid-cycle LH surge suggesting the presence of ovulation. Although LH kits help to determine the fertile period, they do not improve the chance of natural conception. It could be useful for couples not having regular sexual intercourse. It indicates the fertile period, but their repetitive use may become expensive and frustrating. Reliability and ease of use may also vary among different products, and false-positive LH tests have been estimated to occur in 7% of cases (17).
- Endometrial biopsy and histological dating have been used to evaluate ovulation. However, the results are not clearly related to fertility status. Their use is limited (18,19) and they have been abandoned as routine tests (4,20,21).
- Mid-luteal serum progesterone testing is an easy method and is the most commonly used test to confirm ovulation. It is usually done on day 21 of a 28-day cycle or seven days before the commencement of menses. Yet the progesterone concentration fluctuates widely even among normal women and may impair interpretation. Values greater than 3.0 ng/mL are presumptive that ovulation has occurred (22). Some authors reported that serum progesterone levels greater than 10 ng/mL correlate with a normal "in-phase" endometrial histology (23). Whether this value is correlated with luteal function is unclear.
- US plays a role in confirming ovulation; however, it is time consuming and costly. Serial US examinations evaluating follicular growth, appearance of the corpus luteum, and luteal-appearing changes in endometrial lining could show indirect signs of ovulation.
- For practical purposes, menstrual history may be all that is required in women with regular cycles in order to confirm ovulation (3). Still, NICE guidelines do recommend measuring mid-luteal progesterone in women undergoing infertility investigation even in the presence of regular cycles (4).

Other hormonal tests

Women with oligo-ovulation or anovulation must be investigated further with other hormonal evaluations. They could have hypogonadotropic hypogonadism (World Health Organization [WHO] type 1), PCOS (WHO type 2), or ovarian failure (WHO type 3) (Figure 37.2) (4). Thyroid disorders or hyperprolactinemia require specific treatment. PCOS is the most common cause of oligo-anovulation.

Hormonal tests include evaluation of ovarian reserve, prolactin measurement, and thyroid function. Although

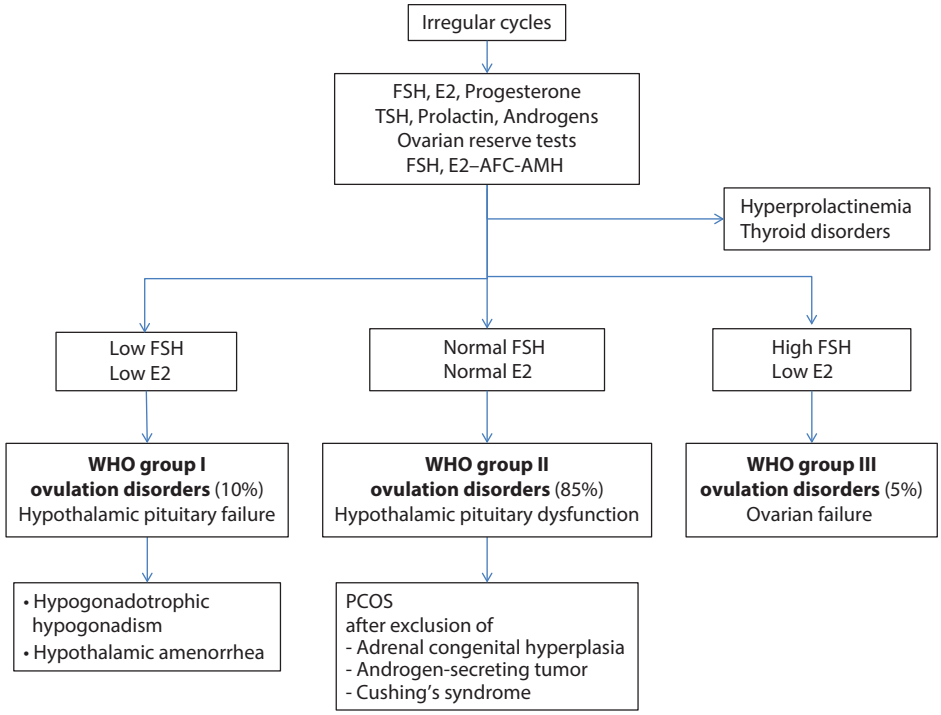

Figure 37.2 Tests for ovulation function. *Abbreviations*: AFC, antral follicular count; AMH, anti-Mullerian hormone; E2, estradiol; FSH, follicle-stimulating hormone; PCOS, polycystic ovary syndrome; TSH, thyroid stimulating hormone; WHO, World Health Organization.

the latter two serum tests are done routinely in many fertility centers, they are not universally recommended (4). Although the prevalence of thyroid disease is not higher among infertile women (4), those with abnormal thyroid stimulating hormone (TSH) values usually have ovulatory dysfunction (24). Although controversial, screening and treatment of subclinical thyroid dysfunction seem to improve pregnancy outcomes (25,26).

Women with signs and symptoms of hyperandrogenism require further investigations (serum testosterone, δ4-androstenedione, DeHydroEpiAndrosterone-Sulfate (DHEA-S) and 17-hydroxy-progesterone) to rule out the presence of late-onset congenital adrenal hyperplasia, Cushing syndrome, or androgen-producing tumors.

Ovarian reserve

Ovarian reserve evaluation is an essential component in the infertility workup. The main goal is to evaluate the fertility potential and predict ovarian response to controlled ovarian stimulation. In addition, it helps clinicians to choose the optimal stimulation strategy and to avoid iatrogenic complications, such ovarian hyperstimulation syndrome. Evaluating the ovarian reserve also facilitates appropriate patient counseling (27).

Ovarian reserve tests offer a quantitative rather than a qualitative evaluation of the ovaries. Their value is limited in the prediction of ongoing pregnancy, both for spontaneous conceptions or those achieved by ART (27). Age remains the best predictor of pregnancy following *in vitro* fertilization (IVF) (28). For this reason, withholding IVF purely on the basis of ovarian reserve tests is controversial and considered inappropriate (27,29).

The main tests for ovarian reserve include day-3 serum follicle-stimulating hormone (FSH) and estradiol (E2), serum anti-Mullerian hormone (AMH) and antral follicular count (AFC). Other ovarian tests such as serum inhibin B or isolated E2, ovarian volume, ovarian flow measurement, and clomiphene citrate challenge test are not recommended. Their predictive values are considered inferior to other ovarian markers (3,4,30).

Day-3 serum FSH and E2

FSH is downregulated by E2, and these hormonal markers should be interpreted together. Indeed, elevated E2 could otherwise falsely normalize FSH. Early follicular-phase FSH is an indirect marker of ovarian reserve and there is high intra- and inter-cycle variability. Sensitivity of FSH to predict poor ovarian response is better at very high threshold levels (31). If several values are obtained in the same patient, the highest value is considered to be prognostic (32). The upper threshold of FSH varies between 8.9 and 25 IU/L (4,31).

Antral follicle count

AFC has been described as the sum of all follicles 2–10 mm in the largest diameter measured by transvaginal US (33). AFC should be performed during the early follicular phase of the cycle (33). It is a direct marker of ovarian reserve. While AFC has good intra- and inter-cycle reliability in experienced centers, reproducibility may be limited in less experienced clinics (3). One of the three criteria of PCOS

in the Rotterdam criteria (34) is the presence of 12 or more antral follicles of 2–9 mm per ovary (35). According to NICE, an AFC greater than 16 is predictive of a high response to ovarian stimulation, while an AFC lower than 4 is predictive of a low response (4).

Anti-Mullerian hormone

AMH is a dimeric glycoprotein member of the TGF-β superfamily and is produced in ovaries. AMH expression is absent in primordial follicles and appears in the granulosa cells of primary follicles. The strongest staining of AMH is observed in pre-antral and small antral follicles. It is found in growing follicles until they become dominant (36). AMH is a direct ovarian reserve marker and may also represent different stages of growing follicles.

Since cyclic variation of AMH is minimal (37), blood sampling for AMH can be obtained at any time during the cycle. AMH seems to demonstrate less intra- and inter-cycle variability than AFC (38). Yet there has been no international standard for the AMH assay. Further, different assays may produce different absolute values (27). Normal values of AMH are described by several normograms (39,40). According to NICE, AMH levels greater than 3.5 ng/mL are predictive of a high ovarian response, while a level under 0.75 ng/mL is predictive of low response (4). Although controversial, some authors have advocated the use of AMH as a diagnostic criterion for PCOS (41).

Day-3 FSH and E2 are the most commonly used screening tests, but AFC and AMH appear to be more sensitive and specific (29) in the prediction of poor ovarian response (28). AFC and AMH are highly correlated (42). They have comparable performance in the prediction of excessive and poor ovarian response to stimulation (28,43). Thus, combining these two tests does not improve the prediction of poor response (28). The Bologna criteria for poor ovarian reserve include at least one abnormal ovarian test: AFC <5–7 follicles or AMH <0.5–1.1 ng/mL (44).

Cervix

The postcoital test (PCT) evaluates motile sperm in the cervical mucus around ovulation time and within hours following intercourse. It was the traditional method for identifying cervical factor infertility and indirectly identifying male factor infertility. However, it has poor inter- and intra-observer reproducibility (45). Moreover, there is no consensus on the definition of cervical infertility, and current treatments for unexplained infertility are able to overcome cervical factors. Since the PCT is a poor predictor of conception (46) and has a limited influence on treatment strategy, this test has been abandoned as a routine part of the fertility evaluation (3,4).

Screening for and treating cervical chlamydia infection (in both partners if screening is positive) is recommended before uterine instrumentation that could reactivate or introduce upper tract dissemination (4).

Uterus

Intrauterine abnormalities including endometrial polyps, submucosal myoma, adhesions, or a uterine septum interfering with fertility and compromising pregnancy rates in assisted reproduction.

The first-line diagnostic tool to evaluate uterine cavity is two-dimensional transvaginal US. It is inexpensive, easy to perform, and well tolerated by patients. Its sensitivity in detecting intrauterine lesions ranges from 56% to 89% (47,48). US has less diagnostic value in differentiating submucosal fibroids in the presence of multiple fibroids, endometrial polyps within a thick endometrium, and synechiae or uterine malformations.

Hysterosalpingography (HSG) evaluates tubal patency and, to a certain extent, is an assessment of the uterine cavity. However, intrauterine defects could also be due to air bubbles, mucus, or menstrual debris. False-negative findings may be the result of excessive contrast media obliterating shadows caused by small lesions. Compared to hysteroscopy, HSG has a lower sensitivity and specificity and high rates of false-positive and false-negative results (49,50). HSG is therefore a poor test for uterine cavity evaluation.

Hysterosonography (sonohysterography) is a combination of US with saline or contrast media infusion into the uterine cavity. Extension of this procedure to assess the patency of the fallopian tubes following examination of the uterine cavity is called hysterosalpingo-contrast sonography (HyCoSy). Hysterosonography improves delineation of the uterine cavity and is considered to be more accurate than US and HSG. It has high sensitivity (78%–100%) and specificity (71%–91%) for detecting intrauterine lesions (48,51,52). As with US, it is more precise for diagnosing polyps or submucosal fibroids than endometrial hyperplasia or structural abnormalities (48,51,52). Three-dimensional hysterosonography could also be performed and seems to be comparable with hysteroscopy for diagnosing intrauterine lesions (53,54).

Hysteroscopy remains the most accurate test (48,51,52) and is considered the gold standard for evaluation of the uterine cavity (ASRM). Since hysteroscopy is an invasive method for evaluation of the uterine cavity, it is usually reserved for further evaluation and treatment of already-suspected anomalies using imaging techniques (3,4,55). Hysteroscopy using a small-diameter hysteroscope allows this procedure to be conducted in the office setting, and polypectomy or adhesiolysis can be performed in the same setting (56,57).

Hysteroscopy allows visualization of the uterine cavity but not the uterine contour. Accordingly, diagnosing congenital uterine anomalies using hysteroscopy alone is insufficient. It should be investigated by magnetic resonance imaging (MRI), three-dimensional US, or a combination of laparoscopy and hysteroscopy. MRI is an accurate and noninvasive test, but it is costly. Laparoscopy combined with hysteroscopy is also accurate, but is invasive. Three-dimensional US seems to be a good compromise, as it is highly correlated with the results of MRI, laparoscopy, and hysteroscopy, particularly when performed during the luteal phase, as the thick endometrial lining enhances cavity visualization (58).

Fallopian tubes

There are several techniques to evaluate tubal integrity (3).

Hysterosalpingography

HSG is radiographic evaluation of the fallopian tubes that is performed by injecting radiocontrast of either oil-based or water-soluble media into the uterine cavity via the cervix. Contraindications to HSG include contrast allergy, pregnancy, and active pelvic infection. It should be performed in the early follicular phase to ensure the absence of pregnancy and to facilitate maximum uterine visibility.

Post-HSG infection can occur in 0.3%–3.1% of cases, particularly in the presence of abnormal tubes (59).

HSG findings of "proximal tubal occlusion" are usually due to tubal spasm, collection of debris, or a mucus plug inside the proximal tubes. Such findings should be followed up with additional tests such selective tubal catheterization. HSG sensitivity and specificity rates are 65% and 83%, respectively (60). HSG is more specific for detecting distal as opposed to proximal occlusion (60), and has a high correlation (94%) with laparoscopic findings (61).

Chlamydia trachomatis serology screening has been advocated for patients at high risk of tubal damage and to increase the accurate prediction of tubal disease in conjunction with HSG. However, this test has been shown to have limited clinical value (3). Negative serology and a normal HSG indicate a low probability of tubal disease on laparoscopy examination (<5%) (62). On the other hand, patients with positive serology have a higher risk for tubal pathology (63).

Hysterosalpingo-contrast sonography

HyCoSy shows intratubal flow of contrast media. The presence of fluid in the cul-de-sac after uterine instillation implies patency of at least one tube (64). Pain induced by HyCoSy and its complications are comparable to HSG (65). Although HyCoSy might have been considered inferior to HSG for evaluating tubal patency (61), it has been shown to be as reliable as HSG in low-risk patients (66,67).

Laparoscopy

Laparoscopy with chromopertubation has long been considered as the "gold standard" for evaluating tubal patency. Its advantages include the feasibility to diagnose and treat conditions that decrease fertility, including endometriosis or periadnexal adhesions. However, it is an invasive procedure that requires general anesthesia. The risk of major complications is low (<1%) (68). Laparoscopy is indicated when there is evidence or strong suspicion of endometriosis, pelvic/adnexal adhesions, or significant tubal disease requiring treatment. In the era of ART, today laparoscopy is rarely performed in the workup of infertility.

HSG and HyCoSy are the first-line tests to evaluate the fallopian tubes in infertile women (4). These procedures are generally well tolerated, inexpensive, and capable of demonstrating tubal patency at rates as high as 80% (66). The choice between these two techniques depends on availability, operator experience, and whether the patient is allergic to contrast media or iodine. Laparoscopy for diagnostic purposes is rarely needed.

MALE INVESTIGATION

Basic male investigation begins with a detailed history and physical examination. Semen analysis and a serum hormonal profile represent the first-line laboratory investigations. The goal of these investigations is to identify the underlying causes of male factor infertility that can be corrected to enhance the fertility status. More importantly, a thorough male fertility evaluation may reveal serious associated conditions including testis cancer, osteoporosis/osteopenia, and genetic and hormone disorders that can have significant health consequences or even be life threatening.

History

A general history should include the developmental history such as congenital malformation of the genitalia, cryptorchidism, and delayed onset of puberty. Previous history of herniorrhaphy, particularly in childhood, may result in inadvertent damage to the vas deferens that has not been recognized. A history of mumps orchitis (particularly in adolescence), sexually transmitted infections, genitourinary surgeries, instrumentation, or trauma should be obtained. Symptoms of the lower urinary tract and erectile and ejaculatory functions should also be carefully reviewed.

A systematic review of related organ system function such as pulmonary disease and upper respiratory infections may suggest genetic conditions such as Young's syndrome, Kartagener's syndrome (immotile cilia syndrome; primary ciliary dyskinesia), or cystic fibrosis (CF). A history of a metabolic or neurological condition may be related to impaired erectile and ejaculatory function. History of gonadotoxic treatment should also be recorded. Use of medication, alcohol, drugs, and occupational and environmental exposure to toxins such as heat and chemicals that can act as endocrine disruptors are elements to be recorded as well.

Physical examination

A thorough physical examination should focus on general signs, such as secondary sex characteristics that reflect normal androgenization (hair distribution, absence of gynecomastia, and skeletal muscle development), and on the genitalia.

Genital examination includes localization of the penile urethral meatus and palpation of the testes for their presence, size, and consistency. Testicular cancer risk is increased significantly among men with infertility and is the most common type of cancer for young reproductive males. Proper testicular examination may facilitate diagnosis (69). Testicular size can be assessed by using testis-shaped models of defined sizes (Prader orchidometer) and may be indicative of spermatogenesis. The normal range is 12–30 mL (70). Small testes are related to testicular dysfunction or hypogonadism.

Size, texture, position, and orientation of the epididymis and the bilateral presence of the vasa should also be carefully examined. Congenital bilateral absence of vas deferens (CBAVD) suggests the presence of mutation of the CF transmembrane conductance regular gene (*CFTR*). Cysts or nodularity of the epididymis suggest congenital or inflammatory changes that can lead to obstruction.

Examination of the spermatic cord in the upright position is important to evaluate the presence of varicoceles. Varicocele is classified into three grades: (I) palpable only with Valsalva maneuver; (II) palpable even without Valsalva maneuver; and (III) detectable by visual inspection. Digital rectal examination can detect cysts in the seminal vesicles and prostatic adenoma and neoplasia.

Laboratory investigations

Semen analysis

The first-line laboratory investigation for male infertility includes semen analysis performed according to the WHO criteria (71). Bacteriological semen analysis is usually done at the same time in order to explain potential semen anomalies, to screen for *C. trachomatis*, and to identify and treat an infection that could impair ART.

There are inter-laboratory and intra-individual variations in semen analysis (72). Moreover, a single abnormal test is highly sensitive for semen abnormalities, but may falsely label semen parameters as "abnormal" in up to 10% of males (73). Thus, abnormal semen analysis results should be repeated at least one month later to confirm the diagnosis (74).

Semen samples should be ideally collected by masturbation after two to five days of abstinence (5). In exceptional circumstances, semen may be produced at home or during sexual intercourse using a special condom. How the sample was produced, difficulties in semen production, and any partial loss of the sample should be reported.

Aspermia is the absence of semen and can be related to retrograde ejaculation or anejaculation due to psychological or neurological causes. In the case of retrograde ejaculation, a post-orgasm urine analysis may be performed, with specific preparation (such as alkalinization of urine) to evaluate sperm quality.

Semen analysis assesses parameters including volume, pH, sperm concentration, vitality, motility, and morphology. The main reference values of semen analysis according to the WHO are summarized in Table 37.1.

The bulk of the semen volume is made up of secretions from the male accessory gland in the reproductive tract, mainly seminal vesicles and prostate. Low semen volume may be associated with the absence or blockade of the seminal vesicles or the ejaculatory duct in the prostate. In men with CBAVD, low semen volume is often seen due to the poor development of the seminal vesicles. Low semen volume can also be the result of a collection problem, androgen deficiency, obstruction to the ejaculatory duct, or partial retrograde ejaculation. High semen volume may reflect exudation in cases of active inflammation of the accessory organs.

Table 37.1 Reference values of semen analysis according to the World Health Organization

Criteria	Reference value
Volume	≥1.5 mL
pH	≥7.2
Total sperm number	≥39 million/ejaculate
Sperm concentration	≥15 million/mL
Total motility	≥40%
Progressive motility	≥32%
Normal morphology	≥4%
Vitality	≥58%

The pH of semen reflects the balance of pH from various accessory gland secretions, with the seminal vesicle secretions being alkaline and prostatic secretions being acidic. A pH of less than 7 in a sample with low volume and azoospermia strongly suggests ejaculatory duct obstruction or CBAVD.

In the absence of obstruction in the excurrent ductal system, the spermatozoa concentration in semen and the extrapolated total number of spermatozoa per ejaculate can both reflect testicular capacity in sperm production. The total number of spermatozoa per ejaculate may be affected by the completeness of semen collection or accidental spillage of the sample, while the concentration of spermatozoa in semen is influenced by the volume of the secretions from accessory glands. The total number of spermatozoa per ejaculate and the sperm concentration have been shown to correlate to both time to pregnancy (TTP) and pregnancy rate (75).

Although there is no agreed definition of severe oligozoospermia, the limit of 5 million/mL is generally accepted. Azoospermia is defined by the absence of spermatozoa identified in the sample and cryptozoospermia by the identification of spermatozoa only in the sediment of the semen post-centrifugation. Azoospermia is classified based on its etiology as obstructive azoospermia (OA) or non-obstructive azoospermia (NOA). Biochemical assays of semen may reflect function or blockade of the accessory sex organs and excurrent ductal system.

There are specific markers for each accessory gland that are beyond the scope of this chapter but are not used widely. For example, seminal level of fructose, which is produced by seminal vesicles, will be low in cases of ejaculatory duct obstruction and seminal vesicle agenesis or dysfunction.

Sperm motility is graded as progressive motility (PR; spermatozoa moving actively regardless of the speed), non-progressive motility (NP; motility with an absence of progression), and immotile (71). Previous categorization of sperm PR as rapid or slow is no longer used because of the difficulties in objectively defining the speed of forward progression (71). However, when discussing sperm motility, it is important to specify total motility (PR + NP) or

PR. While we have the ability to overcome abnormally motile sperm with ARTs, the importance of progressive sperm motility in fertilizing oocytes *in vivo* has long been established (76).

Sperm vitality is an important variable, especially for samples with less than 40% progressively motile spermatozoa. The percentage of dead spermatozoa cannot exceed the percentage of immotile spermatozoa. Sperm viability is assessed using a dye test or a hypo-osmotic swelling (HOS) test (71). Viable non-motile sperm identified using a HOS test can be used for intracytoplasmic sperm injection (ICSI).

Morphological anomalies in spermatozoa could be identified in the head, neck, mid-piece, and tail. Morphological anomalies are commonly found in more than one part of a spermatozoon. Defective spermatogenesis and some epididymal pathologies may contribute to an increased percentage of abnormal morphology of spermatozoa. Spermatozoa with abnormal morphology generally have a lower fertilizing potential, depending on the types of anomalies, and may also have abnormal DNA. Unfortunately, assessment of sperm morphology is associated with a number of technical difficulties related to variations in interpretation or poor performance in external quality control assessments.

Identification of non-sperm cells, such as epithelial cells or rounds cells (germ cells or leukocytes), may be indicative of a pathology of the efferent ducts (ciliary tufts), testicular damage (immature germ cells), or inflammation of the accessory glands (leukocytes). If the estimate of the round cell concentration exceeds 10^6 per mL, their nature should be assessed (71). Special staining assessing their peroxidase activities could indicate that the round cells are leukocytes. Excessive numbers of leukocytes in the ejaculate may be associated with inflammation or infection. Leukocytes can impair sperm motility and DNA integrity through oxidative stress.

The WHO semen reference values were revised in 2010 (71). It is interesting to understand how these criteria were chosen. To begin with, the study population from whom the reference values are derived may not necessarily be an ideal representative sample of the whole population because of selection bias due to the embarrassing nature of participating in reproductive studies (77,78). The subset of fertile men with TTP values of less than 12 months was selected to provide reference values for human semen, since infertility is currently defined as a failure to conceive after at least 12 months of unprotected intercourse.

Data from 1953 semen samples from five studies done in eight countries in three continents in accordance to the WHO criteria were combined and analyzed (79). Since there is no reason to believe that high sperm numbers or percentages of progressively motile or morphologically normal spermatozoa are harmful to fertility (80) and that "too high" values appear to be clinically irrelevant, one-sided lower reference limits seem appropriate for the various semen parameters. Many lower reference limits for semen variables have been proposed, but it is widely accepted that 95% of the data should be included in the reference interval when establishing the reference limits. Hence, a one-sided distribution at a reference limit of fifth percentiles (with 95% confidence intervals) was chosen for all semen parameters.

The interpretation of semen parameters should also be done with caution. First, all males from the reference population—even those under the fifth percentiles—were fertile with TTP values of <12 months. Thus, not all men with semen parameters below the reference values can be labeled with certainty as being "infertile." Second, the measurements made on the whole population of ejaculated spermatozoa cannot define the fertilizing capacity of the few that reach the site of fertilization. Thus, one should not use semen parameters to "predict" success of fertility even in the setting of assisted reproduction. Nevertheless, semen analysis provides essential information that can guide clinicians to additional investigations and management aiming at improving the fertility status of couples experiencing difficulty to conceive.

Antisperm autoantibodies

Antisperm autoantibodies (ASAs) can be suspected when urological history is suggestive (hernia or testicular surgery, testicular trauma, torsion, orchitis, or vasectomy), with isolated asthenospermia on initial semen analysis, or when agglutination of sperm (motile spermatozoa sticking to each other) is noted in semen analysis. ASAs can be found in the serum, in the seminal plasma, or bound directly to sperm. These antibodies may form following a breach of the testicular barrier, leading to contact between the immune system and testicular cells. ASAs are more frequent among infertile males and may decrease the likelihood for conception by impairing sperm penetration of cervical mucus, zona pellucida interaction, and oocyte fusion (81). Autoimmune infertility is a controversial issue and the standard for clinical interpretation of the presence of ASAs is not established (81,82). As the clinical utility of ASAs is uncertain, ASA tests should not be part of the routine male fertility evaluation (4,5,29).

Sperm DNA fragmentation

Sperm chromatin quality or integrity, often loosely referred to as "DNA integrity," can be modified during spermatogenesis and transport through the reproductive tract (83). Although high levels of damage in DNA integrity often correlate with poor semen parameters and infertile men, DNA damage is also found in men with normal semen parameters (84). Damage in sperm DNA integrity can occur due to gonadotoxin, heat exposure, radiation, and varicoceles. A number of laboratory evaluations commonly used in basic science research have been gaining popularity in clinical research and practice for evaluating sperm DNA integrity. These include: (1) sperm chromatin structure assay (SCSA), which defines abnormal chromatin structure as increased susceptibility of sperm DNA to acid-induced denaturation *in situ*; (2) terminal deoxynucleotide transferase-mediated dUTP

nick-end labeling (TUNEL) assay; and (3) single-cell gel electrophoresis assay (Comet). Some investigators have suggested threshold values used to define and abnormal test for SCSA (25%–27%) and TUNEL assay (>36%) (5). Low DNA fragmentation is significantly associated with increased likelihood of pregnancy *in vivo* and after intrauterine insemination (85). Damage to sperm DNA integrity may contribute to poor reproductive performance in some couples and risk of spontaneous recurrent miscarriage. But this association is not strong enough to correctly predict outcomes of assisted reproduction, including IVF or ICSI (86), and so to provide a clinical indication for the routine use of this test (5,87).

Ultrasound

US can be done scrotally to evaluate scrotal and inguinal pathologies (e.g., varicoceles and testicular mass) or transrectally to assess the prostate, ejaculatory ducts, and seminal vesicles (for cystic lesions or obstruction). US is not done routinely in male fertility evaluation. Its goal is to confirm a pathology that was suspected during physical examination or was suggested based on semen and hormonal analysis.

Endocrine tests

If the semen analysis indicates a low number or concentration of sperm, or in cases of male sexual dysfunction, further endocrine tests should be requested. Serum FSH and total testosterone measurements should be performed in all cases of oligozoospermia. This will help distinguish between pituitary–hypothalamic axis dysfunction, testicular dysfunction, and reproductive tract obstruction. Additional hormonal evaluation such as LH, prolactin, and TSH should be requested if the clinical findings suggest a specific pathology (5).

Low levels of FSH, LH, and testosterone in the context of low sperm concentrations suggest hypogonadotropic hypogonadism. Though it is not a common cause of male infertility, this endocrinopathy may be a result of Kallmann's syndrome or acquired causes as hyperprolactinemia and hemochromatosis. If testicular failure mainly impairs the spermatogenesis and not endocrine function, testosterone, FSH, and LH levels may be within normal limits. In the case of complete testicular failure, FSH and LH will be elevated whereas testosterone will be normal or low.

Genetic testing

NOA and severe oligozoospermia

Males having abnormal spermatogenesis related to testicular failure, such as in NOA or severe oligozoospermia (<5 million/mL), are at increased risk for having genetic abnormalities compared to fertile men (5,88). Genetic testing including karyotype analysis and Y-chromosome microdeletion is recommended in these circumstances before performing ICSI (5). A karyotype analysis can diagnose numeric chromosomal abnormalities (e.g., Klinefelter's syndrome [KS]) or other chromosomal structure abnormalities (e.g., Robertsonian or reciprocal translocations). KS is the most common chromosomal abnormality: Non-mosaic KS accounts for 11% of azoospermia cases and mosaic KS accounts for 0.5% of severe oligozoospermia cases (89). If the karyotype is abnormal, there is an increased risk of sperm chromosomal aneuploidy, and genetic counseling including preimplantation genetic diagnosis should be discussed with the couples prior to assisted reproduction.

The short arm (Yp) of the Y chromosome contains sex determination genes (*SRY*), and the long arm (Yq) contains genes that are important for spermatogenesis. Y chromosome microdeletions in three specific regions of the Yq, termed AZFa, AZFb, and AZFc (AZF – "azoospermic factor") can severely impair spermatogenesis. AZFa is located on proximal Yq11 (Yq11.21), while AZFb and AZFc are located on distal Yq11 (q11.23) (90). AZF microdeletions in men with oligozoospermia have been approximated at 4% and may reach as high as 18% in azoospermic patients (88). The vast majority of the AZF mutations arise *de novo*. AZFc is the most common deletion, seen in 60% of all Y chromosome microdeletions. About a third of men with a microdeletion in only the AZFc region may have severe oligozoospermia, while the majority are azoospermic. Testicular sperm extraction in more than half of azoospermic men with AZFc deletions may have sperm recovered for ICSI (91).

If sperm is extracted and ICSI performed, vertical transmission of the mutation and inherited infertility in male offspring are inevitable. Thus, genetic counseling is important for these men. Deletions involving AZFa or the entire region of AZFb are generally azoospermic (91). The histological findings in AZFa-deletion men are usually Sertoli cell-only syndrome, while those with AZFb deletions tend to have germ cells that arrest at the primary spermatocyte stage. The prognosis for sperm recovery by testicular sperm extraction is extremely poor and other options such as the use of donor sperm or adoption, if appropriate, should be discussed.

Obstructive azoospermia

CBAVD is a common cause of primary OA in healthy men with no prior history of genitourinary disorders. There is a strong association between CBAVD and mutations of the *CFTR* gene. CF is a serious autosomal recessive condition. Almost all men with CF exhibit CBAVD. Of men with CBAVD, more than 50% are heterozygous for the *CFTR* gene mutation or carry compound heterozygous mutations including milder coding mutations for the *CFTR* gene (92). The *CFTR* mutation is also linked to congenital unilateral agenesis of the vas deferens (CUAVD) and with congenital epididymal obstruction (93).

In case of agenesis of the vas deferens related to *CFTR* mutation, a history of non-severe pulmonary diseases or asthma may or may not be present. The cumulative carrier frequency varies according to ethnicity. A carrier frequency as high as 1 in 25 is seen in men who are

Northern European descendants or Ashkenazi Jewish. CBAVD can be viewed as the mildest phenotype within the *CFTR* gene mutation spectrum. Concerning the genitalia, fibrous cord-like vas may be palpable, only the seminal vesicles and proximal vas may be missing, or asymmetry may be apparent (85). *CFTR* mutations should be tested in all OA patients. More than 1800 mutations have been detected, but only a few dozen prevalent CF-associated mutations are routinely screened. This means that a negative result does not exclude an unknown mutation.

CFTR screening of the female partner is also essential, but even then a negative result leaves a small residual risk of a CF-affected offspring. Where *CFTR* mutations are found in both partners, preimplantation genetic diagnosis may be proposed to the couple to prevent the birth of a child with CF. Importantly, the etiology of some conditions of CBAVD and CUAVD may also not be related to a *CFTR* mutation, especially when CBAVD is associated with urinary tract malformations (up to 20% of cases) (93). Patients with unilateral renal agenesis and CBAVD/CUAVD may have a non-*CFTR* mutation-mediated gene that leads to abnormal development of the entire mesonephric duct at a very early stage in embryo development (<7 weeks) (94). Since this condition could have an autosomal dominant form of inheritance with incomplete penetrance and variable expression, genetic counseling is recommended in this circumstance as well (94).

In summary, men with NOA or severe oligozoospermia should be offered karyotype evaluation and Y chromosome analysis. CBAVD/CUAVD further warrants *CFTR* mutation screening and genetic counseling.

CONCLUSION

Infertility is a difficult situation for a couple. Basic investigations beginning with a detailed history and physical examination are the first step of infertility management. Each member of the couple should undergo basic infertility investigations including evaluation of the uterine cavity, the fallopian tubes, ovarian function and reserve, and semen analysis. These investigations could create anxiety. Our goal as physicians is to provide education, counseling, and assistance, including emotional support, during the initial investigations and later during the treatment.

REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. *Fertil Steril* 2008; 90(5 Suppl): S60.
2. Thonneau P, Marchand S, Tallec A et al. Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988–1989). *Hum Reprod* 1991; 6(6): 811–6.
3. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: A committee opinion. *Fertil Steril* 2015; 103(6): e44–50.
4. NICE: National Institute for Health and Clinical Excellence. Fertility assessment and treatment for people with fertility problems. *NICE Clinical Guideline* 2013; 156. www.nice.org.uk/guidance/cg156.
5. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: A committee opinion. *Fertil Steril* 2015; 103(3): e18–25.
6. Hull MG, Glazener CM, Kelly NJ et al. Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)* 1985; 291(6510): 1693–7.
7. Chan P, Fisch P, Hemmings R, St-Michel P, Gref P. (eds.). *Les activités de procréation médicalement assistée démarche clinique et thérapeutique*, Quebec College of Medicine, Quebec 2015.
8. Souter VL, Penney G, Hopton JL, Templeton AA. Patient satisfaction with the management of infertility. *Hum Reprod* 1998; 13(7): 1831–6.
9. Tulandi T, Platt R. The art of taking a history. *Fertil Steril* 2004; 81(1): 11–2.
10. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: A systematic review and meta-analysis. *Hum Reprod* 1998; 13(6): 1532–9.
11. Waylen AL, Metwally M, Jones GL, Wilkinson AJ, Ledger WL. Effects of cigarette smoking upon clinical outcomes of assisted reproduction: A meta-analysis. *Hum Reprod Update* 2009; 15(1): 31–44.
12. Munster K, Schmidt L, Helm P. Length and variation in the menstrual cycle—A cross-sectional study from a Danish county. *Br J Obstet Gynaecol* 1992; 99(5): 422–9.
13. Collins JA. Diagnostic assessment of the infertile female partner. *Curr Probl Obstet Gynecol Fertil* 1988; 11: 6–42.
14. Creinin MD, Keverline S, Meyn LA. How regular is regular? An analysis of menstrual cycle regularity. *Contraception* 2004; 70(4): 289–92.
15. Luciano AA, Peluso J, Koch EI, Maier D, Kuslis S, Davison E. Temporal relationship and reliability of the clinical, hormonal, and ultrasonographic indices of ovulation in infertile women. *Obstet Gynecol* 1990; 75(3 Pt 1): 412–6.
16. Guermandi E, Vegetti W, Bianchi MM, Uglietti A, Ragni G, Crosignani P. Reliability of ovulation tests in infertile women. *Obstet Gynecol* 2001; 97(1): 92–6.
17. McGovern PG, Myers ER, Silva S et al. Absence of secretory endometrium after false-positive home urine luteinizing hormone testing. *Fertil Steril* 2004; 82(5): 1273–7.
18. Coutifaris C, Myers ER, Guzick DS et al. Histological dating of timed endometrial biopsy tissue is not related to fertility status. *Fertil Steril* 2004; 82(5): 1264–72.
19. Murray MJ, Meyer WR, Zaino RJ et al. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. *Fertil Steril* 2004; 81(5): 1333–43.

20. Haney AF. Endometrial biopsy: A test whose time has come and gone. *Fertil Steril* 2004; 82(5): 1295–6; discussion 301–2.
21. Kazer RR. Endometrial biopsy should be abandoned as a routine component of the infertility evaluation. *Fertil Steril* 2004; 82(5): 1297–8; discussion 300–2.
22. Wathen NC, Perry L, Lilford RJ, Chard T. Interpretation of single progesterone measurement in diagnosis of anovulation and defective luteal phase: Observations on analysis of the normal range. *Br Med J (Clin Res Ed)* 1984; 288(6410): 7–9.
23. Jordan J, Craig K, Clifton DK, Soules MR. Luteal phase defect: The sensitivity and specificity of diagnostic methods in common clinical use. *Fertil Steril* 1994; 62(1): 54–62.
24. Lincoln SR, Ke RW, Kutteh WH. Screening for hypothyroidism in infertile women. *J Reprod Med* 1999; 44(5): 455–7.
25. Velkeniers B, Van Meerhaeghe A, Poppe K, Unuane D, Tournaye H, Haentjens P. Levothyroxine treatment and pregnancy outcome in women with subclinical hypothyroidism undergoing assisted reproduction technologies: Systematic review and meta-analysis of RCTs. *Hum Reprod Update* 2013; 19(3): 251–8.
26. Spencer L, Bubner T, Bain E, Middleton P. Screening and subsequent management for thyroid dysfunction pre-pregnancy and during pregnancy for improving maternal and infant health. *Cochrane Database Syst Rev* 2015; 9: CD011263.
27. Dewailly D, Andersen CY, Balen A et al. The physiology and clinical utility of anti-Mullerian hormone in women. *Hum Reprod Update* 2014; 20(3): 370–85.
28. Broer SL, van Disseldorp J, Broeze KA et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: An individual patient data approach. *Hum Reprod Update* 2013; 19(1): 26–36.
29. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: A committee opinion. *Fertil Steril* 2015; 103(3): e9–17.
30. Sills ES, Alper MM, Walsh AP. Ovarian reserve screening in infertility: Practical applications and theoretical directions for research. *Eur J Obstet Gynecol Reprod Biol* 2009; 146(1): 30–6.
31. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006; 12(6): 685–718.
32. Roberts JE, Spandorfer S, Fasouliotis SJ, Kashyap S, Rosenwaks Z. Taking a basal follicle-stimulating hormone history is essential before initiating *in vitro* fertilization. *Fertil Steril* 2005; 83(1): 37–41.
33. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: Practical recommendations for better standardization. *Fertil Steril* 2010; 94(3): 1044–51.
34. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; 19(1): 41–7.
35. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: International consensus definitions. *Hum Reprod Update* 2003; 9(6): 505–14.
36. Weenen C, Laven JS, Von Bergh AR et al. Anti-Mullerian hormone expression pattern in the human ovary: Potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod* 2004; 10(2): 77–83.
37. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y. Stable serum levels of anti-Mullerian hormone during the menstrual cycle: A prospective study in normo-ovulatory women. *Hum Reprod* 2007; 22(7): 1837–40.
38. van Disseldorp J, Lambalk CB, Kwee J et al. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. *Hum Reprod* 2010; 25(1): 221–7.
39. Almog B, Shehata F, Suissa S et al. Age-related normograms of serum antimullerian hormone levels in a population of infertile women: A multicenter study. *Fertil Steril* 2011; 95(7): 2359–63, 63.e1.
40. Nelson SM, Messow MC, Wallace AM, Fleming R, McConnachie A. Nomogram for the decline in serum antimullerian hormone: A population study of 9,601 infertility patients. *Fertil Steril* 2011; 95(2): 736–41.e1–3.
41. Dewailly D, Gronier H, Poncelet E et al. Diagnosis of polycystic ovary syndrome (PCOS): Revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. *Hum Reprod* 2011; 26(11): 3123–9.
42. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 2003; 18(2): 323–7.
43. Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. *Hum Reprod Update* 2011; 17(1): 46–54.
44. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of "poor response" to ovarian stimulation for *in vitro* fertilization: The Bologna criteria. *Hum Reprod* 2011; 26(7): 1616–24.
45. Jette NT, Glass RH. Prognostic value of the postcoital test. *Fertil Steril* 1972; 23(1): 29–32.
46. Oei SG, Helmerhorst FM, Bloemenkamp KW, Hollants FA, Meerpoel DE, Keirse MJ. Effectiveness of the postcoital test: Randomised controlled trial. *BMJ* 1998; 317(7157): 502–5.

47. Bonnamy L, Marret H, Perrotin F, Body G, Berger C, Lansac J. Sonohysterography: A prospective survey of results and complications in 81 patients. *Eur J Obstet Gynecol Reprod Biol* 2002; 102(1): 42–7.
48. Grimbizis GF, Tsolakidis D, Mikos T et al. A prospective comparison of transvaginal ultrasound, saline infusion sonohysterography, and diagnostic hysteroscopy in the evaluation of endometrial pathology. *Fertil Steril* 2010; 94(7): 2720–5.
49. Roma Dalfo A, Ubeda B, Ubeda A et al. Diagnostic value of hysterosalpingography in the detection of intrauterine abnormalities: A comparison with hysteroscopy. *AJR Am J Roentgenol* 2004; 183(5): 1405–9.
50. Gaglione R, Valentini AL, Pistilli E, Nuzzi NP. A comparison of hysteroscopy and hysterosalpingography. *Int J Gynaecol Obstet* 1996; 52(2): 151–3.
51. Bingol B, Gunenc Z, Gedikbasi A, Guner H, Tasdemir S, Tiras B. Comparison of diagnostic accuracy of saline infusion sonohysterography, transvaginal sonography and hysteroscopy. *J Obstet Gynaecol* 2011; 31(1): 54–8.
52. Soares SR, Barbosa dos Reis MM, Camargos AF. Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine cavity diseases. *Fertil Steril* 2000; 73(2): 406–11.
53. Makris N, Kalmantis K, Skartados N, Papadimitriou A, Mantzaris G, Antsaklis A. Three-dimensional hysterosonography versus hysteroscopy for the detection of intracavitary uterine abnormalities. *Int J Gynaecol Obstet* 2007; 97(1): 6–9.
54. Abou-Salem N, Elmazny A, El-Sherbiny W. Value of 3-dimensional sonohysterography for detection of intrauterine lesions in women with abnormal uterine bleeding. *J Minim Invasive Gynecol* 2010; 17(2): 200–4.
55. Crosignani PG, Rubin BL. Optimal use of infertility diagnostic tests and treatments. The ESHRE Capri Workshop Group. *Hum Reprod* 2000; 15(3): 723–32.
56. Bettocchi S, Nappi L, Ceci O, Selvaggi L. Office hysteroscopy. *Obstet Gynecol Clin North Am* 2004; 31(3): 641–54, xi.
57. Pundir J, El Toukhy T. Uterine cavity assessment prior to IVF. *Womens Health (London, England)* 2010; 6(6): 841–7; quiz 7–8.
58. Caliskan E, Ozkan S, Cakiroglu Y, Sarisoy HT, Corakci A, Ozeren S. Diagnostic accuracy of real-time 3D sonography in the diagnosis of congenital Mullerian anomalies in high-risk patients with respect to the phase of the menstrual cycle. *J Clin Ultrasound* 2010; 38(3): 123–7.
59. Stumpf PG, March CM. Febrile morbidity following hysterosalpingography: Identification of risk factors and recommendations for prophylaxis. *Fertil Steril* 1980; 33(5): 487–92.
60. Swart P, Mol BW, van der Veen F, van Beurden M, Redekop WK, Bossuyt PM. The accuracy of hysterosalpingography in the diagnosis of tubal pathology: A meta-analysis. *Fertil Steril* 1995; 64(3): 486–91.
61. Darwish AM, Youssef AA. Screening sonohysterography in infertility. *Gynecol Obstet Invest* 1999; 48(1): 43–7.
62. Meikle SF, Zhang X, Marine WM, Calonge BN, Hamman RF, Betz G. *Chlamydia trachomatis* antibody titers and hysterosalpingography in predicting tubal disease in infertility patients. *Fertil Steril* 1994; 62(2): 305–12.
63. den Hartog JE, Lardenoije CM, Severens JL, Land JA, Evers JL, Kessels AG. Screening strategies for tubal factor subfertility. *Hum Reprod* 2008; 23(8): 1840–8.
64. Habibaj J, Kosova H, Bilali S, Bilali V, Qama D. Comparison between transvaginal sonography after diagnostic hysteroscopy and laparoscopic chromopertubation for the assessment of tubal patency in infertile women. *J Clin Ultrasound* 2012; 40(2): 68–73.
65. Ayida G, Kennedy S, Barlow D, Chamberlain P. A comparison of patient tolerance of hysterosalpingocontrast sonography (HyCoSy) with Echovist-200 and x-ray hysterosalpingography for outpatient investigation of infertile women. *Ultrasound Obstet Gynecol* 1996; 7(3): 201–4.
66. Saunders RD, Shwayder JM, Nakajima ST. Current methods of tubal patency assessment. *Fertil Steril* 2011; 95(7): 2171–9.
67. Maheux-Lacroix S, Boutin A, Moore L et al. Hysterosalpingosonography for diagnosing tubal occlusion in subfertile women: A systematic review with meta-analysis. *Hum Reprod* 2014; 29(5): 953–63.
68. Chapron C, Querleu D, Bruhat MA et al. Surgical complications of diagnostic and operative gynaecological laparoscopy: A series of 29,966 cases. *Hum Reprod* 1998; 13(4): 867–72.
69. Garner MJ, Turner MC, Ghadirian P, Krewski D. Epidemiology of testicular cancer: An overview. *Int J Cancer* 2005; 116(3): 331–9.
70. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. *Lancet* 2004; 364(9430): 273–83.
71. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th Edition. Cambridge, U.K.: Cambridge University Press, 2010.
72. Alvarez C, Castilla JA, Martinez L, Ramirez JP, Vergara F, Gaforio JJ. Biological variation of seminal parameters in healthy subjects. *Hum Reprod* 2003; 18(10): 2082–8.
73. Opsahl MS, Dixon NG, Robins ER, Cunningham DS. Single vs. multiple semen specimens in screening for male infertility factors. A comparison. *J Reprod Med* 1996; 41(5): 313–5.
74. Male Infertility Best Practice Policy Committee of the American Urological Association, Practice Committee of the American Society for Reproductive Medicine. Report on optimal evaluation of the infertile male. *Fertil Steril* 2006; 86(5 Suppl 1): S202–9.
75. Slama R, Eustache F, Ducot B et al. Time to pregnancy and semen parameters: A cross-sectional study among fertile couples from four European cities. *Hum Reprod* 2002; 17(2): 503–15.

76. Pusch HH. The importance of sperm motility for the fertilization of human oocytes *in vivo* and *in vitro*. *Andrologia* 1987; 19(5): 514–27.
77. Tielemans E, Burdorf A, te Velde E, Weber R, van Kooij R, Heederik D. Sources of bias in studies among infertility clients. *Am J Epidemiol* 2002; 156(1): 86–92.
78. Handelsman DJ. Sperm output of healthy men in Australia: Magnitude of bias due to self-selected volunteers. *Hum Reprod* 1997; 12(12): 2701–5.
79. Cooper TG, Noonan E, von Eckardstein S et al. World Health Organization reference values for human semen characteristics. *Hum Reprod Update* 2010; 16(3): 231–45.
80. Tournaye H, Staessen C, Camus M, Verheyen G, Devroey P, Van Steirteghem A. No evidence for a decreased fertilizing potential after *in-vitro* fertilization using spermatozoa from polyzoospermic men. *Hum Reprod* 1997; 12(10): 2183–5.
81. Francavilla F, Santucci R, Barbonetti A, Francavilla S. Naturally-occurring antisperm antibodies in men: Interference with fertility and clinical implications. An update. *Front Biosci* 2007; 12: 2890–911.
82. Zini A, Fahmy N, Belzile E, Ciampi A, Al-Hathal N, Kotb A. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: Systematic review and meta-analysis. *Hum Reprod* 2011; 26(6): 1288–95.
83. Sakkas D, Alvarez JG. Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril* 2010; 93(4): 1027–36.
84. Schulte RT, Ohl DA, Sigman M, Smith GD. Sperm DNA damage in male infertility: Etiologies, assays, and outcomes. *J Assist Reprod Genet* 2010; 27(1): 3–12.
85. Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. *Reprod Biomed Online* 2006; 12(4): 466–72.
86. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with *in vitro* fertilization? *Fertil Steril* 2008; 89(4): 823–31.
87. Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: A guideline. *Fertil Steril* 2013; 99(3): 673–7.
88. Foresta C, Garolla A, Bartoloni L, Bettella A, Ferlin A. Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. *J Clin Endocrinol Metab* 2005; 90(1): 152–6.
89. Van Assche E, Bonduelle M, Tournaye H et al. Cytogenetics of infertile men. *Hum Reprod* 1996; 11(Suppl 4): 1–24; discussion 5–6.
90. Vogt PH, Edelmann A, Kirsch S et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 1996; 5(7): 933–43.
91. Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. *Fertil Steril* 2010; 94(5): 1753–6.
92. Dork T, Dworniczak B, Aulehla-Scholz C et al. Distinct spectrum of *CFTR* gene mutations in congenital absence of vas deferens. *Hum Genet* 1997; 100(3–4): 365–77.
93. Lissens W, Mercier B, Tournaye H et al. Cystic fibrosis and infertility caused by congenital bilateral absence of the vas deferens and related clinical entities. *Hum Reprod* 1996; 11(Suppl 4): 55–78; discussion 79–80.
94. McCallum T, Milunsky J, Munarriz R, Carson R, Sadeghi-Nejad H, Oates R. Unilateral renal agenesis associated with congenital bilateral absence of the vas deferens: Phenotypic findings and genetic considerations. *Hum Reprod* 2001; 16(2): 282–8.

Prognostic testing for ovarian reserve

38

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FEMALE REPRODUCTIVE AGING

Age-related subfertility and ovarian reserve

With the postponement of childbearing in Western societies, rates of subfertility related to advanced female age have increased considerably (1). A higher proportion of couples therefore depends on assisted reproduction technologies (ARTs) to achieve a pregnancy. The increase of subfertility with advanced female age is mainly based on changes in ovarian function referred to as decreased or diminished ovarian reserve. Ovarian reserve can be defined as the quantity as well as the quality of the remaining oocytes in both ovaries at a given age. Declines in follicle numbers dictate the occurrence of irregular cycles and ultimately the cessation of menstrual bleeding (i.e., menopause), while oocyte quality decay results in decreasing fertility, defined as the capacity to conceive and give birth to a child (Figure 38.1) (2).

Variability of reproductive aging

There is substantial individual variation in the onset of menopause, varying roughly between 40 and 60 years, with a mean age of 51 years. This variation has shown to be rather constant over time and populations worldwide (3–5). Female fecundity is believed to decrease after the age of 31 years, a decrease that may accelerate after 37 years of age, leading to sterility at a mean age of 41 years of age (6). As is the case with menopause, the rate of decline in fertility may vary considerably between women of the same age. This implies that a woman at the age of 35 years either may be close to natural sterility or have a normal fertility comparable to a 25-year-old. The decrease of female fertility is believed to exhibit the same range of variation as for the occurrence of menopause (7). This implies that age at menopause, which is determined by the size of the remaining follicle pool, is considered a proxy variable for age at loss of natural fertility, with a fixed time period of 10 years in between. The correct prediction of menopause in an individual woman would therefore provide valuable information regarding a woman's fertile lifespan and hence aid in preventing future subfertility (Figure 38.2).

Still, the putative relationship between quantity of follicles and quality at the oocyte level may well be much more complicated. The variation in fecundity within female age groups is notable, while within quantity groups, defined according to markers such as the antral follicle count (AFC) or anti-Mullerian hormone (AMH) level, fertility is highly influenced by the age of the female. Unfortunately, studies that address the variation of female fertility depending on both age and quantitative ovarian reserve status are lacking, due to the fact that simple tests of qualitative ovarian reserve (i.e., embryo quality) are not present at the current time (8).

Natural and assisted fertility decline

The human species can be considered as relatively subfertile compared to other animals (9,10). The average monthly fecundity rate of approximately 20% implies that among human couples trying to conceive, many exposure months may be needed to achieve their goal, especially if monthly fecundity has dropped with increasing female age (11).

The proportion of infertile couples (by definition the failure to achieve a vital pregnancy within one year) will amount to 10%–20% in the age group of women over 35 years, compared to only 4% for women in their twenties. These infertility rates may rise to 30%–50% for only moderately fertile women of age 35 years and over, which may lead to trying to conceive for several years without any result (11,12). The maintenance of regular menstrual cycles until an age when natural fecundity has already been reduced to approximately zero means that women are largely unaware that this process is taking place.

The age-related decline in female fertility has also been shown in numerous reports concerning *in vitro* fertilization (IVF) programs. After a mean female age of approximately 34 years, the chance of producing a live birth in IVF programs decreases steadily and reduces to less than 10% per cycle in women over 40 years of age (Figure 38.3). The chance of a live birth after IVF depends on both the quantitative and qualitative ovarian reserve. A reduced quantitative ovarian reserve is expressed by a poor response to ovarian stimulation. The qualitative aspect is best expressed by female age. A young woman with a poor response to ovarian hyperstimulation may have a reduced quantitative ovarian reserve, but as the quality aspect of her ovarian reserve is still good, she will still have reasonable pregnancy prospects. By contrast, an older woman with a poor response has a reduced quantitative and qualitative ovarian reserve and therefore her prospects of becoming pregnant after ART use are very poor (8,13).

Ovarian reserve prediction

The knowledge and insights into the process of ovarian aging imply that for ovarian reserve testing prior to IVF, female age remains the predictor of first choice. The availability of a test to be capable of providing reliable information regarding a woman's individual ovarian reserve within a certain age category would enable the clinician to provide an individually tailored treatment plan. For instance, a reliable test would allow counselling of women with a low ovarian reserve regarding their

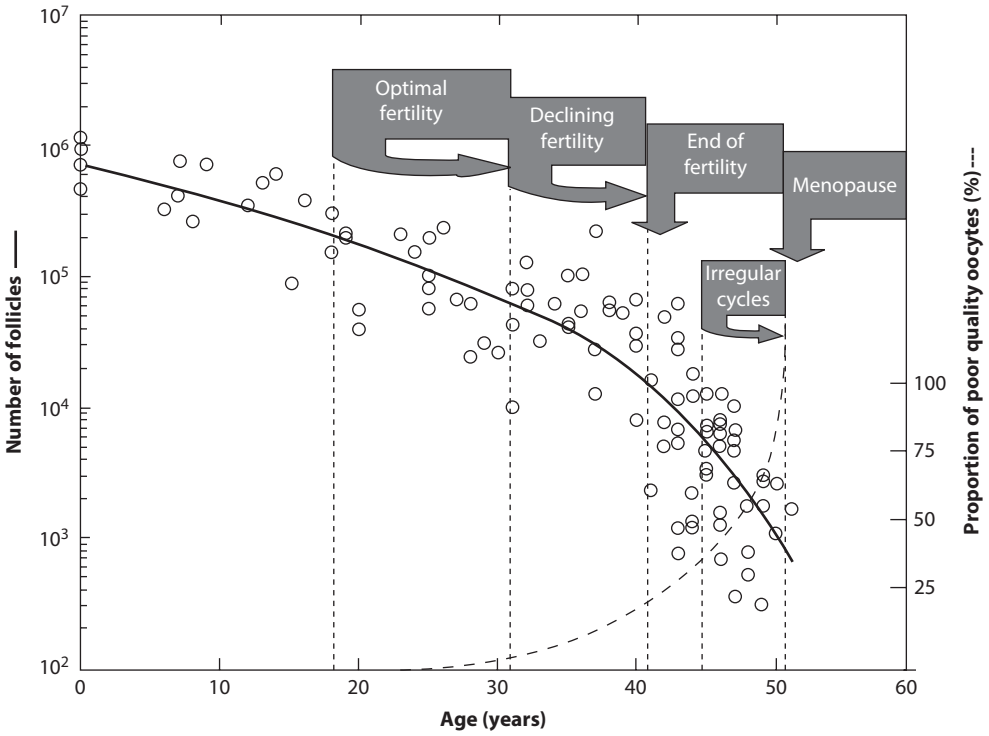

Figure 38.1 Quantitative (solid line) and qualitative (dotted line) declines of the ovarian follicle pool, which is assumed to dictate the onset of important reproductive events. (Adapted from de Bruin JP, te Velde ER. Female reproductive aging: Concepts and consequences. In: Tulandi T, Gosden RG, eds. *Preservation of Fertility*. London, UK: Taylor & Francis, 2004, p. 3.)

chances of conceiving or of preserving oocytes. In the case of older infertile women seeking treatment, the test could allow older women with a still sufficient quantitative ovarian reserve to start IVF treatment, while for such cases with an exhausted reserve, refusal of IVF could be proposed. Ultimately, the observed response to maximal ovarian stimulation may provide further information on the reserve capacity of the ovaries. In the following two sections, the biological rationale behind ovarian reserve testing and the accuracy and clinical value of several of these tests will be discussed.

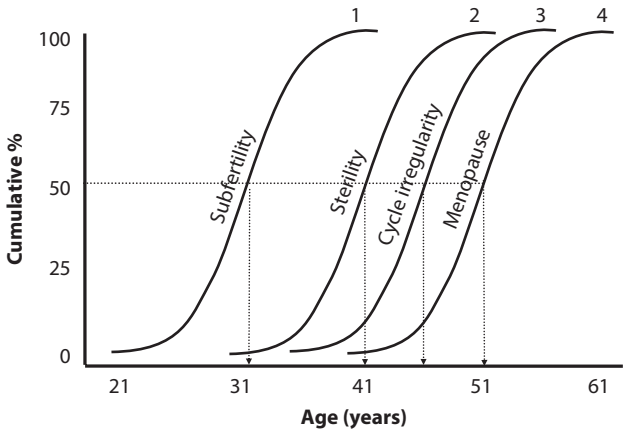

Figure 38.2 Variations in age at the occurrence of specific stages of ovarian aging. (For explanation of the background of data, see Te Velde ER, Pearson PL. *Hum Reprod Update* 2002; 8: 141–54, with permission.)

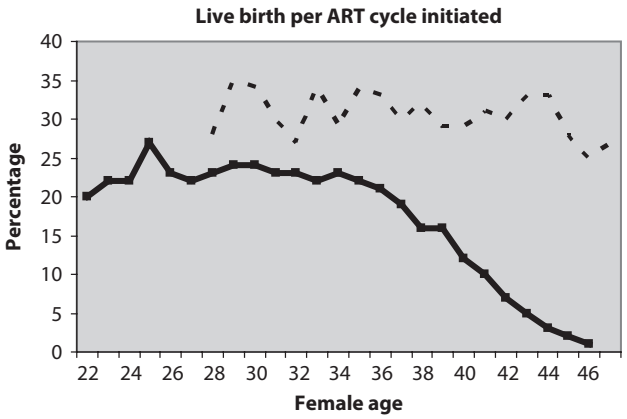

Figure 38.3 Effect upon average singleton live birth rates of female age, showing a steady decrease after the age of 34 years. The dotted line represents the average singleton live birth rate after oocyte donation as a function of the recipient age. It underlines the potential of oocyte donation in the treatment of women who remained unsuccessful in previous *in vitro* fertilization treatment. Data were drawn from the 2003 CDC ART report (http://www.cdc.gov/art/). *Abbreviation*: ART, assisted reproduction technology.

THE PHYSIOLOGICAL BACKGROUND TO OVARIAN RESERVE TESTING

Follicle quantity

In the scenario of IVF treatment, ovarian reserve can be considered normal in conditions where stimulation with the use of exogenous gonadotropins will result in the development of some 5–15 follicles and the retrieval of a corresponding number of healthy oocytes at follicle puncture (14,15). With such a yield, the chances of producing a live birth through IVF are considered optimal (16). In addition to the number of recruitable follicles, which determines the ovarian reserve status, follicle sensitivity to follicle-stimulating hormone (FSH) and the pharmacodynamics of FSH may also determine a woman's extent of ovarian response to stimulation, although thorough science in this field is scarce.

Female age

In general, as outlined before, age of the woman is a simple way of obtaining information on the extent of her ovarian reserve, regarding both quantity as well as quality (17). However, because of the substantial variation between women of the same age category, female age is not sufficient.

It would therefore be useful to identify young women with clearly accelerated ovarian aging or older women with still adequate ovarian reserve. If it would be possible to identify such women, fertility management could be effectively individualized. For instance, stimulation dose or treatment scheme could be adjusted (18), counselling against initiation of IVF treatment or pertinent refusal could be effected, or treatment could be initiated early before the reserve has diminished too far.

Tests and their valuation

Most tests examined in the literature are evaluated by their capacity to predict some defined outcome related to ovarian reserve. The preferred or gold standard outcome of prediction studies would be live birth after a series of ART exposure cycles, but other outcomes (especially oocyte yield or follicle number and pregnancy after one IVF/intracytoplasmic sperm injection [ICSI] cycle) are in fact the most common. As the occurrence of pregnancy in a single exposure to IVF and embryo transfer will be dependent on many other factors besides ovarian reserve, like laboratory performance and transfer technique, focus has been mostly upon the capacity of these tests to predict the ovarian response. Indeed, most if not all ovarian reserve tests (ORTs) relate to the size of the follicle cohort that is at any time responsive to FSH. The AFC assessed by transvaginal ultrasonography provides direct visual assessment of the cohort (19). The endocrine marker AMH, which is produced by the granulosa cells surrounding the antral follicles, provides a direct marker of quantity (20,21).

Baseline FSH, which has been extensively studied in the past decades, provides the most indirect marker. FSH levels will become increased with advancing age due to a reduction in the release of inhibin B and estradiol, thereby reducing the negative feedback on FSH release from the pituitary (22). High FSH levels therefore represent small cohort sizes. Endocrine challenge tests in which the growth of antral follicles is stimulated by endogenous or exogenous FSH and response is assessed in terms of output of estradiol or inhibin B are also principally related to cohort size (20). However, they are considered as too laborious for screening purposes and do not add much predictive value compared to static tests like AMH or the AFC (23,24).

The same may be true for the clomiphene citrate (CC) challenge test, in which a CC-induced rise in FSH levels is counteracted by the release of estradiol and inhibin B from growing antral follicles. The size of the antral follicle cohort will determine the degree of subsequent FSH suppression. Like the other challenge tests, the CC challenge test does not provide much additional information compared to basal FSH (Figure 38.4) (25,26).

THE CLINICAL VALUE OF OVARIAN RESERVE TESTING

ART treatment outcome prediction

Poor-response prediction

A poor response to stimulation, defined as a low number of mature follicles developed or oocytes obtained after ovarian hyperstimulation, will generally be interpreted as a proof of diminished ovarian reserve and reduced prognosis for pregnancy. For that reason, poor-response prediction has been studied extensively, although mainly in relatively small studies. In 2011, an international project was undertaken to combine all of these smaller studies and merge them into one large summary database. With all of these data combined, a more robust analysis could be performed and more solid answers regarding the value of ovarian reserve testing could be given. Such a study setup is called an individual patient data meta-analysis (IPD-MA) and this is regarded as the gold standard for test evaluations.

The IPD-MA for response and pregnancy outcome prediction after ART treatment included 5705 women undergoing their first IVF cycle. It appeared that the AFC and AMH are superior over the other ORTs, especially basal FSH, in the prediction of a poor response. AMH and the AFC are adequate predictors of a poor ovarian response to ovarian hyperstimulation in IVF, with areas under the curve–receiver–operator characteristic curve (AUC–ROC) of 0.78 and 0.76, respectively (Figure 38.5) (27).

Due to the large body of data, multivariable analyses could also be performed, studying the added value of the ORT to patient characteristics such as female age. These multivariable analyses showed that a model with age, AFC, and AMH had a significantly higher predictive accuracy than a model based alone (AUC–ROC 0.80 vs. 0.61, $p \leq 0.001$), thereby confirming that AFC and AMH have added value to female age in the prediction of a poor response. Interestingly, AMH alone yielded an accuracy

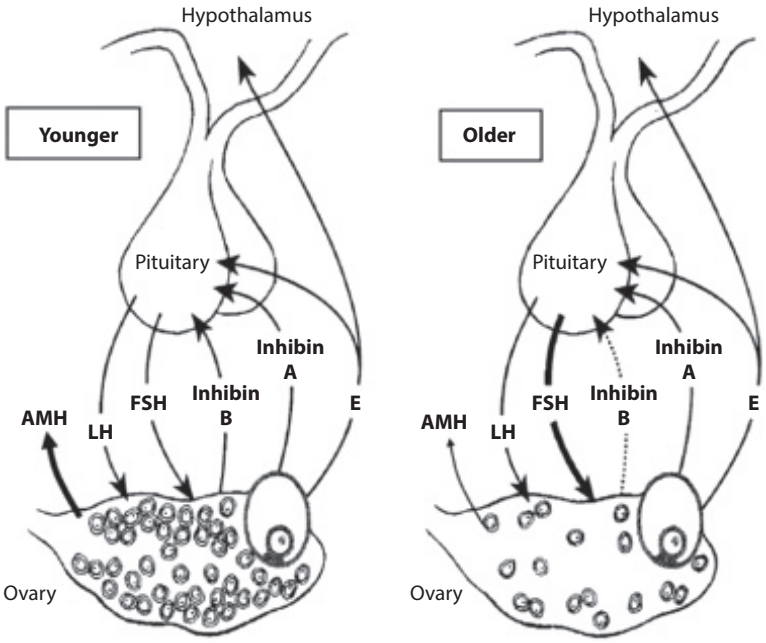

Figure 38.4 Illustration of the changes in follicle reserve with increasing female age and the effects of these quantitative changes upon several endocrine factors. *Abbreviations*: AMH, anti-Mullerian hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone. (Adapted from Soules MR et al. *Am J Human Biol* 1998; 30: 193–204.)

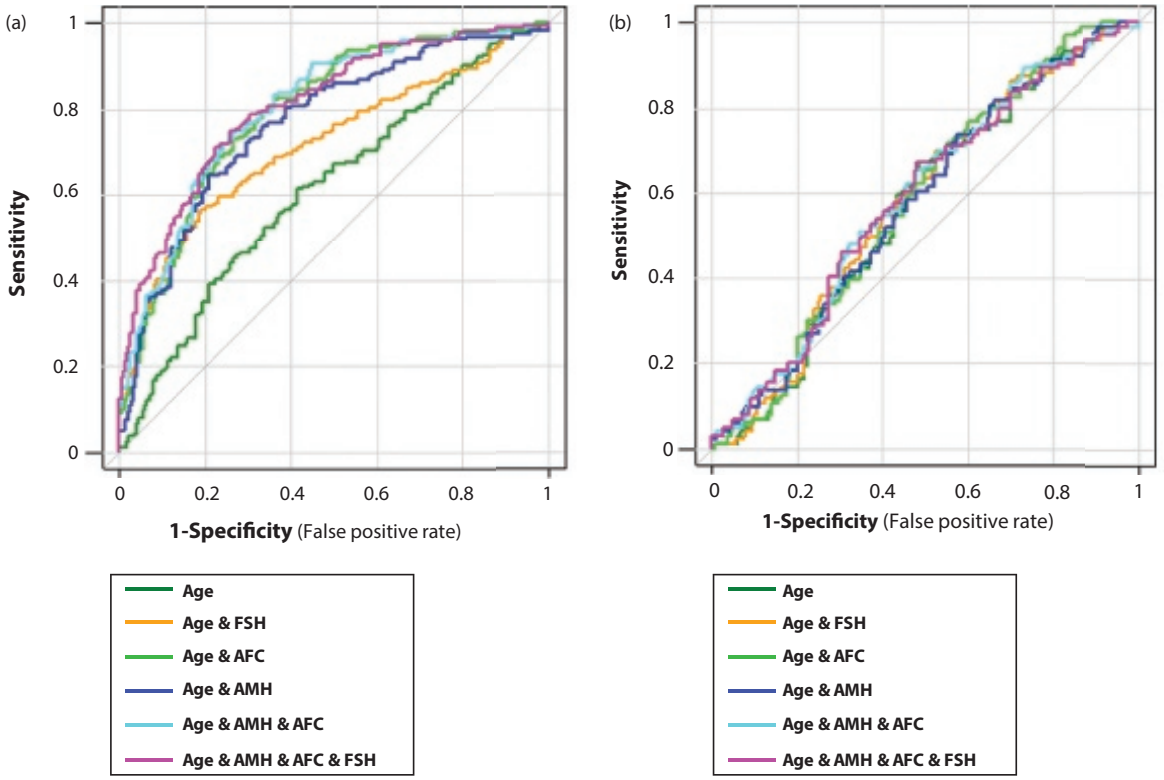

Figure 38.5 Receiver–operator characteristic (ROC) curves of age and ovarian reserve tests (ORTs) in the prediction of poor response and ongoing pregnancy. (a) Poor-response prediction based on age and ORT. The ROC curves of age or age combined with one or more ORT are depicted. The ROC curves for "Age + AMH," "Age + AMH + AFC," and "Age + AMH + AFC + FSH" run toward the upper left corner, indicating a good capacity to discriminate between normal and poor responders at certain cutoff levels. (b) Ongoing pregnancy prediction based on age and ORT. The ROC curves for age or age combined with one or more ORT run almost parallel to or even cross the x/y line, indicating that the tests are useless for pregnancy prediction. *Abbreviations*: AFC, antral follicle count; AMH, anti-Mullerian hormone; FSH, follicle-stimulating hormone. (Adapted from Broer SL et al. *Hum Reprod Update* 2013; 19: 26–36.)

that is comparable to all multivariable models, suggesting that a single measurement of AMH would be sufficient (27).

If poor response was to be the endpoint of interest, then the clinical value of these tests would be satisfactory. Unfortunately, though, no proven strategy to prevent the occurrence of poor response is currently known. Also, a poor response may not always imply a poor prognosis, especially in younger women (28). The same may be true for "poor responders" after the application of mild stimulation protocols (29). In poor responders to a first IVF cycle, it has become increasingly clear that any adaptation in the treatment protocol in a second cycle will improve neither the subsequent response nor the prognosis for pregnancy where randomized trials are concerned.

However, some new expectations are emerging from the use of androgens or growth hormone, although larger studies may be needed here (30,31). All of this may indicate that in predicted poor responders prior to starting IVF, the expectations of adapted management may also be marginal.

Prior to start of the first IVF cycle, ORTs could be used to determine the FSH dosage of the first IVF cycle. So far, only a few studies exist on the effect of adapting the dosage of FSH based on prior ORTs to obtain an optimal number of oocytes and improve prospects for pregnancy. A first study showed that predicted poor responders, based on an AFC of below 5, did not benefit from a higher starting dosage of gonadotropins in the first IVF treatment cycle (32). Also, in a pseudo-randomized trial, it was demonstrated that there is no proven clinical value of increasing the dosage FSH in patients with predicted low ovarian reserve (33). However, in contrast, another study indicated that an individualized dose regimen in IVF cases with normal basal FSH levels did increase the proportion of appropriate ovarian responses during controlled ovarian hyperstimulation (34). Even a higher ongoing pregnancy rate in the individualized dose group was reported.

Recently, the first study using AMH as an indicator for FSH dosage did show an increase in the ovarian response, but an effect on the cumulative ongoing pregnancy rate could not be found (35). These findings together indicate the need for larger studies providing the final answer to the question of whether a predicted poor responder will or will not benefit from the use of higher dosages of FSH. Currently, we are awaiting the results of the OPTIMIST trial (trial number: NTR2657), which studied the effects of dosage alterations in a large group of IVF patients, and the results of the ESTHER trial (trial number: NCT01956110) where human FSH was compared to recombinant FSH and dosages adjusted to AMH.

Excessive-response prediction

Due to the promising results of poor-response prediction, the possibility of excessive-response prediction has become an area of study, especially since excessive responders may be in jeopardy due to high patient discomfort, reduced pregnancy rates, and ovarian hyperstimulation syndrome risks (36,37). In view of these drawbacks, elimination of exaggerated ovarian response in stimulation protocols will improve safety, success, and cost factors of ART programs.

The international collaboration has extended to all studies also assessing excessive response. This IPD-MA included 4786 women undergoing their first IVF cycle. This study showed that both AMH and the AFC are accurate predictors of excessive response to ovarian hyperstimulation, with AUC-ROC values for AMH and the AFC of 0.81 and 0.79, respectively (38).

Again, multivariable analyses were performed in order to study the added value of the ORTs on patient characteristics. These analyses showed an increase in the AUC-ROC from 0.61 to 0.85 when, besides age, the ORTs of the AFC an AMH were added, thereby confirming the added value of the AFC and AMH. For excessive-response prediction, the combination of the AFC with AMH is superior to a single ORT (Figure 38.6) (38).

The clinical value of excessive-response prediction will depend on the consequences to this prediction. To date, excessive responders in a first cycle may benefit from dose adaptation in a subsequent cycle. In this respect, the Arce trial has highlighted that a mitigated response may very well reveal the same number of good-quality blastocysts as a more maximal response, with the same success rates (35). The possible beneficial effect of individualized dose regimens on prior predicted excessive responders has also been demonstrated by the results of the CONSORT study (39). Based on an algorithm for individualizing the FSH dosage using FSH, body mass index, age, and the AFC, excessive responses could be clearly prevented, without an obvious reduction in pregnancy prospects.

Pregnancy prediction

The IPD-MA also studied the value of ORTs for the prediction of ongoing pregnancy after IVF. For these analyses, 5705 women undergoing their first IVF cycle could be included. The predictive ability for the occurrence of pregnancy after IVF was very small.

Again, it was studied whether the combination of ORTs with patient characteristics could improve predictive accuracy. In these multivariable analysis, it became clear that ORTs do not have any added value in the prediction of ongoing pregnancy to female age alone (Figure 38.5). Age alone has a moderate AUC-ROC of 0.57. When combining age with AFC and AFC, the AUC-ROC is 0.59 (27). Neither combination of ORTs could improve this accuracy. Therefore, ORTs are not useful in the prediction of ongoing pregnancy after IVF.

This finding should not be regarded as a surprise, as most tests relate to the quantitative aspects of the ovarian reserve that are constantly present (i.e., antral follicle cohort size), while the quality perspective is only tested against a single exposure, which certainly will not be a good expression of a couple's fertility potential (this can only be tested properly in a series of ART cycles).

However, recent studies have noted that, although ovarian reserve markers may not predict pregnancy, they can be

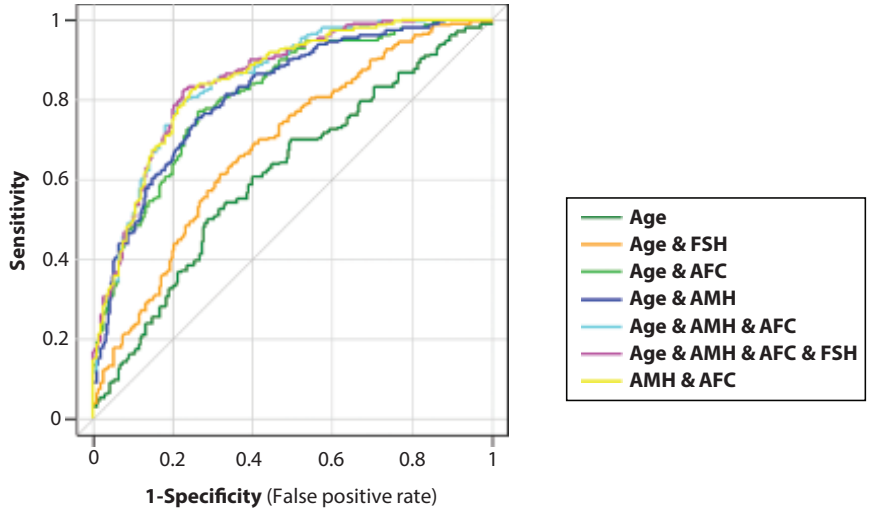

Figure 38.6 Receiver–operator characteristic (ROC) curves of age and ovarian reserve tests in the prediction of an excessive response. The ROC curves of age and age combined with one or more ovarian reserve test are depicted. The ROC curves for "Age + AMH," "Age + AFC," "Age + AMH + AFC," and "Age + AMH + AFC + FSH" run toward the upper left corner of the ROC space, indicating a good capacity to discriminate between normal and excessive responders at certain cutoff levels. *Abbreviations*: AFC, antral follicle count; AMH, anti-Mullerian hormone; FSH, follicle-stimulating hormone. (Adapted form Broer SL et al. *Fertil Steril* 2013; 100: 420–9.)

used to make a moderate distinction between patients with a good and poor prognosis. The success of IVF was found to mainly depend on maternal age and serum AMH concentrations (40). Specifically, in older women, ORTs such as AMH or the AFC could help with identifying couples where refraining from treatment is the best advice, such as in favor of egg donation or adoption (Table 38.1) (8).

First cycle poor response

Testing for ovarian reserve may also be possible by using the quantity of the ovarian response to ovarian hyperstimulation in the first ART cycle. As stated above, a poor response to stimulation will generally be interpreted as a proof of diminished ovarian reserve and reduced prognosis for pregnancy. The Bologna criteria have been defined as a consensus regarding the criteria of a poor responder. The criteria state that after a poor response to ovarian hyperstimulation a woman can be classified as a poor responder (i.e., with a diminished ovarian reserve) when at least two of the following three features are present: (i) advanced maternal age or any other risk factor for poor ovarian response (POR); (ii) a previous POR; and (iii) an abnormal ORT. Two episodes of POR after maximal stimulation are sufficient to define a patient as a poor responder in the absence of advanced maternal age or abnormal ORT (41).

Poor responders in IVF/ICSI treatment also experience an earlier transition into menopause compared to normal responders, confirming the relationship between response and fertility potential. Still, a poor response may also be caused by conditions like submaximal stimulation

Table 38.1 Predicted one-year probability of achieving a live birth according to a simplified model based on the data of all of the patients

	AMH (μg/L)					Total no.
Age (years)	**0–1**	**1–2**	**2–3**	**3–5**	**5–25**	**of patients**
0–30	0.44 (0.39–0.48)	0.54 (0.50–0.58)	0.56 (0.53–0.60)	0.68 (0.65–0.70)	0.68 (0.65–0.71)	67
n	3	14	15	16	19	
30–35	0.41 (0.35–0.45)	0.51 (0.46–0.55)	0.53 (0.49–0.57)	0.64 (0.61–0.67)	0.64 (0.61–0.67)	182
n	34	43	48	37	20	
35–40	0.32 (0.26–0.38)	0.41 (0.36–0.46)	0.43 (0.38–0.48)	0.53 (0.49–0.57)	0.54 (0.50–0.57)	192
n	61	61	30	22	18	
40–45	0.16 (0.08–0.23)	0.21 (0.14–0.27)	0.22 (0.15–0.28)	0.29 (0.22–0.34)	0.29 (0.22–0.34)	46
n	23	9	7	5	2	
Total no. of patients	121	127	100	80	59	487

Source: From Hamdine O et al. *Fertil Steril* 2015; 104: 891–8, with permission.
Note: Probability values are presented with 95% confidence intervals. *Abbreviation*: AMH, anti-Mullerian hormone.

in obese women, carrying of an FSH receptor polymorphism, or simply by chance. In such poor responders, prospects in the actual and subsequent cycles are not so unfavorable that refusal of treatment is justified. Only in case of a poor response in a woman that could be defined as diminished ovarian reserve according to the Bologna criteria does prognosis for subsequent cycles become cumbersome enough to consider further denial of treatment (13,42–44). If the policy would be to allow any couple with female age under 40 years to proceed to ART use, then a poor response combined with an appropriate ORT may be the best policy to direct further management.

Applicability of ORTs in ART practice

To date, it has been demonstrated that ORTs are adequate predictors of a poor and excessive response. However, the clinical applicability will rely on future studies that demonstrate whether adjustment of clinical management can be justified based on these predictions and whether these adjustments would be cost-effective.

Moreover, ORTs do not predict pregnancy after ART use and cannot be used for this objective. However, counseling on the basis of prognosis level from age and AMH/AFC is interesting, although much of the information comes from female age, and adding tests could only be useful for counseling in certain subgroups, like older women.

Reproductive lifespan prediction

The new challenge for ORTs lies in the possibility of identifying women with a reduced reproductive lifespan at such a stage in their lives that adequate action can be taken. Ideally, this could imply that these tests can be used to determine who will achieve a spontaneous pregnancy within a certain timeframe and who will be in need of ART treatment. Also, and more realistically, such tests performed at a younger age could be used to predict the age at which a woman will become menopausal. The relationship between menopausal age and the end of natural fertility has been hypothesized to be fixed (Figure 38.2) (7). Therefore, based on reproductive lifespan forecasting, individualized preventive infertility management could become worthwhile.

Moreover, a woman's age at menopause is also related to various other general health issues. A late menopause age is associated with reduced all-cause morbidity and mortality, whereas women with an early menopause are at increased risk for osteoporosis, bone fractures, and cardiovascular risks. Therefore, prediction of menopause could not only be valuable regarding fertility, but also for preventive strategies for general health (5).

Current fecundity prediction

In many Western countries, the average age of women giving birth to their first child is approaching 30 years. This means that a significant proportion of women when starting to try to conceive will already exhibit a reduced possibility of spontaneous pregnancy.

So far, three studies have been performed to assess the value of ovarian reserve testing in predicting spontaneous pregnancies. One study in 100 unselected women (aged 30–44 years) aiming to achieve a spontaneous pregnancy showed a good correlation between initial AMH levels and natural fertility in a six-month follow-up period (45). However, these findings could not be confirmed in a second study, where no correlation was found between low AMH levels and reduced fecundability in women in their mid-twenties (46). In a follow-up period up to 12 months, a third study showed that AMH levels did not predict time to ongoing pregnancy (47). Therefore, to date there is no role for ovarian reserve testing in the prediction of actual fecundity.

Menopause prediction

As it is hypothesized that there is a fixed time interval between age at menopause and natural sterility, several studies have been undertaken regarding the role of ORTs in predicting menopause and thereby predicting age at natural sterility. If these tests were to be accurate, this may motivate some women to start a family at a younger age, or apply fertility-preservation techniques such as oocyte freezing. Alternatively, ovarian reserve testing could reassure others that postponing childbearing will not interfere with a woman's chances to achieve a pregnancy later on.

Individualized forecasting of menopausal age has mostly been studied using age in relation to cycle status. Together it builds to a comprehensible predictor, although age does not differentiate well for young women, since a regular cycle at 20, 25, or 30 years of age does not provide any additional information to the expected menopausal age. FSH accurately reflects current reproductive status; however, the capacity to predict future changes in reproductive status is weak (48).

The AFC has been shown to be an accurate univariate predictor of time to menopause; however, when correcting for age, only a non-significant trend for adding the AFC was found (48).

AMH has been studied more extensively in relation to menopause prediction. There are several studies consistently showing AMH to be associated with menopausal age, even after correction for age (48,49–53). Age-specific AMH levels can be used to predict the age range in which menopause will occur (Figure 38.7 and Table 38.2). Moreover, AMH has been shown to have added value on top of other patient characteristics, such as body mass index and smoking (51). Also, it is a more accurate predictor of time to menopause than mother's age at menopause (52).

However, the prediction models lack the capacity to predict the extreme ages of menopause (very young and very late) (53). Specifically, these extreme ages at menopause are the most valuable to predict, as these have the main clinical value regarding the fertility lifespan and general health implications. Larger datasets are needed to study the ability of AMH to predict these menopausal ages. Moreover, the prediction intervals remain wide. To use these predictions, the interval needs to be much smaller in order to have clinical implications. Studies

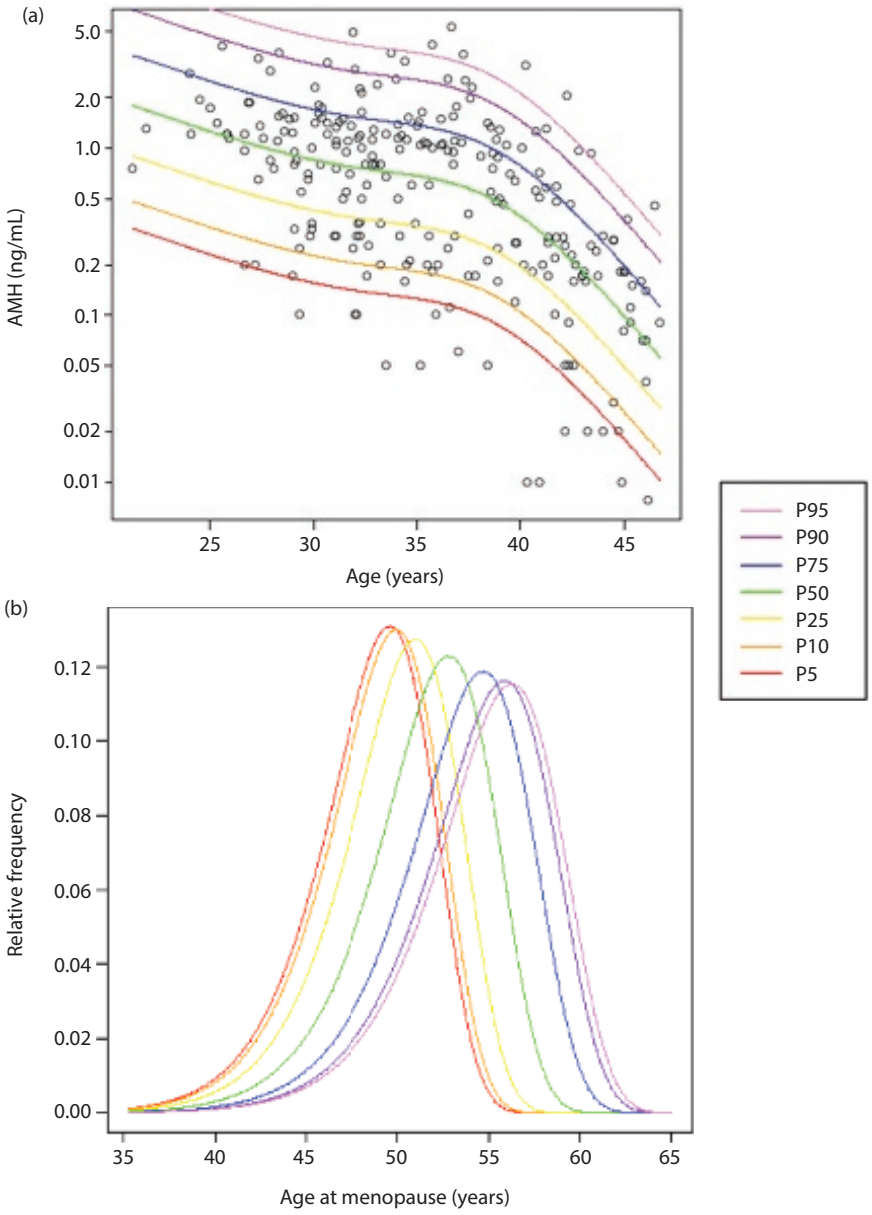

Figure 38.7 Nomograms for the relationship between age-specific AMH concentrations and the distribution of age at menopause. (a) The AMH levels measured at entry of the study for women at a given age are shown, measured approximately 11 years before cycle status assessment. The lines represent the upper margins of the different percentiles of AMH. Women can thus be placed in a percentile category based on their AMH concentration at a given age. (b) The variation of age at menopause for different percentiles of AMH. *Abbreviation*: AMH, anti-Mullerian hormone; P, percentile. (From Broer SL et al. *J Clin Endocrinol Metab* 2011; 96: 2532–9, with permission.)

measuring AMH levels consecutively and therefore relating the decrease in AMH, or studies adding other factors, such as the mother's menopausal age or genetic factors, should be undertaken in order to narrow these prediction intervals. Furthermore, the most recent studies with a follow-up time of up to 14 years also demonstrate that with extended time between AMH measurements and menopause, the predictive strength of AMH may become decreased (50,54).

Thus, although AMH is a very promising factor in the prediction of menopause, it is currently not applicable for predicting menopause or the end of natural fertility in day-to-day clinical practice.

SUMMARY

Age-related fertility decline varies considerably among women. Therefore, chronological female age, though informative for pregnancy prospects in assisted reproduction, will not always correctly express a woman's reproductive potential. Currently, ORTs have been shown to be accurate predictors of the quantitative aspects of the ovarian reserve and thereby of the response to ovarian

Table 38.2 Characteristics of the studies included in the meta-analysis regarding anti-Mullerian hormone and prediction of menopause

First author (year)	Patients (n)	MP at FU (n)	Outcome variable	Analytical method	Results		
					HR	C-statistic	Other
Sowers (2010)	50	50	TTM	Generalized estimating equations and mixed model analysis	—	—	$_{log}$AMH 1 unit lower, age of menopause 1.75 years earlier ($\pm$0.14)
Tehrani (2011)	266	63	ANM	Accelerated failure time modeling and AUC	—	—	Acceptable agreement between observed and predicted ANM (bias −0.3; 95% CI −4 to 3 years); individual ANM predictions
Broer (2011)	281	48	TTM and ANM	Cox regression analysis + C-statistic	HR 9.2; 95% CI 2.5–34; $p < 0.001$ 1 unit AMH = 0.89 ng/mL	90%	—
Freeman (2012)	401	198	TTM	Cox regression analysis	HR 5.6; 95% CI 4.7–6.7; $p < 0.0001$ 1 unit = 1 SD_{log}AMH	—	—
Tehrani (2013)	1015	277	ANM	Accelerated failure time modeling and AUC + C-statistic	—	92%	Good agreement between observed and predicted ANM; individual ANM predictions
Dólleman (2015)	1163	527	TTM	Cox regression analysis + C- statistic	HR 9.1; SD 0.03	89%	—
Nair (2015)	716	207	TTM	Discrete time hazard regression in three-year intervals from baseline	HR 0–3 years: 8.1 HR 3–6 years: 2.3 HR 6–9 years: 1.6	—	—

Source: From Depmann M et al. *Menopause* 2016; 23: 224–32, with permission.

Abbreviations: AMH, anti-Mullerian hormone; AUC, area under the curve; MP, reached menopause; FU, follow-up; ANM, age at natural menopause; TTM, time to menopause; HR, hazard ratio (percentage increase in chance of MP occurring during FU per [unit] decrease of AMH); CI: confidence interval; C-statistic, percentage of correctly predicted MP occurring during FU.

hyperstimulation. However, they are not accurate predictors of the qualitative aspect of the ovarian reserve and thus are not good predictors of pregnancy after IVF.

For the prediction of the reproductive lifespan, mainly AMH has been studied. AMH is not applicable for the prediction of fecundity. For the prediction of menopause and thereby the end of natural fertility, there is consistent evidence that AMH is a good predictor; however, due to wide prediction intervals, AMH is currently not applicable in day-to-day clinical practice for these purposes.

REFERENCES

1. Stephen EH, Chandra A. Declining estimates of infertility in the United States: 1982–2002. *Fertil Steril* 2006; 86: 516–23.
2. Te Velde ER, Pearson PL. The variability of female reproductive aging. *Hum Reprod Update* 2002; 8: 141–54.
3. Morabia A, Costanza MC. International variability in ages at menarche, first livebirth, and menopause. World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives. *Am J Epidemiol* 1998; 148: 1195–205.
4. Thomas F, Renaud F, Benefice E, de Meeus T, Guegan JF. International variability of ages at menarche and menopause: Patterns and main determinants. *Hum Biol* 2001; 73: 271–90.
5. Daan NM, Fauser B. Menopause prediction and potential implications. *Maturitas* 2015; 82: 257–65.
6. van Noord-Zaadstra BM, Looman CWN, Alsbach H et al. Delaying childbearing: Effect of age on fecundity and outcome of pregnancy. *BMJ* 1991; 302: 1361–5.
7. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: Mechanisms and clinical consequences. *Endocr Rev* 2009; 30: 465–93.
8. Hamdine O, Eijkemans MJ, Lentjes EW, Torrance HL, Macklon NS, Fauser BC, Broekmans FJ. Antimüllerian hormone: Prediction of cumulative live birth in gonadotropin-releasing hormone antagonist treatment for *in vitro* fertilization. *Fertil Steril* 2015; 104: 891–8.
9. Viudes-de-Castro MP, Vicente JS. Effect of sperm count on the fertility and prolificity rates of meat rabbits. *Anim Reprod Sci* 1997; 46: 313–19.
10. Moce E, Lavara R, Vicente JS. Influence of the donor male on the fertility of frozen–thawed rabbit sperm after artificial insemination of females of different genotypes. *Reprod Domest Anim* 2005; 40: 516–21.
11. Evers JL. Female subfertility. *Lancet* 2002; 360: 151–9.
12. Menken J, Trussell J, Larsen U. Age and infertility. *Science* 1986; 233: 1389–94.
13. Oudendijk JF, Yarde F, Eijkemans MJ, Broekmans FJ, Broer SL. The poor responder in IVF: Is the prognosis always poor?: A systematic review. *Hum Reprod Update* 2012; 18: 1–11.
14. Fasouliotis SJ, Simon A, Laufer N. Evaluation and treatment of low responders in assisted reproductive technique: A challenge to meet. *J Assist Reprod Genet* 2000; 17: 357–73.
15. Popovic-Todorovic B, Loft A, Lindhard A et al. A prospective study of predictive factors of ovarian response in "standard" IVF/ICSI patients treated with recombinant FSH. A suggestion for a recombinant FSH dosage normogram. *Hum Reprod* 2003; 18: 781–7.
16. van der Gaast MH, Eijkemans MJ, van der Net JB et al. Optimum number of oocytes for a successful first IVF treatment cycle. *Reprod Biomed Online* 2006; 13: 476–80.
17. Templeton A, Morris JK, Parslow W. Factors that affect outcome of *in-vitro* fertilisation treatment. *Lancet* 1996; 348: 1402–6.
18. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: A systematic review. *Hum Reprod Update* 2003; 9: 61–76.
19. Hendriks DJ, Mol BW, Bancsi LF, te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after *in vitro* fertilization: A metaanalysis and comparison with basal follicle-stimulating hormone level. *Fertil Steril* 2005; 83: 291–301.
20. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006; 12: 685–718.
21. Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM. Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technique cycles. *Fertil Steril* 2002; 77: 468–71.
22. Klein NA, Houmard BS, Hansen KR et al. Age-related analysis of inhibin A, inhibin B, and activin a relative to the intercycle monotropic follicle-stimulating hormone rise in normal ovulatory women. *J Clin Endocrinol Metab* 2004; 89: 2977–81.
23. Hendriks DJ, Broekmans FJ, Bancsi LF et al. Single and repeated GnRH agonist stimulation tests compared with basal markers of ovarian reserve in the prediction of outcome in IVF. *J Assist Reprod Genet* 2005; 22: 65–73.
24. Kwee J, Elting MW, Schats R et al. Comparison of endocrine tests with respect to their predictive value on the outcome of ovarian hyperstimulation in IVF treatment: Results of a prospective randomized study. *Hum Reprod* 2003; 18: 1422–7.
25. Hendriks DJ, Mol BW, Bancsi LF, Te Velde ER, Broekmans FJ. The clomiphene citrate challenge test for the prediction of poor ovarian response and nonpregnancy in patients undergoing *in vitro* fertilization: A systematic review. *Fertil Steril* 2006; 86: 807–18.
26. Jain T, Soules MR, Collins JA. Comparison of basal follicle-stimulating hormone versus the clomiphene citrate challenge test for ovarian reserve screening. *Fertil Steril* 2004; 82: 180–5.
27. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, Eijkemans MJ, Mol BW,

Broekmans FJ, IMPORT study group. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: An individual patient data approach. *Hum Reprod Update* 2013; 19: 26–36.
28. Lashen H, Ledger W, Lopez-Bernal A, Barlow D. Poor responders to ovulation induction: Is proceeding to *in-vitro fertilization worthwhile? Hum Reprod* 1999; 14: 964–9.
29. *Verberg* MF, Eijkemans MJ, Macklon NS et al. Predictors of low response to mild ovarian stimulation initiated on cycle day 5 for IVF. *Hum Reprod* 2007; 22: 1919–24.
30. Kyrou D, Kolibianakis EM, Venetis CA et al. How to improve the probability of pregnancy in poor responders undergoing *in vitro* fertilization: A systematic review and meta-analysis. *Fertil Steril* 2009; 91: 749–66.
31. Mochtar MH, Van DV, Ziech M, van Wely M. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2007; 2: CD005070.
32. Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, Te Velde ER. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: A randomized controlled trial. *Hum Reprod* 2005; 20: 611–15.
33. Lekamge DN, Lane M, Gilchrist RB, Tremellen KP. Increased gonadotrophin stimulation does not improve IVF outcomes in patients with predicted poor ovarian reserve. *J Assist Reprod Genet* 2008; 25: 515–21.
34. Popovic-Todorovic B, Loft A, Bredkjaeer HE et al. A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a "standard" dose of 150 IU/day in "standard" patients undergoing IVF/ICSI treatment. *Hum Reprod* 2003; 18: 2275–82.
35. Arce JC, Andersen AN, Fernández-Sánchez M, Visnova H, Bosch E, García-Velasco JA, Barri P, de Sutter P, Klein BM, Fauser BC. Ovarian response to recombinant human follicle-stimulating hormone: A randomized, antimüllerian hormone-stratified, dose-response trial in women undergoing *in vitro* fertilization/intracytoplasmic sperm injection. *Fertil Steril* 2014; 102: 1633–40.
36. Fauser BC, Diedrich K, Devroey P. Predictors of ovarian response: Progress towards individualized treatment in ovulation induction and ovarian stimulation. *Hum Reprod Update* 2008; 14: 1–14.
37. Sunkara SK, Rittenberg V, Raine-Fenning N et al. Association between the number of eggs and live birth in IVF treatment: An analysis of 400 135 treatment cycles. *Hum Reprod* 2011; 26: 1768–74.
38. Broer SL, Dólleman M, van Disseldorp J, Broeze KA, Opmeer BC, Bossuyt PM, Eijkemans MJ, Mol BW, Broekmans FJ, IPD-EXPORT Study Group. Prediction of an excessive response in *in vitro* fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: An individual patient data meta-analysis. *Fertil Steril* 2013; 100: 420–9.
39. Olivennes F, Howles CM, Borini A et al. Individualizing FSH dose for assisted reproduction using a novel algorithm: The CONSORT study. *Reprod Biomed Online* 2009; 18: 195–204.
40. La Marca A, Nelson SM, Sighinolfi G et al. Anti-Mullerian hormone-based prediction model for a live birth in assisted reproduction. *Reprod Biomed Online* 2011; 22: 341–9.
41. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of "poor response" to ovarian stimulation for *in vitro* fertilization: The Bologna criteria. *Human Reprod* 2011; 26: 1616–24.
42. de Boer EJ, den TI, Te Velde ER, Burger CW, van Leeuwen FE. Increased risk of early menopausal transition and natural menopause after poor response at first IVF treatment. *Hum Reprod* 2003; 18: 1544–52.
43. Lawson R, El Toukhy T, Kassab A et al. Poor response to ovulation induction is a stronger predictor of early menopause than elevated basal FSH: A life table analysis. *Hum Reprod* 2003; 18: 527–33.
44. Hendriks DJ, Te Velde ER, Looman CW, Bancsi LF, Broekmans FJ. Expected poor ovarian response in predicting cumulative pregnancy rates: A powerful tool. *Reprod Biomed Online* 2008; 17: 727–36.
45. Steiner AZ, Herring AH, Kesner JS, Meadows JW, Stanczyk FZ, Hoberman S, Baird DD. Antimüllerian hormone as a predictor of natural fecundability in women aged 30–42 years. *Obstet Gynecol* 2011; 117: 798–804.
46. Hagen CP, Vestergaard S, Juul A et al. Low concentration of circulating antimüllerian hormone is not predictive of reduced fecundability in young healthy women: A prospective cohort study. *Fertil Steril* 2012; 98: 1602–08.
47. Depmann M, Broer SL, Eijkemans MJC, van Rooij IAJ, Scheffer GJ, Heimensem J, Mol BW, Broekmans FJM. Anti-Müllerian hormone does not predict time to pregnancy: Results of a prospective cohort study. *Gynecol Endocrinol* 2017; 10: 1–5. PMID: 28393651.
48. Broer SL, Eijkemans MJ, Scheffer GJ et al. Antimullerian hormone predicts menopause: A long-term follow-up study in normoovulatory women. *J Clin Endocrinol Metab* 2011; 96: 2532–9.
49. Tehrani FR, Shakeri N, Solaymani-Dodaran M, Azizi F. Predicting age at menopause from serum antimullerian hormone concentration. *Menopause* 2011; 18: 766–70.
50. Nair S, Slaughter JC, Terry JG et al. Anti-mullerian hormone (AMH) is associated with natural menopause in a population-based sample: The CARDIA Women's Study. *Maturitas* 2015; 81: 493–8.

51. Dólleman M, Verschuren WM, Eijkemans MJ, Broekmans FJ, van der Schouw YT. Added value of anti-Müllerian hormone in prediction of menopause: Results from a large prospective cohort study. *Hum Reprod*. 2015; 30: 1974–81.
52. Dólleman M, Depmann M, Eijkemans MJ et al. Anti-Mullerian hormone is a more accurate predictor of individual time to menopause than mother's age at menopause. *Hum Reprod*. 2014; 29: 584–91.
53. Depmann M, Broer SL, van der Schouw YT, Tehrani FR, Eijkemans MJ, Mol BW, Broekmans FJ. Can we predict age at natural menopause using ovarian reserve tests or mother's age at menopause? A systematic literature review. *Menopause* 2016; 23: 224–32.
54. Depmann M, Eijkemans MJC, Broer SL, Scheffer GJ, van Rooij IAJ, Laven JSE, Broekmans FJM. Does AMH predict menopause? Results of a prospective ongoing cohort study. *Hum Reprod* 2016; 31: 1579–87.

Drugs used for ovarian stimulation

39

Clomiphene citrate, aromatase inhibitors, metformin, gonadotropins, gonadotropin-releasing hormone analogs, and recombinant gonadotropins

ZEEV SHOHAM and COLIN M. HOWLES

INTRODUCTION

Infertility treatment became available owing to developments in the characterization and purification of hormones. Treatment with urinary-derived human gonadotropins and clomiphene citrate (CC) became available in 1961 and then over the following 35 years advancements in production techniques, including the use of recombinant DNA technology (1), led to the availability of purer and more consistent injectable gonadotropins (for a review, see (2)). The purpose of this chapter is to overview the development, structure, and mode of action of treatments for ovulation induction (OI) and controlled ovarian stimulation (COS) for assisted reproduction technologies (ARTs).

CLOMIPHENE CITRATE

Drug description

CC was synthesized in 1956, and an indisputable therapeutic breakthrough occurred in 1961 when Greenblatt and his group discovered that CC, a nonsteroidal analog of estradiol, exerts a stimulatory effect on ovarian function in women with anovulatory infertility (3). The drug was approved for infertility treatment by the U.S. Food and Drug Administration in 1967.

CC is a triphenylchloroethylene derivative in which the four hydrogen atoms of the ethylene core have been substituted with three phenyl rings and a chloride anion. One of the three phenyl rings bears an aminoalkoxy (OCH_2–CH_2–$N[C_2K_2]_2$) side chain, but the importance of its action on CC remains uncertain. The dihydrogen citrate moiety ($C_6H_8O_7$) accounts for the fact that commercially available preparations represent the dihydrogen citrate salt form of CC. CC is a white or pale yellow odorless powder, unstable in air and light, with a melting point of 116–118°C. It is a triarylethylene compound (1-*p*-diethyl aminoethoxyphenyl-1,2-diphenyl-2-chloroethylene citrate, with a molecular weight of 598.09) that is chemically related to chlorotrianisene, which is a weak estrogen. Structurally, CC is related to diethylstilbestrol, a potent synthetic estrogen. Although this compound is not a steroid, but a triphenylchloroethylene, its steroic configuration bears a remarkable structural similarity to estradiol, and consequently facilitates binding to estrogen receptors (ERs).

CC is available as a racemic mixture of two stereochemical isomers referred to as (*cis*) Zu-clomiphene or the (*trans*) En-clomiphene configuration (Figure 39.1a and 39.1b), the former being significantly more potent. In the commercially available preparations, the isomers are in the ratio of 38% Zu- and 62% En-clomiphene. Limited experience suggests that the clinical utility of CC may indeed be due to its *cis* isomer (4,5). However, it remains uncertain whether *cis*-CC is more effective than CC proper in terms of ovulation and conception rates (6–9). Following the development of a reverse-phase high-performance liquid chromatography (HPLC) assay (10), it was apparent that each isomer exhibited its own characteristic pharmacokinetic profile, the En isomer being absorbed faster and eliminated more completely than the Zu isomer. Although CC tablets contain 62% En isomer and 38% Zu isomer, the observed plasma concentrations of the Zu isomer were much higher than those of the En isomer. Because the Zu isomer is considered more estrogenic than the En isomer, response of the target tissues should vary according to both the relative affinity and the concentrations of each isomer interacting with the relevant ER. Tracer studies of CC with radioactive carbon labeling have shown that the main route of excretion is via the feces, although small amounts are also excreted in the urine. After administration of CC for five consecutive days at a dose of 100 mg daily, the drug could be detected in serum for up to 30 days.

Mechanism of action

Administration of CC is followed in short sequence by enhanced release of pituitary gonadotropins, resulting in follicular recruitment, selection, assertion of dominance, and rupture.

The principal mechanism of CC action is a reduction in the negative feedback of endogenous estrogens due to prolonged depletion of hypothalamic and pituitary ERs (11,12). This action consequently leads to an increase in

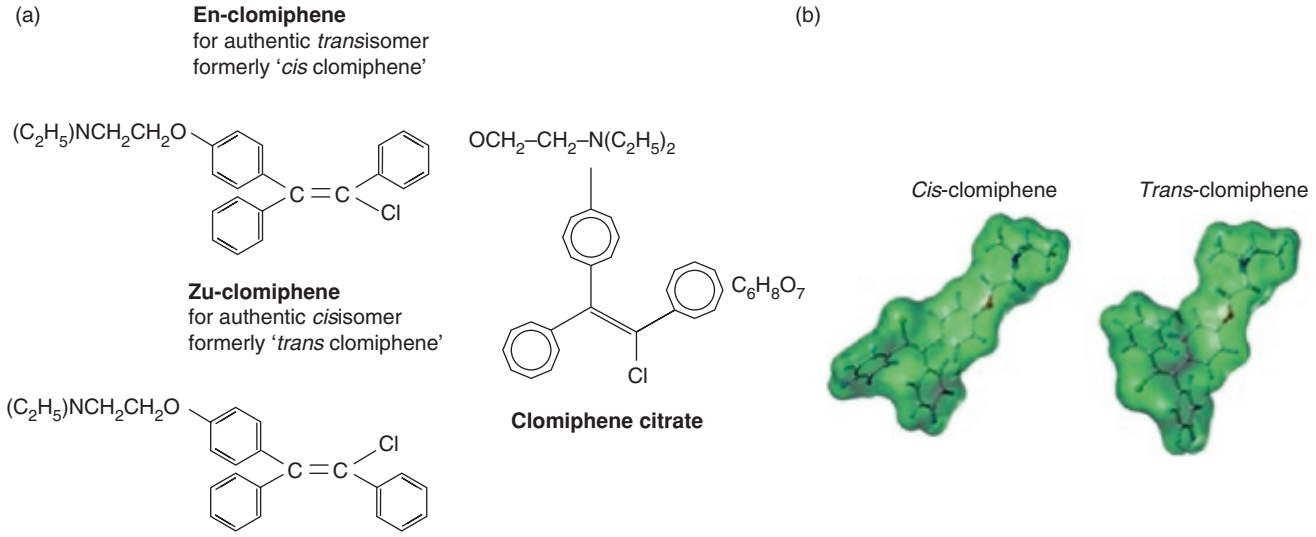

Figure 39.1 (a) Clomiphene citrate is available as a racemic mixture of two stereochemical isomers referred to as (*cis*) Zu-clomiphene or the (*trans*) En-clomiphene configuration, with the former being significantly more potent. In the preparations that are commercially available, the isomers are in a ratio of 38% Zu- and 62% En-clomiphene. (b) The isomeric models in a different configuration.

the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus into the hypothalamic–pituitary portal circulation, engendering an increase in the release of pituitary gonadotropins. Administration of a moderate gonadotropin stimulus to the ovary overcomes the ovulation disturbances and increases the cohort of follicles reaching ovulation (13,14). A marked increase in serum concentrations of luteinizing hormone (LH) in proportion to follicle-stimulating hormone (FSH) may sometimes occur (15), and this temporary change in the LH:FSH ratio appears to bring about some impairment of follicular maturation, resulting in delayed ovulation. Shortly after discontinuation of CC, both gonadotropins gradually decline to the preovulatory nadir, only to surge again at midcycle.

The drug interacts with ER-binding proteins similar to native estrogens and behaves as a competitive ER antagonist (16,17). Importantly, CC does not display progestational, corticotropic, androgenic, or antiandrogenic properties.

Indications and contraindications for treatment

Anovulatory infertility is the most important indication for CC treatment. In addition, treatment is indicated for women with oligomenorrhea, or amenorrhea, who responded to progesterone (P) treatment with withdrawal bleeding. Treatment is ineffective in women with hypogonadotropic hypogonadism (HH; World Health Organization [WHO] group I). Other controversial indications include luteal-phase defect, unexplained infertility, and women undergoing *in vitro* fertilization (IVF) when multiple follicle development is required. Contraindications to CC administration include preexisting ovarian cysts, with suspected malignancy, and liver disease.

Duration of treatment

CC increases secretion of FSH and LH and is administered for a period of five days. In women with normal cycles, administration of CC for more than five days resulted in an initial increase of serum FSH concentration that lasted for five to six days, followed by a decline in serum FSH levels, despite continuation of the drug, whereas LH levels remained high throughout the entire treatment period (18,19).

CC is usually administered on day 5 of spontaneous or induced menstruation. This is based on the theory that on day 5 the physiologic decrease in serum FSH concentration provides the means for selection of the dominant follicle. Initiation of the drug on day 2 induces earlier ovulation, which is analogous to the physiologic events of the normal menstrual cycle. The starting dose is usually 50 mg/day, owing to the observation that 50% of pregnancies occur with the 50-mg dose (20). In order to obtain good results, CC therapy should be carefully monitored. Obviously, serial measurements of LH, FSH, estradiol, and P and ultrasound measurements provide the most detailed information on the patient's response to treatment.

Results of treatment

CC induces ovulation in the majority of women. The ovulation rate ranges between 70% and 92%; however, the pregnancy rate is much lower. The discrepancy between the high ovulation rates and relatively low pregnancy rates may be due to the following factors: (1) antiestrogen effects on the endometrium; (2) antiestrogen effects on the cervical mucus; (3) decrease of uterine blood flow; (4) impaired placental protein 14 synthesis; (5) subclinical pregnancy loss; (6) effect on tubal transport; and (7) detrimental effects on the oocytes (21). The Cochrane review (22) of clinical

data regarding the use of CC for unexplained subfertility in women, based on five randomized trials of CC (doses ranging from 50 to 250 mg/day for up to 10 days) compared with placebo or no treatment, showed that the odds ratio (OR) for pregnancy per patient was 2.38 (95% confidence interval [CI] 1.22–4.62). The OR for pregnancy per cycle was 2.5 (95% CI 1.35–4.62). It was concluded from this review that CC appeared to improve pregnancy rates modestly in women with unexplained subfertility.

Side effects and safety

The most common side effects are hot flushes (10%), abdominal distention, bloating or discomfort (5%), breast discomfort (2%), nausea and vomiting (2%), visual symptoms, and headache (1.5%). A rise in basal body temperature may be noted during the five-day period of CC administration. Visual symptoms include spots (floaters), flashes, or abnormal perception. These symptoms are rare, universally disappear upon cessation of CC therapy, and have no permanent effect. The multiple pregnancy rate is approximately 5% and almost exclusively due to twins.

Several reports have associated long-term (>12 months) CC therapy with a slight increase in future risk of ovarian cancer (relative risk [RR] = 1.5–2.5) (23). Owing to these initial reports, the Committee on Safety of Medicines in the U.K. advised doctors to adhere to the manufacturers' recommendations of limiting treatment to a maximum of six months. However, this increased risk has not been confirmed by subsequent reports. Several case reports have linked CC with congenital malformations, especially neural tube defects (24–30). Data available on 3751 births after CC treatment included 122 children born with congenital malformations (major and minor), representing an incidence of 32.5/1000 births (31). This figure is within the range found among the normal population (32).

Summary

CC is one of the most popular drugs for OI because it is easy to administer, highly effective, considered safe, and the cost is minimal.

AROMATASE INHIBITORS

Aromatase, a cytochrome P450-dependent enzyme, acts as the ultimate step in the synthesis of estrogen, catalyzing the conversion of androgens to estrogens (33). The conversion of androgens to estrogens also occurs at peripheral sites, such as in muscle, fat, and the liver (34). Recently, a group of new, highly selective aromatase inhibitors has been approved to suppress estrogen production in postmenopausal women with breast cancer. Aromatase inhibitor is a competitive inhibitor of the aromatase enzyme system, and inhibits the conversion of androgens to estrogens. It inhibits the aromatase enzyme by competitively binding to the heme of the aromatase–cytochrome P450 subunit of the enzyme, resulting in a reduction of estrogen biosynthesis in all tissues where it is present (Figure 39.2). Treatment significantly lowers serum estrone, estradiol, and estrone sulfate, and has not been shown significantly to affect

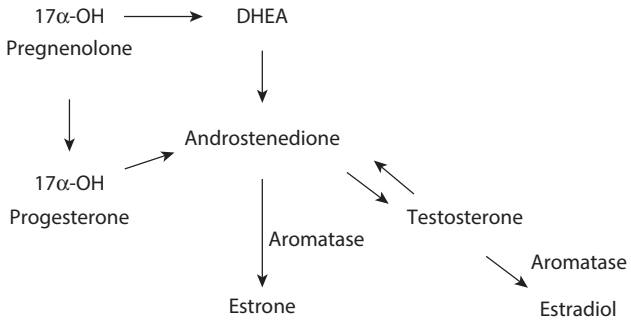

Figure 39.2 Aromatase inhibitor. Aromatase, an enzyme found in the liver, is responsible for the conversion of androgens—androstenedione and testosterone—into estrogens—estrone and estradiol. By inhibiting aromatase, the body produces less estrogen and maintains a higher testosterone state. *Abbreviation*: DHEA, dehyroepiandrosterone sulfate.

adrenal corticosteroid synthesis, aldosterone synthesis, or synthesis of thyroid hormones. Maximum suppression is achieved within 48–78 hours. The first aromatase inhibitor to be developed was aminoglutethimide, but its usage was stopped owing to side effects, one of which was adrenal insufficiency (35). However, this development stimulated the formulation of numerous other aromatase inhibitors that were described as first-, second-, and third-generation inhibitors according to chronologic development. They were further classified as type I (steroid analogs of androstenedione) and type II (nonsteroidal) (Table 39.1).

Pharmacokinetics

Third-generation aromatase inhibitors are administered orally, and have a half-life of approximately 48 hours, which allows once-daily dosing (36,37). These drugs metabolize mainly in the liver, and are excreted through the biliary (85%) and the urinary (11%) systems.

Side effects and safety

Reported side effects are bone pain (20%), hot flushes (18%), back pain (17%), nausea (15%), and dyspnea (14%). These side effects are typically observed after long-term administration.

One major concern is the use of letrozole in OI or COS because of its possible teragenicity as observed in animal models. There was one concerning report (published as an

Table 39.1 The different types and generations of aromatase inhibitors

Generation	Type I	Type II
First	None	Aminoglutethimide
Second	Formestane	Fadrozole
		Rogletimide
Third	Exemestane	Anastrozole
		Letrozole
		Vorozole

abstract only) (38) of an increase in cardiac and bone malformations in letrozole-treated pregnancies. Following the publication of the abstract, the manufacturer, Novartis, wrote to clinicians in the U.S.A. and Canada stating that letrozole was not safe for use in women who were either desiring pregnancy or pregnant. Since this notification, there has been a series of published studies, including a multicenter retrospective analysis of 911 newborns conceived after CC or letrozole treatment (39). This did not show any teratogenic effect of letrozole, and they reported a similar rate of congenital malformation to that seen in women conceiving after treatment with CC. In the most recent paper from Badawy et al. (40), they also stated that there were no observed increases in congenital malformations following the use of letrozole. Subsequently, two large prospective randomized trials have studied letrozole in polycystic ovary syndrome (PCOS) (41) and unexplained infertility (42). They have shown that cumulative rates of teratogenicity with letrozole are <5% and comparable to rates with clomiphene. These results are reassuring and have led one recent reviewer to ask not necessarily for more safety data, but rather for evidence of any harm as manifested by higher rates of congenital abnormalities (43).

Drugs available

Letrozole: this is chemically described as 4,4′-(1H-1,2,4-triazole-1-ylmethylene) dibenzonitrile, with a molecular weight of 285.31 and an empirical formula of $C_{17}H_{11}N_5$.

Anastrozole: the molecular formula is $C_{17}H_{19}N_5$ and it has a molecular weight of 293.4.

Both drugs are approved for the treatment of breast cancer in postmenopausal women.

The first clinical study using an aromatase inhibitor (letrozole: AstraZeneca) for OI was published by Mitwally and Casper in 2001 (44). With letrozole treatment in patients with PCOS, ovulation occurred in 75% and pregnancy was achieved in 25%. Letrozole appears to prevent unfavorable effects on the endometrium that are frequently observed with antiestrogen use for OI. Since the initial observation, several studies have been published on the use of aromatase inhibitors in the treatment of infertile patients (45–47). The same investigators (48) showed that the use of an aromatase inhibitor reduced the FSH dose required for ovarian stimulation, without the undesirable antiestrogenic effects occasionally noted with CC.

A recent meta-analysis of six randomized controlled trials (RCTs) involving 841 patients with PCOS showed no significant differences in pregnancy, abortion, or multiple pregnancy rates between CC and letrozole (49). The authors concluded that letrozole may be as effective as CC for OI in patients with PCOS (49). It is now 16 years since the first successful report of the use of aromatase inhibitors in OI. However, aromatase inhibitors have not been introduced into routine clinical practice (50,51). This may be either because they do not appear to significantly improve pregnancy rates versus current treatment options or simply due to the lack of large, well-designed randomized trials with positive results (50,51).

Two randomized studies have also compared the efficacy and safety of single-dose and multi-dose anastrozole with CC in infertile women with ovulatory dysfunction (52,53). Anastrozole was found to be less effective than CC at inducing ovulation in both studies. Anastrozole has also been shown to have a weaker effect on follicular growth than CC (54).

Aromatase inhibitors have also been investigated for use in ART. Four randomized trials have been published with letrozole in a total of 235 patients with poor ovarian response (55–58). When letrozole was combined with FSH, the gonadotropin dose required was consistently lower than when gonadotropins were used alone. In three trials, pregnancy rates were comparable in the treatment arms (56–58), and in one trial, pregnancy rates were lower in the letrozole arm than in the control arm (55). Only one randomized trial with letrozole has been reported in patients with normal ovarian response undergoing IVF or intracytoplasmic sperm injection (ICSI) (59). This was a pilot study involving 20 patients and showed an increased number of oocytes retrieved, and increased implantation and clinical pregnancy rates when letrozole was added to recombinant human (r-h)FSH (59). However, no significant difference between groups was shown, possibly owing to the small study population.

METFORMIN

The biguanide metformin (dimethylbiguanide) is an oral antihyperglycemic agent widely used in the management of non-insulin-dependent diabetes mellitus. It is an insulin sensitizer that reduces insulin resistance and insulin secretion. Over the last few years there has been increased interest in the use of metformin (at doses of 1500–2500 mg/day) to increase ovulatory frequency, particularly in women described as having PCOS.

There is, however, some recent conflicting evidence regarding the usefulness of metformin in PCOS patients. In a Cochrane systematic review (60), metformin was concluded to be an effective treatment for anovulation in women with PCOS, with it being recommended to be a first-line treatment, and with some evidence of benefit on parameters of the metabolic syndrome. Ovulation rates were higher when combined with clomiphene (76% vs. 46% when used alone). Finally, the authors recommended that it should be used as an adjuvant to general lifestyle improvements, and not as a replacement for increased exercise and improved diet.

Subsequently, both the American Society for Reproductive Medicine Practice Committee (U.S.A.) (61) and the National Institute for Health and Care Excellence (U.K.) (62) have made recommendations for its use in treating anovulatory PCOS. In previously untreated women with PCOS, no superiority of the combination of CC and metformin, rather than CC alone, was demonstrated in a large, Dutch multicenter study (63). In a "head-to-head" study comparing CC with metformin as first-line treatment, although ovulation and pregnancy rates were similar, significantly fewer miscarriages and, therefore,

more live births were achieved with metformin (64). In a meta-analysis of randomized trials in PCOS patients undergoing OI or IVF/embryo transfer (ET) (65), co-administration of metformin with gonadotropins did not significantly improve ovulation (OR = 3.27, 95% CI 0.31–34.72) or pregnancy (OR = 3.46, 95% CI 0.98–12.2) rates. Metformin co-administration in an IVF treatment did not improve the pregnancy rate (OR = 1.29, 95% CI 0.84–1.98), but was associated with a reduction in the risk of ovarian hyperstimulation syndrome (OHSS) (OR = 0.21, 95% CI 0.11–0.41) (65). However, the authors concluded that the review was inconclusive in terms of not being able to exclude an important clinical treatment effect because of the small number of trials and small sample sizes of the individual trials limiting the power of the meta-analysis.

Neveu et al. (66) carried out an observational comparative study to determine which first-line medication (CC or metformin) was more effective in PCOS patients undergoing OI and to verify whether any patient characteristic was associated with a better response to therapy. The authors included 154 patients who had never been treated for OI to avoid confounding effects of a previous fertility treatment. Patients receiving metformin alone had an increased ovulation rate compared with those receiving CC alone (75.4% vs. 50%). Patients on metformin had similar ovulation rates compared with those in the combination group (75.4% vs. 63.4%). Pregnancy rates were equivalent in the three groups. Response to metformin was independent of body weight and dose. Finally, nonsmoking predicted better ovulatory response overall, as well as lower fasting glucose for CC and lower androgens for metformin.

A recent literature review (67) was carried out to establish whether metformin was efficacious when given to CC-resistant PCOS patients (the Medline database was searched from January 1, 1980, to January 1, 2005). When the data from four prospective, double-blind, placebo-controlled trials were pooled, the overall effect of the addition of metformin in the CC patient was $p = 0.0006$, with a 95% CI of OR of 1.81–8.84. In only two trials was the randomization prospective; when the data of these two trials were pooled, the overall effect of the addition of metformin in the CC-resistant patient was $p < 0.0001$, with a 95% CI of OR of 6.24–70.27. Combining all data gave an overall positive effect of $p < 0.0001$, with a 95% CI of OR of 3.59–12.96. The authors concluded that the addition of metformin in the CC-resistant patient is highly effective at achieving OI. In the largest study to date, Legro and colleagues (68) randomized 626 subfertile women with PCOS who had received previous fertility therapy but were not known to be CC resistant to have CC + placebo, extended-release metformin + placebo, or a combination of metformin + CC for up to six months. The dose of extended-release metformin was gradually increased until a maximum dose of 2000 mg/day. Medication was discontinued when pregnancy was confirmed, and subjects were followed until delivery. The primary endpoint of the study was live birth rate. The live birth rate was 22.5% (47 of 209 subjects) in the CC group, 7.2% (15 of 208) in the metformin group, and 26.8% (56 of 209) in the combination therapy group ($p < 0.001$ for metformin vs. both CC and combination therapy; $p = 0.31$ for CC vs. combination therapy). Among pregnancies, the rate of multiple pregnancies was 6.0% in the CC group, 0% in the metformin group, and 3.1% in the combination therapy group. The rates of first-trimester pregnancy loss did not differ significantly among the groups. However, the conception rate among subjects who ovulated was significantly lower in the metformin group (21.7%) than in either the CC group (39.5%, $p = 0.002$) or the combination therapy group (46.0%, $p < 0.001$). With the exception of pregnancy complications, adverse event rates were similar in all groups, though gastrointestinal side effects were more frequent and vasomotor and ovulatory symptoms less frequent in the metformin group than in the CC group. The authors concluded that CC was superior to metformin at achieving live birth in women with PCOS, although multiple births are a complication.

In spite of the non-significant difference in live birth rates between CC and combination therapy, the latter group had superior ovulation rates versus CC or metformin alone (60.4% vs. 49.0% vs. 29.0%; Figure 39.3) (68) and a trend to an improvement in the pregnancy rate (absolute difference = 7.2%) following use of CC + metformin versus CC. There were some important reductions in body mass index (BMI), testosterone, insulin, and insulin resistance in patients treated with the combination versus CC alone.

Some of the differences in results reported in Legro et al. (68) compared with Palomba et al. (64) may have been due to the inclusion of a large percentage of patients with a BMI >30 kg/m^2. However in a *post-hoc* analysis, the largest differences in pregnancy rate and live birth rate in the CC versus CC + metformin groups were found in women with a BMI >34 kg/m^2.

In the last Cochrane systematic review by Tang et al. (69), it is clear that pregnancy and live birth rates are significantly lower when metformin is used alone compared with combination therapy (CC). However, metformin may still be useful as an adjuvant to OI. In a Finnish multicenter study, pregnancy rates increased when metformin was added from three months pretreatment and in subsequent combination therapy in the obese subgroup with PCOS (70).

To conclude, whereas the adverse features of PCOS can be ameliorated with lifestyle intervention, such as diet and exercise, some further short-term benefits related to ovulation may be derived from medication with metformin. Further studies are warranted to examine the role of metformin in managing the long-term metabolic implications of PCOS.

Pharmacokinetics

Metformin is administered orally and has an absolute bioavailability of 50%–60%, and gastrointestinal absorption is apparently complete within six hours of ingestion. Metformin is rapidly distributed following absorption and does not bind to plasma proteins. No metabolites or

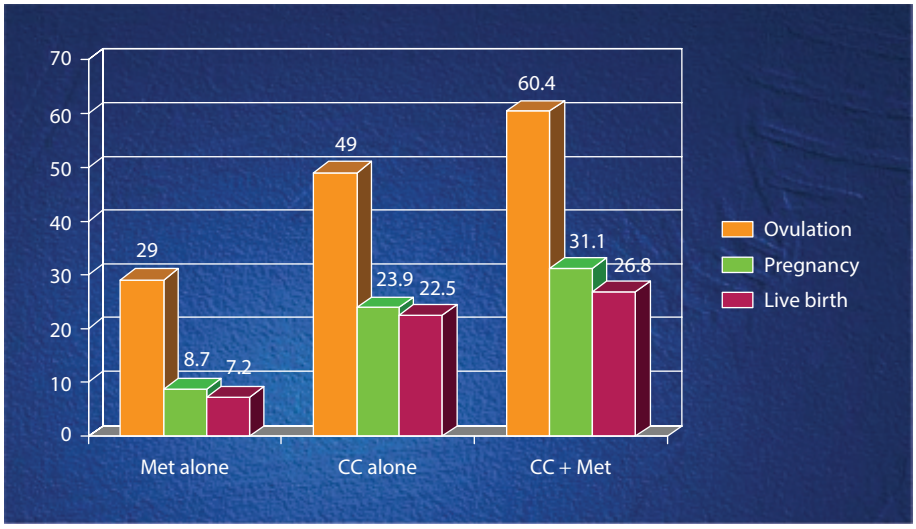

Figure 39.3 Ovulation, pregnancy, and live birth rates (%) in polycystic ovary syndrome patients treated with Met alone, CC alone, or Met + CC. *Abbreviations*: Met, metformin, CC, clomiphene citrate. (Reproduced from Legro RS et al., *N Engl J Med* 2007; 356: 551–66, with permission.)

conjugates of metformin have been identified. Metformin undergoes renal excretion and has a mean plasma elimination half-life after oral administration of between 4.0 and 8.7 hours. Food decreases the extent of and slightly delays the absorption of metformin.

Side effects and safety

In one U.S. double-blind clinical study of metformin in patients with type 2 diabetes, the most reported adverse reactions (reported in >5% patients) following metformin use were diarrhea (53%), nausea/vomiting (25.5%), flatulence (12.1%), asthenia (9.2%), indigestion (7.1%), abdominal discomfort (6.4%), and headache (5.7%). Overall, metformin use in women of reproductive age has an assured safety record (71).

GONADOTROPINS

Human chorionic gonadotropin: The LH surge surrogate

Owing to inconsistency of the spontaneous LH surge in COS, and its inefficacy in patients being treated with GnRH agonists, human chorionic gonadotropin (hCG) has been uniformly adopted by all successful ovarian stimulation programs to effect the final triggering of ovulation. When preovulatory follicles are present, administration of hCG is followed by granulosa cell luteinization, a switch from estradiol to P synthesis, resumption of meiosis and oocyte maturation, and subsequent follicular rupture 36–40 hours later. These processes will occur only if the follicle is of appropriate size and granulosa and theca cell receptivity is adequate, depending on LH receptor status.

hCG has been used as a surrogate LH surge because of the degree of homology between the two hormones. Both LH and hCG are glycoproteins with a molecular weight of approximately 30 kDa, and both have almost identical α-subunits and a high cysteine content (Figure 39.4). Most importantly, they have the same natural function (i.e., to induce luteinization and support lutein cells). Major differences include the sequence of the β-subunit, the regulation of secretion of both hormones, and the pharmacokinetics of clearance of hCG as opposed to LH (Table 39.2) (72,73).

The plasma metabolic clearance rate of hCG is slower than that of LH (i.e., a rapid disappearance phase in the first five to nine hours after intramuscular [i.m.] injection and a slower clearance rate in the 1–1.3 days after administration) (Figure 39.5) (74). The calculated initial and terminal half-lives of recombinant hCG are 5.5 + 1.3 and 3.1 + 3.0 hours, respectively, as opposed to 1.2 + 0.2 and 10.5 + 7.9 hours, respectively, for r-hLH, as determined after intravenous (i.v.) administration of the drugs (73). By day 10 after administration, <10% of the originally administered hCG was measurable (75). Some authors have advocated the presence of a serum factor directed against hCG preparations, which significantly prolongs the half-life of hCG administration to women who have received repeated courses of gonadotropins (76). Others have not found such a correlation (75). Ludwig et al. suggested that the main differences between LH and hCG lie within the N-linked oligosaccharides and the C-terminal sequence, in which the latter, and especially the O-linked oligosaccharides in this peptide, are responsible for the longer half-life of hCG compared with LH (77).

It is of interest that hCG does not inhibit the subsequent spontaneous LH surge by the intact pituitary, confirming that an ultrashort loop feedback of LH (here hCG) with its own secretion is not functional (78–80).

It has been found that elevated P levels immediately after hCG administration subsequently induce pituitary LH surges in CC/human menopausal gonadotropin (hMG) cycles (78).

The long serum half-life of hCG is likely to be an undesirable characteristic in clinical practice. Residual hCG may be mistaken for early detection of *de novo* synthesis

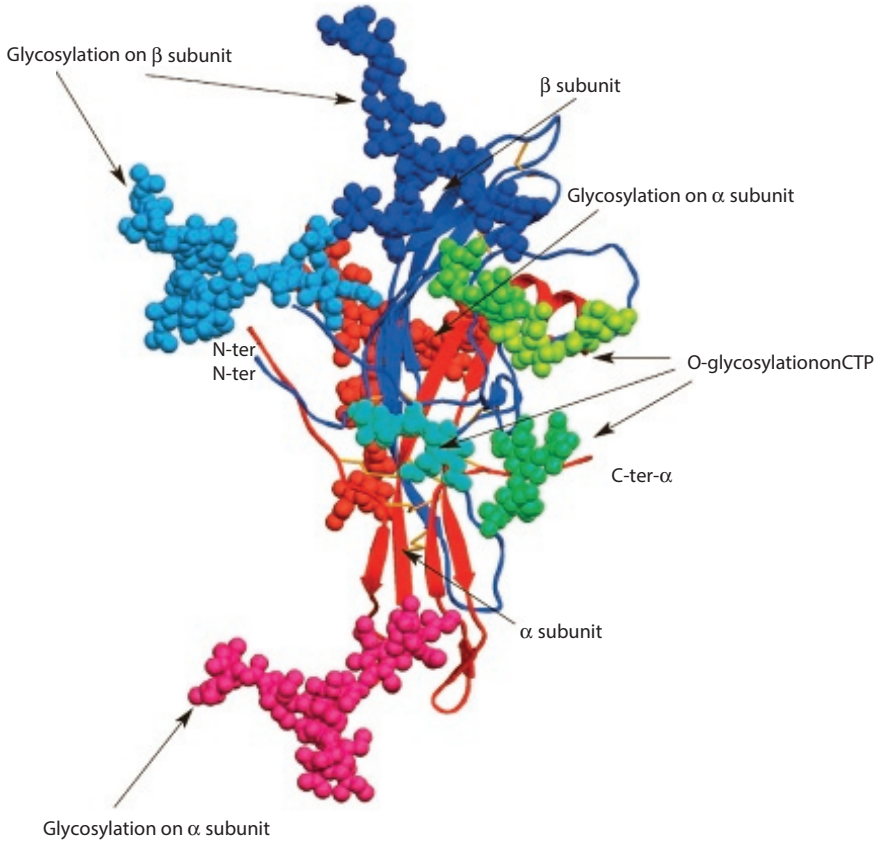

Figure 39.4 Human chorionic gonadotropin (hCG) model. Computerized model of hCG with full glycosylation and CTP. *Abbreviation*: CTP, cytidine triphosphate. (This model was created and provided by the scientific department of Serono Laboratories, U.S.A.)

Table 39.2 Luteinizing hormone (LH) and human chorionic gonadotropin (hCG) pharmacokinetics and characteristics. Pharmacokinetics of recombinant human LH (rLH), urinary human menopausal gonadotropin (u-hMG), urinary hCG (u-hCG), and recombinant hCG (r-hCG)

Test drug	r-hLH	u-hMG	u-hCG	r-hCG
Subjects (n)	12	12	12	12
Route	i.v.	i.v.	i.v.	i.v.
Dose (IU)	300	300	5000	5000
C_{max}[a] (IU/l)	32.1 ± 5.0	24.0 ± 4.2	906 ± 209	1399 ± 317
$t_{1/2}$ (1)[a] (h)	0.8 ± 0.2	0.7 ± 0.2	5.5 ± 1.3	4.7 ± 0.8
$t_{1/2}$[a] (h)	10.5 ± 7.9	12.4 ± 12.3	31 ± 3	28 ± 3

Source: Modified from le Cotonnec JY et al. *Fertil Steril* 1998; 69: 189–94; Trinchard-Lugan et al. *Reprod Biomed Online* 2002; 4: 106–15.
Note: Results are expressed as mean ± SD.
[a] Based on serum concentrations measured with immunoradiometric assay (mean ± SD).
Abbreviations: i.v., intravenous; C_{max}, maximum concentration; $t_{1/2}$ (1), initial half-life; $t_{1/2}$, terminal half-life.

of hCG by a newly implanted pregnancy. Additional consequences of hCG administration are the sustained luteotropic effect, development of multiple corpora lutea, and supraphysiologic levels of estradiol and P synthesis. Sustained high-level stimulation of the corpora lutea may lead to OHSS, a major complication of gonadotropin therapy (81). Administration of hCG results in an increase in LH-like activity, but does not reconstitute the midcycle physiologic FSH surge. Another disadvantage of hCG versus the physiologic LH surge is that of higher luteal-phase levels of estradiol and P induced by supraphysiologic hCG concentrations. Excessive levels of circulating estradiol have been implicated in the relatively high rates of implantation failure and early pregnancy loss observed in ovarian stimulation programs (82,83). Another possible disadvantage of the prolonged activity of hCG is that of small-follicle, delayed ovulation, which could be the cause of the development of multiple pregnancies.

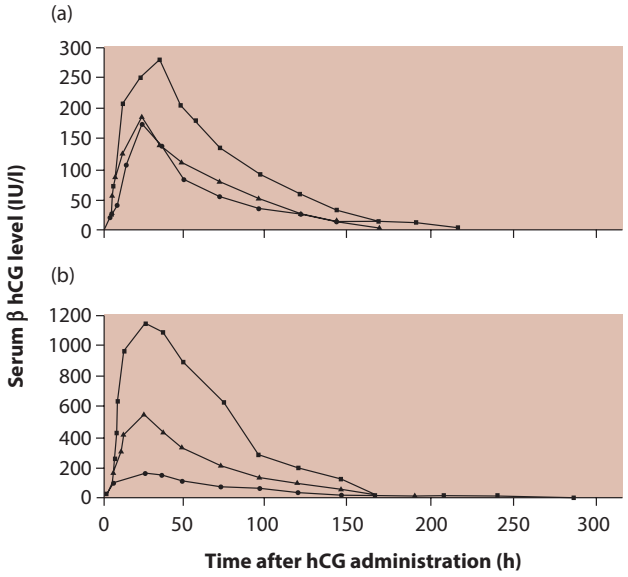

Figure 39.5 Pharmacokinetics of serum β-hCG in two hypogonadotropic women: (a) the first woman; (b) the second woman. Three regimens of hCG injections were applied in each woman: 10,000 IU administered subcutaneously or intramuscularly, and 5000 IU administered intramuscularly. *Abbreviation*: hCG, human chorionic gonadotropin. (Modified from Weissman et al. *Gynecol Endocrinol* 1996; 10: 273–6.)

Almost universal use of GnRH agonists and pituitary desensitization protocols has made the fear of untimely LH surges relatively obsolete; hence, the timing of the LH-like stimulus with hCG has been given greater flexibility. Tan et al. (84) actually showed that there was no difference in cycle outcome with random timing of hCG administration over a three-day period. Unfortunately, invalidation of the pituitary mechanism that releases us from an inappropriate LH surge has also made us completely dependent on hCG, with all its inherent problems, for the final stage of ovulation triggering.

Another issue requiring clarification is the minimal effective dose of hCG in order to trigger oocyte maturation and ovulation. In a study examining the minimal effective dose of hCG in IVF (85), dosages of 2000, 5000, and 10,000 IU of urinary hCG (u-hCG) were administered to 88, 110, and 104 women, respectively. No differences in oocyte recovery were noted when comparing the groups that received 5000 and 10,000 IU. However, a significantly lower number of oocytes were aspirated in the 2000-IU group, compared with the 5000- and 10,000-IU groups.

With the development of recombinant technology, r-hCG became available for clinical use, and is as efficacious as u-hCG with the benefit of improved local tolerance (75,86,87). A study in IVF (87) showed that r-hCG 250 μg is at least as effective as 5000 IU of u-hCG. The use of a higher dose of r-hCG, such as 500 μg, resulted in the retrieval of more oocytes, but also a three-fold increase of OHSS. The local reaction at the injection site was significantly better than to the urinary product of equal dose (77). A total of 33 different nongonadotropin proteins have been recently identified (using classical proteomic analyses) as contaminants in two commercially available preparations of u-hCG (88). Moreover, human prion peptides were detected in u-hCG (but were not identified in r-hCG) (88).

Gonadotropins: Historical overview

In 1927, Aschheim and Zondek discovered a substance in the urine of pregnant women with the same action as the gonadotropic factor in the anterior pituitary (89). They called this substance gonadotropin or "prolan." Furthermore, they believed that there were two distinct hormones, prolan A and prolan B. They subsequently used their findings to develop the pregnancy test that carries their names. In 1930, Zondek reported that gonadotropins were also present in the urine of postmenopausal women (90), and in the same year, Cole and Hart found gonadotropins in the serum of pregnant mares (91). This hormone, pregnant mare serum gonadotropin, was found to have a potent gonadotropic effect in animals. However, it was only in 1937 that Cartland and Nelson were able to produce a purified extract of this hormone (92). It was not until 1948, as a result of the work of Stewart, Sano, and Montgomery, that gonadotropins in the urine of pregnant women were shown to originate from the chorionic villi of the placenta, rather than the pituitary. It was subsequently designated "chorionic gonadotropin" (93). After years of experimental tests, it gradually became apparent that the pituitary factor was needed for the production of mature follicles, and that chorionic gonadotropin could induce ovulation only when mature follicles were present (94). Within years, it became apparent that the use of gonadotropin extracts from non-primate sources was of limited clinical value owing to the development of antibodies that neutralized their therapeutic effect. In 1947, Piero Donini, a chemist at the Pharmaceutical Institute, Serono, in Rome tried to purify hMG from postmenopausal urine. This purification method was based on a method used by Katzman et al., published in 1943 (95). The first urine extract of gonadotropin contained LH and FSH and was named Pergonal, inspired by the Italian words "per gonadi" (for the gonads) (96). The approval to sell Pergonal was first granted by the Italian authorities in 1950 (Table 39.3). Only in 1961, with Pergonal treatment, was the first pregnancy achieved in a patient with secondary amenorrhea, which resulted in the birth (in 1962 in Israel) of the first normal baby girl (97). Urinary FSH (Metrodin) and highly purified FSH became available with the development of new technologies using specific monoclonal antibodies to bind the FSH and LH molecules in the hMG material in such a way that unknown urinary proteins could be removed. Metrodin has a specific activity of 100–200 IU of FSH/mg of protein, whereas Metrodin-HP (highly purified) has an activity of approximately 9000 IU/mg of protein.

Table 39.3 Milestones of development in infertility treatment

Year	Development
1927	The discovery of pituitary hormone controlling ovarian function
1959	Purification and clinical use of pituitary and urine gonadotropins
1960	Clinical use of clomiphene citrate
1966	Use of clomiphene citrate and gonadotropin becomes common practice
1970	Development of radioimmunoassay for measuring hormone levels
1978	Ultrasound imaging of ovarian follicles
1984	Use of gonadotropin-releasing hormone agonists in infertility treatment
1985	Further purification of urinary gonadotropins
1990	Use of recombinant gonadotropins

Human menopausal gonadotropin

hMG contains an equivalent amount of 75 IU FSH and 75 IU LH *in vivo* bioactivity. Cook et al. (98) demonstrated that hMG preparations also contain up to five different FSH isohormones and up to nine LH species. These differences may cause discrepancies in patients' responses, which are occasionally observed when using various lots of the same preparation.

FSH, which is the major active agent, accounts for <5% of the local protein content in extracted urinary gonadotropin products (99). The specific activity of these products does not usually exceed 150 IU/mg protein. The different proteins found in various hMG preparations include tumor necrosis factor binding protein I, transferrin, urokinase, Tamm–Horsfall glycoprotein, epidermal growth factor, and immunoglobulin-related proteins (100). Local side effects, such as pain and allergic reactions, have been reported and attributed to immune reactions related to nongonadotropin proteins (101).

Technological improvements in recent years have resulted in the introduction of highly purified (HP)-hMG, which can be administered subcutaneously (s.c.). Highly purified hMG contains more hCG and less LH than does traditional hMG (102). Accordingly, hMG and HP-hMG induce different follicular development profiles (102). A total of 34 co-purified proteins were recently identified in HP-hMG products (88). Importantly, human prion peptides were also detected in hMG and HP-hMG (88,103). The identification of human prion proteins in commercially available formulations has prompted careful examination of the risk of transmission of prion disease by urinary gonadotropins (88).

Information is scarce regarding the metabolism of gonadotropin hormones. It was shown that purified preparations of hFSH, hLH, and hCG injected (i.v.) in humans had serum half-lives (as determined by bioassays) of 180–240 minutes, 38–60 minutes, and 6–8 hours, respectively.

Measuring levels of gonadotropins by *in vivo* bioassays serves to compare biologic effects of gonadotropin preparations in a quantitative manner in animals. In the extensively used Steelman–Pohley assay (104), 21-day-old female Sprague–Dawley rats are injected s.c. for three days and their ovaries weighed on the fourth day. Disadvantages of this assay are that its sensitivity is too low to detect small amounts of FSH in the serum, reproducibility is poor (+20% variation), and the procedure is cumbersome. The reliance on this assay, in effect, signifies that an ampoule of hMG, which appears to have 75 IU of FSH, may actually contain between 60 and 90 IU. Circulating levels of the gonadotropins measured at any given moment represent the balance between pituitary release and metabolic clearance. After i.v. injection, the initial half-life of urinary FSH was demonstrated to be approximately two hours (105), and the true terminal (elimination) half-life appeared to be 17 ± 5 hours. After i.m. injection of urinary FSH preparations, the half-life was estimated to be approximately 35 hours (75).

Purified FSH

Further purification of hMG substantially decreased LH-like activity, leading to a commercial purified FSH (pFSH) preparation. Metrodin was introduced in the mid-1980s and is a product from the same source as hMG, but the LH component has been removed by immunoaffinity chromatography (Figure 39.6).

Apart from obtaining a more purified product, the rationale of developing a pFSH preparation was that OI using gonadotropins in patients with elevated endogenous LH serum levels could, theoretically, preferably be performed without exogenously administered LH. It was also

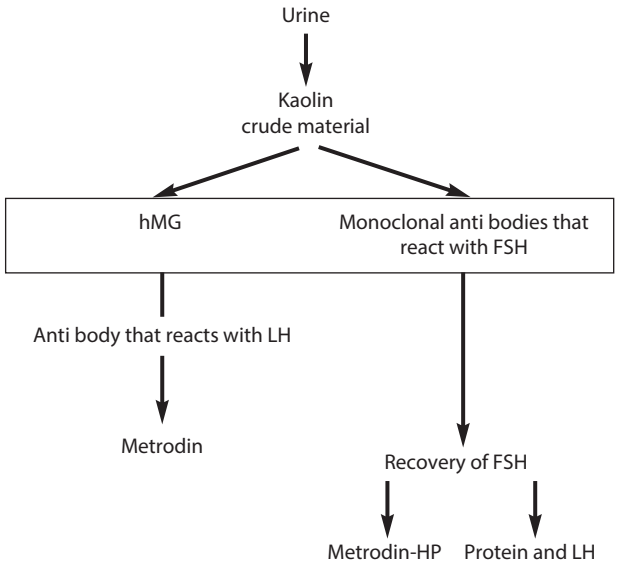

Figure 39.6 Schematic presentation of the production of hMG and the purification of urinary FSH and HP-FSH. *Abbreviations*: FSH, follicle-stimulating hormone; hMG, human menopausal gonadotropin; HP, high-purity; LH, luteinizing hormone.

suggested that FSH alone could increase folliculogenesis (106). Furthermore, it was speculated that LH in gonadotropin preparations could be responsible for the high incidence of complications in patients with elevated serum LH levels (107,108). However, other studies (109,110) have indicated that the effectiveness of gonadotropin preparations and the occurrence of OHSS were not dependent on the LH:FSH ratio (75), albeit the administration of pFSH to patients with PCOS did result in decreased LH levels compared with hMG (111).

The desirable goal of having an FSH preparation of high purity led to the development of an immunopurified product (Metrodin-HP) of >95% purity (112).

Recombinant human gonadotropins (FSH, LH, and chorionic gonadotropin)

Following the development of highly purified urinary FSH, considerable improvements have facilitated both separation of FSH from hLH and its production using recombinant technology. Early technology focused on the production of biological molecules in bacterial cells (usually *Escherichia coli*). However, the structural complexity of human gonadotropins such as FSH and the need for post-translational modification of the molecule by protein folding and glycosylation made functional protein production impossible in prokaryotes. Thus, a mammalian cell culture system was employed, with functional molecules being produced in Chinese hamster ovary (CHO) cells.

The world's first r-hFSH (follitropin-α) preparation for clinical use was produced by Serono Laboratories in 1988, and was licensed for marketing in the European Union as GONAL-f in 1995. An r-hFSH (follitropin-β; Puregon) product was also licensed by Organon Laboratories in 1996. The genes for the other gonadotropins have also been transfected into mammalian cell lines, and r-hLH and r-hCG are now commercially available (r-hLH as Luveris, Merck, Germany; r-hCG as Ovidrel/Ovitrelle, Merck; and r-hFSH and r-hLH in a 2:1 ratio, Pergoveris, Merck). However, the following description of manufacturing techniques and physicochemical properties will focus on r-hFSH (follitropin-α).

The production of hFSH by recombinant technology required isolation and cloning of genes for two subunits, the α-subunit—which is also common to hLH and hCG—and a hormone-specific β-subunit. Appropriate vectors were prepared and transfected into suitable immortalized mammalian cell lines. The cell line originally chosen by Serono Laboratories was well established (CHO–DUKX), and already being used to produce proteins such as recombinant human erythropoietin. These cells are normally dihydrofolate reductase deficient, and therefore sensitive to tetrahydrofolate analogs such as methotrexate. Cells were co-transfected with the human α and β FSH genes and then treated with methotrexate, in order to select successfully transfected cells that could express the newly introduced genes.

A stable line of transformed cells was selected, which secreted high quantities of r-hFSH. These cell lines were used to establish a master cell bank (MCB), which now serves as the source of working cell banks (WCBs). The MCB consists of individual vials containing identical cells, which are cryopreserved until required. Thus, a continuous supply of r-hFSH with guaranteed consistency from WCB to WCB is now available by expansion of cells recovered from a single vial of the MCB (1). MCBs and WCBs are routinely tested for sterility, mycoplasma, and viral contamination.

Quantifying and standardizing gonadotropin content

Traditionally, quantification of hFSH, LH, and hCG for clinical use has involved the use of *in vivo* bioassays. For hFSH, a number of bioassays have been assessed for this purpose, but one of the most robust and specific remains the Steelman–Pohley *in vivo* assay, first developed in the 1950s (104). FSH activity is quantified by rat ovarian weight gain, and FSH vials or ampoules are subsequently filled according to the desired bioactivity, measured in IUs. However, the assay has a number of limitations: it is time consuming, cumbersome, uses large numbers of rats (which is of ethical concern), and is limited in its precision—the European Pharmacopoeia defines an activity range (80%–125% of the target value) within which an FSH batch is acceptable for clinical use.

Recent advances in the manufacturing process for the r-hFSH follitropin-α, however, enable high batch-to-batch consistency in both isoform profile and glycan species distribution (113,114). The most significant advantage of this over other commercially available gonadotropins is that it permits FSH to be quantified reliably by protein content (mass in μg) rather than by biologic activity.

The coefficient of variation for an *in vivo* bioassay is typically ±20%, compared with less than 2% for physicochemical analytic techniques, such as size-exclusion HPLC (SE-HPLC) (113,114). As a result, Merck now quantify their r-hFSH (GONAL-f), r-hLH, and r-hCG protein by SE-HPLC, a precise and robust assay that results in a significant improvement in batch-to-batch consistency (115).

Physicochemical consistency of r-hFSH: Glycan mapping and isoelectric focusing

Glycan mapping provides a fingerprint of the glycan species of r-hFSH and an estimation of the degree of sialylation of the oligosaccharide chains. For each r-hFSH batch, intact glycan species are released by hydrazinolysis and labeled with a fluorescent derivative. As each glycan molecule is labeled with a single molecule of the dye, the response coefficient is the same for all glycan species, which are separated and detected by anion exchange chromatography and fluorimetry. Results are expressed as the relative percentage of the glycan species grouped as a function of their charge, which is related to the number of sialic acids they carry. The hypothetical charge number, *Z*, is defined as the sum of the percentage areas under the curve in the neutral, mono-, di-, tri-, and tetra-sialylated

glycan regions, multiplied by their corresponding charge (115). The *Z* number was demonstrated to be a very precise estimate of the degree of sialylation, with a coefficient of variation of 2% or better.

Evaluation of GONAL-f batch data over time has demonstrated a highly consistent glycoform distribution, which reflects the high consistency of its molecular profile (113,114,116). The second physicochemical technique, isoelectric focusing, is performed in a gel matrix across a pH range of 3.5–7.0. After scanning the gel, the pI values and band intensities of the sample isoforms are compared with the reference standard. The distribution of the main bands from GONAL-f has remained similar to the reference standard over time, indicating a high consistency of isoform distribution (113).

Follitropin-α filled by mass

Between-batch analysis of the ratio of GONAL-f bioactivity, measured in IU using the Steelman–Pohley assay, and protein content, measured in μg by SE-HPLC, has demonstrated a stable, normal distribution of specific activity with no bioreactor run effect (113). Similarly, drug substance production data over time also confirmed the well-controlled behavior and consistency of the GONAL-f manufacturing process (113,114). The highly consistent physicochemical and biologic properties of the product now permit FSH quantification by SE-HPLC, and vials or ampoules can be filled by mass (FbM) rather than by specific bioactivity. This product is referred to as GONAL-f FbM (Merck).

Once the physicochemical consistency of GONAL-f FbM had been demonstrated, the clinical relevance of the improved manufacturing process was assessed. A total of 131 women were enrolled into a multicenter, double-blind, randomized, parallel-group study comparing the efficacy and safety of four batches each of GONAL-f FbM and GONAL-f filled and released by IU (FbIU) in stimulating multiple follicular development prior to IVF (117). Adequate levels of ovarian stimulation were achieved with both preparations, resulting in a large number of embryos. The clinical pregnancy rate per treated cycle was 30.3% with the FbM preparation compared with 26.2% with FbIU. Both preparations showed similar levels of adverse events. However, it is the consistency of clinical response between batches that is of particular importance to physicians. The study demonstrated that the improved manufacturing process for the FbM over the FbIU preparation was associated with an improvement in the consistency of ovarian response ($p < 0.039$), including significantly improved between-batch consistency in the clinical pregnancy rate ($p < 0.001$). Compared with GONAL-f FbIU, the FbM preparation reduced the between-batch variability in clinical outcome.

Similar results were also demonstrated in larger studies in ART and OI of GONAL-f FbM versus FbIU (118–122). In a retrospective study by Balasch et al. (118), the clinical results during the introduction of GONAL-f FbM were compared with standard GONAL-f FbIU. The study included the last 125 patients treated with GONAL-f FbIU and the first 125 patients receiving GONAL-f FbM for ART ovarian stimulation. The patient demographics, oocyte yield, the number of metaphase II oocytes, and the fertilization rates were similar in both groups of patients. However, embryo quality as assessed on day 2 and implantation rates were significantly higher (18.6% vs. 28.6%, $p = 0.008$) in the r-hFSH FbM group. Accordingly, in spite of the mean number of embryos transferred being significantly lower in the r-hFSH FbM group, there was a trend for higher clinical pregnancy rates (44% vs. 35.2%) in this group of patients. In a large U.K. multicenter observational study carried out using GONAL-f FbM in 1427 ART patients (119), the safety and efficacy of GONAL-f FbM was confirmed in routine clinical practice. The patients' mean age was 34.3 years and an average of 10.3 oocytes were retrieved. Only 2.7% of the patients who started FSH therapy did not receive hCG. The incidence of severe OHSS was 0.4% and the clinical pregnancy rate per cycle was 29.2%.

In the OI study (120), following use of GONAL-f FbM versus FbIU, fewer patients required an adjustment in the FSH dose (37% vs. 60%) and there were fewer canceled cycles (13% vs. 21%) during treatment using a chronic low-dose protocol. Hence, the quality of gonadotropin preparation may play an important role in the consistency of the clinical response, including a reduction in the cycle cancellation (122).

Introduction of biosimilar follitropin-α preparations

Twenty years after the launch of the first r-hFSH preparations (follitropin-α and -β), the field of reproductive medicine is at another very important crossroads, the introduction of "biosimilar" FSH preparations, which take innovation at a device level to a new high. For example, Bemfola (follitropin-α, Finox AG, Switzerland), which became commercially available in March 2014 in the EU, is available in a single daily dose pen device. It is a biosimilar (i.e., a medicine that has been demonstrated, through an exhaustive series of physicochemical, *in vitro*, and *in vivo* tests and confirmatory Phase I (123) and Phase III studies (124)) to be similar/equivalent in quality, safety, and efficacy to the reference medicinal product GONAL-f by the European Medicines Agency (EMA). In other words, it bears essentially the same active pharmaceutical ingredient, to be used at the same dose, via the same route for the same indications as the reference product GONAL-f. It has been postulated—incorrectly—that as a biosimilar FSH has a different FSH isoform profile than the originator FSH, it will have different therapeutic efficacy and safety (125). Actually, slight variability due to post-translational modifications can occur in any originator product batch (126). It is therefore expected based on the reference product batches that the glycosylation pattern of a biosimilar and reference product will not be identical. This is not a new discussion in reproductive medicine. At the European launch in 1996 of follitropin-α (GONAL-f) and follitropin-β (Puregon/Follistim), efforts were made to differentiate the two products based on "significant

differences" in their respective isoform profiles (127). Both products differed in terms of mammalian cell line employed, the method of gene transfection, purification procedure, and formulation, but eventually numerous comparative studies, registries, and retrospective studies demonstrated that the two products were exactly the same in terms of efficacy (oocytes, embryos, pregnancies, and live births) and safety (incidence of OHSS) (128–130). Interestingly based on these differences in structure, between GONAL-f and Puregon/Follistim, the latter would never have been considered comparable and hence registered under the biosimilars regulatory pathway.

Into the reproductive therapeutic arena, another biosimilar follitropin-α (Ovaleap, TEVA, The Netherlands) has recently become commercially available. Doses of the product are delivered using a multidose pen device similar to that used for follitropin-β (Puregon).

Follitropin-δ

The most recent recombinant FSH (FE 999049) to enter clinical development has been derived using a cell line of human fetal retinal origin. The amino acid sequences of the α- and β-subunits of are identical to that of natural human FSH, but the sialic acid content of the FSH molecule is higher. Studies in healthy women volunteers comparing the pharmacokinetic and pharmacodynamic properties of FE 999049 to follitropin-α showed that FE 999049 has a longer elimination half-life (30 vs. 24 hours) and induces a higher ovarian response when administered at equal doses of biological activity (131). Based on these differences and a Phase II trial (132), an algorithm was developed for dosing based on anti-Mullerian hormone (AMH) and weight (kg) of the IVF patient.

The results of a Phase III study using this dosing algorithm were recently published (133). In this assessor-blind study using a GnRH antagonist protocol, different doses of FE 999049 were administered daily according to an AMH–weight algorithm versus a standard dose of 150 IU per day of follitropin-α in women aged 18–40 years of age. In the FE999049 arm the dose was fixed, but with follitropin-α the dose could be increased up to a maximum of 450 IU from day 6 of stimulation. A total of 40% of women recruited in both arms were aged 35 years or older.

In spite of a fixed starting dose of 150 IU follitropin-α in all patients irrespective of their age (and hence AMH) compared to an individualized approach of FE 999049 dosed according to AMH and weight, the main efficacy and safety results were similar and there were no significant differences in oocytes retrieved (10.4 ± 6.5 vs. 10 ± 5.6), clinical pregnancies (31.6% vs. 30.7%), incidence of moderate/severe OHSS (1.4% vs. 1.4%), or hospitalization due to OHSS (0.9% vs. 0.3%). However, the authors reported under safety outcomes a significantly higher number of "preventive interventions" for follitropin-α (30 vs. 15; $p = 0.005$). It would have been more relevant for current clinical practice if the study design had allowed individualized dosing with follitropin-α, as this would have given a more balanced assessment of the relative merits of follitropin-δ. In December 2016, follitropin-δ (Rekovelle, Ferring Pharmaceuticals, U.K.) was granted marketing authorization in EU member countries for use in COS for the development of multiple follicles in women undergoing ART use, such as an IVF or ICSI cycle.

Corifollitropin-α

The range of recombinant gonadotropins available for the treatment of subfertility has been expanded through protein engineering. A FSH molecule has been engineered to possess an extended half-life and duration of therapeutic action. This long-acting protein, designated FSH–C-terminal peptide (FSH–CTP, corifollitropin-α), was first described by Bouloux and colleagues in 2001 (134). FSH–CTP consists of the α-subunit of r-hFSH together with a hybrid β-subunit made up of the β-subunit of hFSH and the C-terminal part of the β-subunit of hCG. FSH–CTP has a longer half-life than standard r-hFSH. FSH–CTP initiates and sustains follicular growth for one week, so one dose can replace the first seven daily injections of gonadotropin in COS. A single dose of FSH–CTP induces multi-follicular growth accompanied by a dose-dependent rise in serum inhibin-B (135). The first live birth resulting from a stimulation cycle with FSH–CTP was reported in 2003 (136), and further studies have been carried out in subfertile patients undergoing ART and OI (137–142). FSH–CTP is now approved for use in Europe in ART cycles in combination with a GnRH antagonist.

Two large studies were conducted to demonstrate the non-inferiority of FSH–CTP to r-hFSH (follitropin-β) (140,141). A multicenter, randomized, double-blind, double-dummy clinical trial involving 34 centers and 1506 patients weighing 60–90 kg was initially performed (ENGAGE study) (141). Patients undergoing ART cycles in a standard GnRH antagonist protocol received a single dose of FSH–CTP 150 μg or daily doses of r-hFSH 200 IU during the first week of stimulation. Ongoing pregnancy rates per cycle initiated were not significantly different for FSH–CTP or r-hFSH (38.9% vs. 38.1%, respectively; estimated difference 0.9; $p = 0.71$). The reported incidence of moderate/severe OHSS was 4.1% with corifollitropin-α versus 2.7% with follitropin-β (141).

A further study was conducted to evaluate the efficacy and safety of FSH–CTP in women with low body weight. The ENSURE study was a multicenter, randomized, double-blind, double-dummy clinical trial involving 19 centers and 396 patients weighing <60 kg undergoing ART (141). Patients undergoing ART in a standard GnRH antagonist protocol received a single dose of FSH–CTP 100 μg or daily doses of r-hFSH 150 IU during the first week of stimulation. The primary endpoint—the mean (standard deviation [SD]) number of oocytes retrieved per started cycle—was 13.3 (7.3) with FSH–CTP compared with 10.6 (5.9), which was within the predefined equivalence range (–3 to +5 oocytes). The reported incidence of moderate or severe OHSS was 3.4% for corifollitropin-α and 1.6% for follitropin-β (140).

FSH–CTP was developed with the aim of simplifying ART treatment regimens. However, there were concerns regarding the high incidence of OHSS associated with FSH–CTP in published studies and in clinical practice (140,141). Investigators of the multicenter, open-label, Phase III TRUST study (designed to assess the immunogenicity of repeated exposure to FSH–CTP) raised concerns regarding the high rate of severe OHSS among their patients (143). Six of nine patients who received corifollitropin-α at a single center developed severe OHSS three to five days after hCG administration (143). Three patients were hospitalized for several days and one experienced a pulmonary embolism despite appropriate therapy (143). In the TRUST study, 25 patients discontinued treatment after the first or second cycle because of an excessive response to COS or signs or symptoms of OHSS (142). The overall rate of moderate/severe OHSS in the study was 1.8% in cycle 1, 1.0% in cycle 2, and 0% in cycle 3 (142). The effects of FSH–CTP cannot be adjusted to individual patient requirements (143); therefore, careful assessment of patient suitability is required before treatment is commenced.

Because of some of these concerns, the recent focus of research has been in the use of corifollitropin-α in ART patients with a known poor response to FSH (144–148).

In the most recent study (149), the authors examined the effect of corifollitropin-α followed by 300 IU daily hMG in a short flare-up GnRH agonist and also in a long GnRH agonist protocol in poor responders. They found no significant difference in live birth rates and concluded that both of these protocols are feasible options.

Finally, in the most updated Cochrane systematic review (150), the authors concluded that medium doses (150–180 μg) of long-acting FSH were safe and as effective as daily FSH in women with unexplained subfertility. However, there was evidence of a reduced live birth rate in women receiving lower doses (60–120 μg) compared to daily FSH.

OPTIMIZING OUTCOMES OF OVARIAN STIMULATION

Safety profile of gonadotropins

Accumulation of data on 1160 babies born after induction of ovulation with gonadotropins (31) revealed that major and minor malformations were found in 63 infants, representing an overall incidence of 54.3/1000 (major malformations 21.6/1000; minor malformations 32.7/1000). This rate of malformation is not significantly different from that of the general population.

Outcomes achieved with r-hFSH versus hMG

r-hFSH and hMG are the gonadotropins that are most frequently used for COS with IVF/ICSI. Outcomes achieved using these gonadotropins have been compared over many years in numerous retrospective studies, RCTs, and meta-analyses. Accumulating data suggest that all commercially available gonadotropins have similar efficacy and safety profiles (151). Indeed, there appears to be little overall difference between r-hFSH and hMG in outcomes of fresh ART cycles.

In 2003, Al-Inany et al. published a meta-analysis that compared r-hFSH with urinary FSH products (hMG, pFSH, and HP-FSH) in IVF/ICSI cycles using a long GnRH agonist protocol (152). Four of the 20 studies compared hMG with r-hFSH and showed no significant difference between hMG (n = 603 cycles) and r-hFSH (n = 611 cycles) in terms of clinical pregnancy rate per cycle initiated (OR = 0.81, 95% CI 0.63–1.05; p = 0.11) (153–156). A different meta-analysis from 2003 included six RCTs (n = 2030) of women undergoing COS for IVF/ICSI (157). Pooling of data from five RCTs that used a long GnRH agonist protocol showed that hMG resulted in significantly higher clinical pregnancy rates versus r-hFSH (RR = 1.22, 95% CI 1.03–1.44). However, there was no difference between groups in ongoing pregnancy rates or live births (RR = 1.20, 95% CI 0.99–1.45). A related Cochrane systematic review from 2003 also showed no difference in pooled data from four true RCTs in ongoing pregnancy/live birth rate per woman (OR = 1.27, 95% CI 0.98–1.64) (158).

In 2005, Al-Inany et al. published an updated meta-analysis involving eight RCTs and 2031 participants. They showed no significant differences between hMG and r-hFSH in ongoing pregnancy/live birth rate, clinical pregnancy, miscarriage, multiple pregnancy, or moderate/severe OHSS (159). This group published a third meta-analysis in 2008 including 12 trials involving 1453 hMG cycles and 1484 r-hFSH cycles. They showed a significantly higher live birth rate with hMG versus r-hFSH (OR = 1.2, 95% CI 1.01–1.42; p = 0.04) and similar rates of OHSS in each group (OR = 1.21, 95% CI 0.78–1.86; p = 0.39) (160). Also in 2008, Coomarasamy et al. selected seven RCTs that used a long GnRH agonist protocol (161). A significant increase in live births per woman randomized was found in favor of hMG versus r-hFSH (RR = 1.18, 95% CI 1.02–1.38; p = 0.03) (161). In 2009, Al-Inany et al. published a meta-analysis of six trials involving 2371 participants comparing HP-hMG and r-hFSH in women undergoing IVF/ICSI (102). No significant difference in the overall ongoing pregnancy/live birth rate was found between the groups. However, when IVF cycles were analyzed alone, a significantly higher ongoing pregnancy/live birth rate was found in favor of HP-hMG (OR = 1.31, 95% CI 1.02–1.68; p = 0.03) (102).

The largest meta-analysis of r-hFSH and hMG to date was published in 2010, and included data from 16 RCTs involving 4040 patients undergoing fresh ART cycles (162). The primary endpoint of this analysis was the number of oocytes retrieved, which was selected in order to estimate directly the gonadotropin effects during COS. A recent study of more than 400,000 IVF cycles has confirmed that the number of oocytes retrieved is a robust surrogate outcome for clinical success (163). This large meta-analysis showed that r-hFSH resulted in the retrieval of significantly more oocytes versus hMG (p < 0.001), and a significantly lower dose of r-hFSH versus hMG was required (p = 0.01) (162). No significant difference was observed in baseline adjusted pregnancy rates (RR = 1.04; p = 0.49) or in OHSS (RR = 1.47; p = 0.12).

Individualization of ovarian stimulation

The objective of fertility treatment is the same for all women—optimization of outcomes with minimization of risks. It has become clear that the "one-size-fits-all" approach to fertility treatment is too simplistic, as each woman's ovarian response to stimulation is highly variable (164). Indeed, the use of flexible gonadotropin dosing during ovarian stimulation is now believed to be essential to optimizing cycle outcomes (164).

Accurate prediction of extremes of ovarian response prior to COS would allow tailoring of treatment in the first treatment cycle (164,165). Numerous biomarkers predictive of ovarian reserve and response to treatment have been proposed (165–167). Moreover, various algorithms have been developed in order to calculate the optimum FSH starting dose (165,168). The CONSORT treatment algorithm attempted to predict the optimum dose of r-hFSH (follitropin-α) for ART cycles based on individual patient characteristics: age, BMI, basal FSH, and antral follicle count (AFC). This algorithm resulted in an adequate oocyte yield, good pregnancy rate, and low incidence of OHSS. However, cycle cancelation due to an inadequate response occurred frequently in the lowest evaluable dose group (75 IU/day) (165).

Other factors that have been studied as potential predictors of ovarian response to COS include basal FSH, inhibin-B, estradiol, ovarian volume and vascular flow, and AMH. A number of studies have demonstrated the value of AMH, a marker of the total developing follicular cohort and the growth of small follicles in the ovary, in predicting ovarian response (169–173). AMH has been shown to correlate significantly with oocyte yield and live birth (171), as well as to predict excessive response to COS (170). A nomogram for the decline in serum AMH with age has been constructed and will facilitate counseling of patients regarding reproductive potential (174,175). Assessment of ovarian reserve by AMH before the first cycle of COS may provide a useful approach to individualizing treatment.

Efforts have also been made to identify markers that accurately predict response to the OI regimen in order to improve the safety, efficiency, and convenience of treatment for women with WHO group II anovulatory infertility (176,177). The selection of an appropriate starting dose of r-hFSH would allow physicians to individualize established treatment protocols (176). This could potentially shorten the time taken to reach the ovulation triggering threshold and reduce the risk of cycle cancelation because of extreme responses to gonadotropins (176). However, attempts to identify factors predictive of response to OI have had limited success (177,178). A number of investigators have identified BMI as a marker of response to exogenous FSH and ovulation rates (177,179). The importance of BMI as a major determinant of successful ovulation was confirmed in a recent analysis of data from normogonadotropic, oligoovulatory, or anovulatory women undergoing an OI using a chronic low-dose, step-up treatment regimen (176). In addition, AFC and basal serum FSH concentration were shown to be associated with the response to treatment (176).

An individualized approach to ovarian stimulation is likely to result in optimal treatment outcomes (164). Determination of the most appropriate single drug or combination of drugs for ovarian stimulation, the daily dose, and the duration of treatment is expected to enhance safety and cost-efficacy (164). Indeed, the identification of groups of patients who are likely to benefit from each available management strategy is essential (164). Such an approach would incorporate a wide variety of options based on the anticipated ovarian response.

Adjunctive therapies

Supplementation of FSH with LH, growth hormone or androgens may also help to improve the ovarian response, and this is discussed in depth in Chapter 44. The use of supplementary LH has attracted the most interest in recent years. The classic "two-cell–two-gonadotropin" model proposed that both FSH and LH are required for estradiol synthesis. LH binds to theca cells to induce synthesis of androgens, which diffuse out into the circulation and into the granulosa cells where, through the FSH-stimulated action of aromatase, they are converted to estrogen (180). Thus, LH regulates and integrates both granulosa and theca cell function during late preovulatory development. At this stage, FSH and LH work together to induce local production of growth factors needed for the paracrine regulation of follicular maturation.

LH supplementation is needed for healthy follicular development and oocyte maturation in patients with HH. In patients with HH, stimulation with FSH alone was significantly less effective than stimulation with FSH plus LH in a study by the European Recombinant Human LH Study Group (181). Based on these results, a product containing a fixed combination of r-hFSH and r-hLH in a 2:1 ratio (Pergoveris) was developed for follicular maturation in women with severe gonadotropin deficiency (182).

The use of GnRH agonists for pituitary down-regulation in normogonadotropic women undergoing COS may result in LH levels below those that characterize HH. LH-like activity may be provided using hMG. Studies comparing r-hFSH and hMG have been reported earlier in this chapter and generally show little difference in outcomes. Two meta-analyses of studies comparing outcomes in women receiving supplementary r-hLH with those receiving only r-hFSH also showed no differences between treatment groups (183,184). Thus, it is generally accepted that LH supplementation has no benefit in normal responders undergoing COS.

There is, however, some evidence to suggest that LH has benefits in women aged >35 years, and in poor or suboptimal responders to COS (185). A number of studies have suggested that LH supplementation may improve outcomes in cases of advanced maternal age (186–189). However, conflicting data have been reported from other studies (190). LH supplementation may also have benefits for women with a suboptimal response to stimulation, which is characterized by normal follicular development up to cycle days

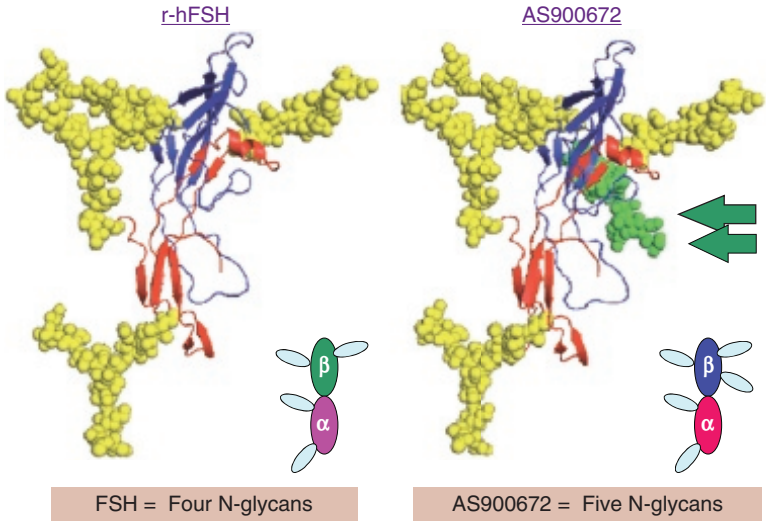

Figure 39.7 Gonadotropin-releasing hormone (GnRH) analog structure. A schematic illustration of native GnRH, GnRH agonist, and GnRH antagonist. Position 6 is involved in enzymatic cleavage, positions 2 and 3 in gonadotropin release, and positions 1, 6, and 10 are important for the three-dimensional structure. *Abbreviation*: r-hFSH, recombinant human follicle-stimulating hormone.

5–7 followed by a plateau of this response on days 8–10. Suboptimal response may be due to LH-β variant polymorphism (191), or polymorphic variants of the FSH receptor (192,193). A significant improvement in fertilization and clinical pregnancy rates has been shown with the addition of r-hLH to r-hFSH in women who required high doses of r-hFSH in previous cycles (194). A number of other studies have also shown evidence of the benefit of LH supplementation in patients with suboptimal response to FSH (195,196).

GONADOTROPIN-RELEASING HORMONE

Introduction

Control of gonadotropin secretion is exerted by hypothalamic release of GnRH, initially known as LH-releasing hormone, but the lack of evidence for a specific FSH-releasing hormone prompted a change in terminology. GnRH is produced and released from a group of loosely connected neurons located in the medial basal hypothalamus, primarily within the arcuate nucleus, and in the preoptic area of the ventral hypothalamus. It is synthesized in the cell body, transported along the axons to the synapse, and released in a pulsatile fashion into the complex capillary net of the portal system of the pituitary gland (197).

GnRH was first isolated, characterized, and synthesized independently in 1971 by Andrew Schally and Roger Guillemin, who were subsequently awarded the Nobel Prize for their achievement (198,199). GnRH is a decapeptide that, similar to several other brain peptides, is synthesized as part of a much larger precursor peptide, the GnRH-associated peptide, that has a 56-amino acid sequence. The structure of GnRH is common to all mammals, including humans, and its action is similar in both males and females. GnRH is a single-chain peptide comprising 10 amino acids with crucial functions at positions 1, 2, 3, 6, and 10. Position 6 is involved in enzymatic cleavage, positions 2 and 3 in gonadotropin release, and positions 1, 6, and 10 are important for the three-dimensional structure (Figure 39.7).

In humans, the critical spectrum of pulsatile release frequencies ranges from the shortest interpulse frequency of approximately 71 minutes in the late follicular phase to an interval of 216 minutes in the late luteal phase (200,201).

GnRH AGONIST

Mechanism of action

Although the exact cellular basis for desensitization of the gonadotroph has not been fully delineated, the extensive use of GnRH agonistic analogs in research facilitated an explosive augmentation of information and knowledge. Acute administration of GnRH agonistic analogs increases gonadotropin secretion (the flare-up effect) and usually requires 7–14 days to achieve a state of pituitary suppression. Prolonged administration of GnRH agonistic analogs leads to down-regulation of GnRH receptors. This phenomenon was first shown in 1978, when Knobil and co-workers published their classic paper demonstrating down-regulation of gonadotropin secretion by sustained stimulation of the pituitary with GnRH (202). The agonist-bound receptor is internalized via receptor-mediated endocytosis (203), with kinetics determined by the potency of the analog. The internalized complex subsequently undergoes dissociation, followed by degradation of the ligand and partial recycling of the receptors (204).

Biosynthesis

Native GnRH has a short plasma half-life and is rapidly inactivated by enzymatic cleavage. The initial concept was to create substances that prolong the stimulation of gonadotropin secretion. Analogs with longer half-lives and higher receptor activities were created by a structural change at the position of enzymatic breakdown of GnRH.

Table 39.4 The structure of gonadotropin-releasing hormone (GnRH) and GnRH agonistic analogs

Compound						6th position				10th position
Amino acid (no.)	1	2	3	4	5	6	7	8	9	10
Native GnRH	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	$GlyNH_2$
Nonapeptides										
Leuprolide						Leu				NHEt
Buserelin						$Ser(O_tBu)$				NHEt
Goserelin						$Ser(O_tBu)$				$AzaGlyNH_2$
Histrelin						D-His(Bzl)				$AzaGlyNH_2$
Decapeptides										
Nafarelin						2Nal				$GlyNH_2$
Triptorelin						Trp				$GlyNH_2$

The first major step in increasing the potency of GnRH was the substitution of glycine number 10 at the C-terminus. While 90% of the biologic activity is lost with splitting of the 10th glycine, it is predominantly restored with the attachment of NH_2-ethylamide to the proline at position 9 (205). The second major modification was the replacement of the glycine at position 6 by D-amino acids, which decreases enzymatic degradation (see Figure 39.7). The combination of these two modifications was found to have synergistic biologic activity. Agonistic analogs with D-amino acids at position 6 and NH_2-ethylamide substituting the Gly10-amide are not only better protected against enzymatic degradation, but also exhibit a higher receptor binding affinity. The affinity could be further increased by introduction of larger, hydrophobic, and more lipophilic amino acids at position 6 (Table 39.4). The increased lipophilicity of the agonist is associated with a prolonged half-life, which may be attributed to reduced renal excretion through increased plasma protein binding, or fat tissue storage of non-ionized fat-soluble compounds (205).

Thus, in all analogs, position 6 is substituted with a D-amino acid or a D-amino acid with different radicals. Insertion of D-amino acid blocks degradation and thus leads to more stability and higher receptor affinity (Table 39.4) (206). The agonists leuprolide (D-Leu6, Pr9-NHEt) and buserelin (D-$Ser(O_tBu)$6, Pr9-NHEt) contain an ethylamide, and goserelin (D-$Ser(O_tBu)$6, Pro9-$AzaGlyNH_2$) and histrelin (Nt-Bzl-D-His6, Pro9-$AzaGlyNH_2$) contain azaglycine at position 10 and are, therefore, nonapeptides. Nafarelin (D-Nal(2)6) and triptorelin (D-Trp6) contain the original Gly10-amide, and are, therefore, decapeptides.

More than 1000 GnRH analogs have been synthesized and tested, but only a few have been introduced into clinical practice. Differences between analogs are mainly related

Table 39.5 Trade names, plasmatic half-life, relative potency, route of administration, and recommended dose for the clinically available gonadotropin-releasing hormone GnRH) analogs

Generic name	Trade name	Half-life	Relative potency	Administration route	Recommended dose
Native GnRH			1	i.v., s.c.	
Nonapeptides					
Leuprolide	Lupron	90 minutes	50–80	s.c.	500–1000 μg/day
			20–30	i.m. depot	3.75–7.5 mg/month
Buserelin	Superfact, Supercur	80 minutes	20–40	s.c.	200–500 μg/day
				Intranasal	300–400 × 3–4/day
Histrelin	Supprelin	<60 minutes	100	s.c.	100 μg/day
Goserelin	Zoladex	4.5 hours	50–100	s.c. implant	3.6 mg/month
Decapeptides					
Nafarelin	Synarel	3–4 hours	200	Intranasal	200–400 × 2/day
Triptorelin	Decapeptyl	3–4.2 hours	36–144	s.c.	100–500 μg/day
				i.m. depot	3.75 mg/month

Abbreviations: i.v., intravenous; s.c., subcutaneous.

to methods of administration and potency. The available data usually describe the relative potency of a certain GnRH agonist compared with native GnRH (Table 39.5). Direct comparison between the clinically available GnRH agonists under identical conditions has never been undertaken. Therefore, translation of data from these models to humans should be performed with caution.

All GnRH agonistic analogs are small polypeptide molecules that need to be administered parenterally, as they would otherwise be susceptible to gastrointestinal proteolysis. The oral and rectal administration of analogs is associated with very low biopotency (0.0%–1% vs. parenteral administration). Intranasal spray is extremely effective, but the bioavailability is only 3%–5%, and the relatively fast elimination kinetics require frequent dosing (two to six times per day) to obtain continuous stimulation and down-regulation (207). For long-term treatment, a depot formulation is available. The drug is formulated as controlled-release depot preparations with the active substance dissolved, or encapsulated, in biodegradable material. i.m. injections provide maintained therapeutic levels for 28–35 days. Thus, monthly injections are sufficient for maintaining down-regulation.

Side effects

Side effects of GnRH agonist therapy are related to the fall in sex hormone serum concentration. As GnRH agonist interacts with GnRH receptors, which are mainly present in the pituitary, no systemic effects are common. The main symptoms of low serum concentrations of estrogen are flushes, decreased libido, impotence, vaginal dryness, reduced breast size, and emotional instability. One of the matters of concern is the effect of estrogen depletion on bone mineral density, as estrogen is of major importance in preventing the development of osteoporosis. A summary of data from different trials (208) showed that GnRH analog therapy caused significant but reversible bone loss. The mechanism appears to be similar to the development of postmenopausal osteoporosis (i.e., high bone turnover with elevated alkaline phosphatase and osteocalcin levels).

Teratogenic effects

There does not appear to be an increased risk of birth defects or pregnancy wastage in human pregnancies exposed to daily low-dose GnRH agonist therapy in the first weeks of gestation. Although placental transfer of GnRH agonists in pregnant rhesus monkeys was demonstrated, no deleterious effects were observed (209). From their toxicology studies in animals, no toxic effects were reported by the drug manufacturers (210). Although several authors claimed a normal outcome of pregnancy following inadvertent administration of a GnRH agonist during early pregnancy (211–213), Ron-El et al. (214) reported the birth of a newborn with a small soft cleft palate. Lahat et al. reported a high incidence of attention deficit hyperactivity disorder in a long-term follow-up of children inadvertently exposed to GnRH agonists in early pregnancy (215). Therefore, as this complication is purely iatrogenic, it should best be avoided.

GnRH ANTAGONIST

Mechanism of action

Antagonist analogs of GnRH have a direct inhibitory, reversible, suppressive effect on gonadotropin secretion. Antagonistic molecules compete for and occupy pituitary GnRH receptors, thus competitively blocking the access of endogenous GnRH and precluding substantial receptor occupation and stimulation. Suppression attained by GnRH antagonists is immediate (no flare-up effect), and, as receptor loss does not occur, a constant supply of antagonists to the gonadotroph is required to ensure that all GnRH receptors are continuously occupied. Consequently, compared with agonistic analogs, a higher dose range of antagonists is required for effective pituitary suppression (Table 39.6).

Synthesis of GnRH antagonists

Over the past three decades, thousands of GnRH analogs, both agonists and antagonists, have been synthesized. The first generation of antagonistic analogs were hydrophilic, and contained replacements for His at position 2 and for Trp at position 3. Inhibitory activity increased after incorporation of a D-amino acid at position 6. However, histamine release also increased, resulting in anaphylactic reactions that prevented their clinical use. In third-generation antagonistic analogs, the undesirable risks of anaphylaxis and edema were eliminated by replacing the D-Arg at position 6 by neutral D-ureidoalkyl amino acids, to produce compounds such as cetrorelix, iturelix, azaline B, ganirelix, abarelix, and antarelix (Table 39.7) (216–222).

Safety and tolerability studies

The introduction of GnRH antagonists into clinical use was delayed owing to the property of the first generation of antagonists to induce systemic histamine release and a subsequent general edematogenic state. Studies in rat mast cells confirmed that incorporation of D-Cit at position 6 of antagonists results in reduced histamine release (223,224). This characteristic of cetrorelix was first assessed in *in vitro* assays that demonstrated effective plasma concentrations to be significantly lower ($<10^3$) than the median effective dose for systemic histamine secretion, and therefore could confidently be regarded as insignificant. Owing to large disparities in such assays, cetrorelix safety was further tested in *in vivo* settings.

Table 39.6 Comparing mechanisms of action of gonadotropin-releasing hormone (GnRH) agonists and antagonists

GnRH antagonist	GnRH agonist
Receptor blockage without receptor activation	Receptor down-regulation
Competitive inhibition	Pituitary desensitization
Immediate and dose-dependent suppression	Initial flare-up
Rapid reversibility	Slow reversibility

Table 39.7 Structure formulation of native gonadotropin-releasing hormone (GnRH) and GnRH antagonists

Name	Amino acid sequence
GnRH	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
First generation	
4F Ant	NAcΔ1, 1Pro-D4FPhe-DTrp-Ser-Tyr-DTrp-Leu-Arg-Pro-GlyNH_2
Second generation	
NalArg	NACD2Nal-D4IFPhe-pTrp-Ser-Tyr-DArg-Leu-Arg-Pro-GlyNH_2
Detirelix	NACD2Nal-D4ClPhe-pTrp-Ser-Tyr-DHarg(Et2)-Leu-Arg-Pro-DAlaNH_2
Third generation	
NalGlu	NACD2Nal-D4C7Phe-D3Pal-Ser-Arg-DGlut(AA)-Leu-Arg-Pro-DAlaNH_2
Antide	NACD2Nal-D4ClPhe-D3Pal-Ser-Lys(Nic)-DDLys(Nic)-Leu-Lys(Isp)Pro-DAlaNH_2
Org30850	NACD4ClPhe-D4ClPhe-DBal-Ser-Tyr-DLys-Leu-Arg-Pro-DAlaNH_2
Ramorelix	NACD2Nal-D4ClPhe-DTrp-Ser-Tyr-DSet(Rha)-Leu-Arg-Pro-AzaglyNH_2
Cetrorelix	NACD2Nal-D4ClPhe-D3Pal-Ser-Tyr-DCit-Leu-Arg-Pro-DAlaNH_2
Ganirelix	NACD2Nal-D4ClPhe-D3Pal-Ser-Tyr-DHarg(Et2)-Leu-Harg(Et2)-Pro-DAlaNH_2
A-75998	NACD2Nal-D4ClPhe-D3Pal-Ser-NMeTyr-DLys(Nic)-Leu-Lys(Isp)-Pro-DAlaNH_2
Azaline B	NACD2Nal-D4ClPhe-D3Pal-Ser-Aph(atz)-DAph(atz)-Leu-Lys(Isp)-Pro-DAlaNH_2
Antarelix	NACD2Nal-D4ClPhe-D3Pal-Ser-Tyr-DHcit-Leu-Lys(Isp)-Pro-DAlaNH_2

Cetrorelix injected at doses of 1.5 mg/kg s.c. and 1 and 4 mg/kg i.v. into rats caused no systemic adverse effects, such as edema, respiratory dysfunction, or cardiovascular compromise. In these animal studies, no teratogenic effects or detrimental influences on implantation rates or on embryonic development were noted when administered in the periconceptional period. Several thousand human patients have been treated with third-generation GnRH antagonists (i.e., ganirelix, cetrorelix, or abarelix) without evidence of systemic or major local skin reactions, and no cessation of therapy was warranted due to side effects (223,225–229). The common side effects observed were injection site reactions and possible nausea, headache, fatigue, and malaise. No drug interactions were demonstrated *in vitro*, with medications metabolized through the cytochrome P450 pathway.

It was suggested that GnRH antagonists may adversely affect oocyte or embryo quality, or the endometrium (230–235). However, most recent evidence suggests that GnRH antagonists do not diminish oocyte or embryo quality or endometrial receptivity (236–238).

Advantages of GnRH antagonists

The use of GnRH antagonists offers a number of potential advantages over agonists (239). Prolonged pretreatment to achieve pituitary down-regulation is not required (240). GnRH antagonists are usually administered only when there is a risk of premature LH surge (usually from days 5–7 of stimulation), so symptoms of hypoestrogenemia are rare (239). Furthermore, lower total doses and fewer days of exogenous gonadotropin stimulation are required versus agonists (241). Consequently, the total cycle duration is shorter and subsequent cycles can be initiated rapidly (242).

A meta-analysis including 45 RCTs and 7511 women to compare GnRH antagonist and long GnRH agonist protocols for COS in ART cycles showed no significant differences in the live birth or ongoing pregnancy rates, but a significantly lower incidence of OHSS with GnRH antagonists (242). Interestingly, the pituitary remains responsive to GnRH stimulation during antagonist co-treatment, so a bolus dose of agonist can be administered (instead of hCG) to trigger final oocyte maturation. This approach may have the potential to reduce further the incidence of OHSS for those at high risk (243,244).

It has been proposed that GnRH antagonist protocols may have particular benefit for patients at the anticipated extremes of ovarian response (172). A prospective cohort study of 538 patients was conducted in order to compare outcomes of GnRH agonist versus antagonist protocols in an attempt to individualize treatment based on baseline AMH level (172). GnRH antagonist co-treatment (vs. agonist co-treatment) resulted in a reduced cycle cancelation rate and treatment burden for poor responders (AMH 1 to <5 pmol/L) (172). However, GnRH antagonists were found to be most advantageous in high responders (AMH ≥15.0 pmol/L) (172). A higher fresh clinical pregnancy rate per oocyte retrieval ($p < 0.001$) was achieved by a profound reduction in excessive response to COS and an increased proportion of fresh ET (vs. agonist co-treatment) (172).

The reduction in treatment burden (in terms of cycle duration and side effects) and a lower risk of OHSS compared with long agonist protocols means that GnRH antagonists are considered to be "patient-friendly" therapies. GnRH antagonists are being used with increasing frequency in COS protocols, and because of their relative advantages, they have replaced GnRH agonists in many clinics as the protocol of first choice.

REFERENCES

1. Howles CM. Genetic engineering of human FSH (Gonal-F). *Hum Reprod* 1996; 2: 172–91.
2. Lunenfeld B. Historical perspectives in gonadotrophin therapy. *Hum Reprod Update* 2004; 10: 453–67.
3. Greenblatt RB, Barfield WE, Jungck EC, Ray AW. Induction of ovulation with MRL/41: Preliminary report. *J Am Med Assoc* 1961; 178: 101–4.
4. Charles D, Klein T, Lunn SF, Loraine JA. Clinical and endocrinological studies with the isomeric components of clomiphene citrate. *J Obstet Gynaecol Br Commonw* 1969; 76: 1100–10.
5. Pandya G, Cohen MR. The effect of cis-isomer of clomiphene citrate (cis-clomiphene) on cervical mucus and vaginal cytology. *J Reprod Med* 1972; 8: 133–8.
6. MacLeod SC, Mitton DM, Parker AS, Tupper WR. Experience with induction of ovulation. *Am J Obstet Gynecol* 1970; 108: 814–24.
7. Murthy YS, Parakh MC, Arronet GH. Experience with clomiphene and cisclomiphene. *Int J Fertil* 1971; 16: 66–74.
8. Van Campenhout J, Borreman E, Wyman H, Antaki A. Induction of ovulation with cisclomiphene. *Am J Obstet Gynecol* 1973; 115: 321–7.
9. Connaughton JF, Jr., Garcia CR, Wallach EE. Induction of ovulation with cisclomiphene and a placebo. *Obstet Gynecol* 1974; 43: 697–701.
10. Mikkelson TJ, Kroboth PD, Cameron WJ et al. Single-dose pharmacokinetics of clomiphene citrate in normal volunteers. *Fertil Steril* 1986; 46: 392–6.
11. Kahwanago I, Heinrichs WL, Herrmann WL. Estradiol "receptors" in hypothalamus and anterior pituitary gland: Inhibition of estradiol binding by SH-group blocking agents and clomiphene citrate. *Endocrinology* 1970; 86: 1319–26.
12. Etgen AM. Antiestrogens: Effects of tamoxifen, nafoxidine, and CI-628 on sexual behavior, cytoplasmic receptors, and nuclear binding of estrogen. *Horm Behav* 1979; 13: 97–112.
13. Dickey RP, Holtkamp DE. Development, pharmacology and clinical experience with clomiphene citrate. *Hum Reprod Update* 1996; 2: 483–506.
14. Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. *Hum Reprod Update* 1997; 3: 359–65.
15. Vandenberg G, Yen SS. Effect of anti-estrogenic action of clomiphene during the menstrual cycle: Evidence for a change in the feedback sensitivity. *J Clin Endocrinol Metab* 1973; 37: 356–65.
16. Clark JH, Markaverich BM. The agonistic–antagonistic properties of clomiphene: A review. *Pharmacol Ther* 1981; 15: 467–519.
17. Clark JH, Peck EJ, Jr., Anderson JN. Oestrogen receptors and antagonism of steroid hormone action. *Nature (London)* 1974; 251: 446–8.
18. Adashi EY. Clomiphene citrate: Mechanism(s) and site(s) of action—A hypothesis revisited. *Fertil Steril* 1984; 42: 331–44.
19. Messinis IE, Milingos SD. Future use of clomiphene in ovarian stimulation. Clomiphene in the 21st century. *Hum Reprod* 1998; 13: 2362–5.
20. Messinis IE, Milingos SD. Current and future status of ovulation induction in polycystic ovary syndrome. *Hum Reprod Update* 1997; 3: 235–53.
21. Out HJ, Coelingh Bennink HJ. Clomiphene citrate or gonadotrophins for induction of ovulation? *Hum Reprod* 1998; 13: 2358–61.
22. Hughes E, Collins J, Vandekerckhove P. Clomiphene citrate for unexplained subfertility in women. *Cochrane Database Syst Rev* 2000: 2: CD000057.
23. Rossing MA, Daling JR, Weiss NS et al. Ovarian tumors in a cohort of infertile women. *N Engl J Med* 1994; 331: 771–6.
24. Anencephaly, ovulation stimulation, subfertility, and illegitimacy [Letter]. *Lancet* 1973; 2: 916–17.
25. Singh M, Singhi S. Possible relationship between clomiphene and neural tube defects. *J Pediatr* 1978; 93: 152.
26. Dyson JL, Kohler HG. Anencephaly and ovulation stimulation. *Lancet* 1973; 1: 1256–7.
27. Sandler B. Anencephaly and ovulation stimulation. *Lancet* 1973; 2: 379.
28. Field B, Kerr C. Ovulation stimulation and defects of neural-tube closure [Letter]. *Lancet* 1974; 2: 1511.
29. Berman P. Congenital abnormalities associated with maternal clomiphene ingestion [Letter]. *Lancet* 1975; 2: 878.
30. James WH. Anencephaly, ovulation stimulation, and subfertility [Letter]. *Lancet* 1974; 1: 1353.
31. Shoham Z, Zosmer A, Insler V. Early miscarriage and fetal malformations after induction of ovulation (by clomiphene citrate and/or human menotropins), *in vitro* fertilization, and gamete intrafallopian transfer. *Fertil Steril* 1991; 55: 1–11.
32. Harlap S. Ovulation induction and congenital malformations. *Lancet* 1976; 2: 961.
33. Goss PE, Gwyn KM. Current perspectives on aromatase inhibitors in breast cancer. *J Clin Oncol* 1994; 12: 2460–70.
34. Harvey HA. Aromatase inhibitors in clinical practice: Current status and a look to the future. *Semin Oncol* 1996; 23: 33–8.
35. Smith IE, Fitzharris BM, McKinna JA et al. Aminoglutethimide in treatment of metastatic breast carcinoma. *Lancet* 1978; 2: 646–9.
36. Lamb HM, Adkins JC. Letrozole: A review of its use in postmenopausal women with advanced breast cancer. *Drugs* 1998; 56: 1125–40.
37. Wiseman LR, Adkins JC. Anastrozole: A review of its use in the management of postmenopausal women with advanced breast cancer. *Drugs Aging* 1998; 13: 321–32.
38. Biljan MM, Hcmmings R, Brassard N. The outcome of 150 babies following the treatment wilh letrozole or lelrozole and gonadotrophins. *Fertil Steril* 2005; 84(Suppl): 1033.

39. Tulandi T, Martin J, Al-Fadhli R et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertil Steril* 2006; 85: 1761–5.
40. Badawy A, Shokeir T, Allam AF et al. Pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate in unexplained infertility. *Acta Obstet Gynecol Scand* 2009; 88: 187–91.
41. Legro RS, Brzyski RG, Diamond MP et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 2014; 371: 119–29.
42. Diamond MP, Legro RS, Coutifaris C et al. Letrozole, gonadotropin, or clomiphene for unexplained infertility. *N Engl J Med* 2015; 373: 1230–40.
43. Legro RS. Ovulation induction in polycystic ovary syndrome: Current options. *Best Pract Res Clin Obstet Gynaecol* 2016 Nov; 37: 152–159.
44. Mitwally MF, Casper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. *Fertil Steril* 2001; 75: 305–9.
45. Mitwally MF, Casper RF. Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. *Fertil Steril* 2002; 77: 776–80.
46. Mitwally MF, Casper RF. Aromatase inhibition for ovarian stimulation: Future avenues for infertility management. *Curr Opin Obstet Gynecol* 2002; 14: 255–63.
47. Fisher SA, Reid RL, Van Vugt DA, Casper RF. A randomized double-blind comparison of the effects of clomiphene citrate and the aromatase inhibitor letrozole on ovulatory function in normal women. *Fertil Steril* 2002; 78: 280–5.
48. Mitwally MF, Casper RF. Aromatase inhibition reduces gonadotrophin dose required for controlled ovarian stimulation in women with unexplained infertility. *Hum Reprod* 2003; 18: 1588–97.
49. He D, Jiang F. Meta-analysis of letrozole versus clomiphene citrate in polycystic ovary syndrome. *Reprod Biomed Online* 2011; 23: 91–6.
50. Papanikolaou EG, Polyzos NP, Humaidan P et al. Aromatase inhibitors in stimulated IVF cycles. *Reprod Biol Endocrinol* 2011; 9: 85.
51. Polyzos NP, Mauri D, Tzioras S. Letrozole in ovulation induction: Time to make decisions. *Hum Reprod Update* 2009; 15: 263–4.
52. Tredway D, Schertz JC, Bock D et al. Anastrozole vs. clomiphene citrate in infertile women with ovulatory dysfunction: A phase II, randomized, dose-finding study. *Fertil Steril* 2011; 95: 1720–4.
53. Tredway D, Schertz JC, Bock D et al. Anastrozole single-dose protocol in women with oligo- or anovulatory infertility: Results of a randomized phase II dose-response study. *Fertil Steril* 2011; 95: : 1725–9.
54. Griesinger G, von OS, Schultze-Mosgau A et al. Follicular and endocrine response to anastrozole versus clomiphene citrate administered in follicular phase to normoovulatory women: A randomized comparison. *Fertil Steril* 2009; 91: 1831–6.
55. Davar R, Oskouian H, Ahmadi S, Firouzabadi RD. GnRH antagonist/letrozole versus microdose GnRH agonist flare protocol in poor responders undergoing *in vitro* fertilization. *Taiwan J Obstet Gynecol* 2010; 49: 297–301.
56. Garcia-Velasco JA, Moreno L, Pacheco A et al. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves *in vitro* fertilization outcome in low responder patients: A pilot study. *Fertil Steril* 2005; 84: 82–7.
57. Goswami SK, Das T, Chattopadhyay R et al. A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: A preliminary report. *Hum Reprod* 2004; 19: 2031–5.
58. Ozmen B, Sonmezer M, Atabekoglu CS, Olmus H. Use of aromatase inhibitors in poor-responder patients receiving GnRH antagonist protocols. *Reprod Biomed Online* 2009; 19: 478–85.
59. Verpoest WM, Kolibianakis E, Papanikolaou E et al. Aromatase inhibitors in ovarian stimulation for IVF/ICSI: A pilot study. *Reprod Biomed Online* 2006; 13: 166–72.
60. Lord JM, Flight IH, Norman RJ. Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. *Cochrane Database Syst Rev* 2003; 3: CD003053.
61. ASRM Practice Committee Guidelines. *Fertil Steril* 2006; 86(Suppl 4): S221–3.
62. *UK National Collaborating Centre for Women's and Children's Health. Fertility: Assessment and Treatment for People with Fertility Problems. Clinical Guideline 11*. UK: National Institute for Clinical Excellence, 2004. https://www.nice.org.uk.
63. Moll E, Bossuyt PM, Korevaar JC, Lambalk CB, van der Veen F. Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: Randomised double blind clinical trial. *BMJ* 2006; 332(7556): 1485.
64. Palomba S, Orio F, Jr., Falbo A et al. Prospective parallel randomized, double-blind, double-dummy controlled clinical trial comparing clomiphene citrate and metformin as the first-line treatment for ovulation induction in nonobese anovulatory women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2005; 90: 4068–74.
65. Costello MF, Chapman M, Conway U. A systematic review and meta-analysis of randomized controlled trials on metformin co-administration during gonadotrophin ovulation induction or IVF in women with polycystic ovary syndrome. *Hum Reprod* 2006; 21: 1387–99.

66. Neveu N, Granger L, St-Michel P, Lavoie HB. Comparison of clomiphene citrate, metformin, or the combination of both for first-line ovulation induction and achievement of pregnancy in 154 women with polycystic ovary syndrome. *Fertil Steril* 2007; 87: 113–20.
67. Siebert TI, Kruger TF, Steyn DW, Nosarka S. Is the addition of metformin efficacious in the treatment of clomiphene citrate-resistant patients with polycystic ovary syndrome? A structured literature review. *Fertil Steril* 2006; 86: 1432–7.
68. Legro RS, Barnhart HX, Schlaff WD et al. Cooperative multicenter reproductive medicine network. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med* 2007; 356: 551–66.
69. Tang T, Lord JM, Norman RJ et al. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligoamenorrhoea and subfertility. *Cochrane Database Syst Rev* 2012; 5: CD003053.
70. Morin-Papunen L, Rantala AS, Unkila-Kallio L et al. Metformin improves pregnancy and live-birth rates in women with polycystic ovary syndrome (PCOS): A multicenter, double-blind, placebo-controlled randomized trial. *J Clin Endocrinol Metab* 2012; 97: 1492–500.
71. Franks S. When should an insulin sensitizing agent be used in the treatment of polycystic ovary syndrome? *Clin Endocrinol (Oxf)* 2011; 74: 148–51.
72. Trinchard-Lugan I, Khan A, Porchet HC, Munato A. Pharmacokinetics and pharmacodynamics of recombinant human chorionic gonadotropin in healthy male and female volunteers. *Reprod Biomed Online* 2002; 4: 106–15.
73. le Cotonnec JY, Porchet HC, Beltrami V, Munafo A. Clinical pharmacology of recombinant human luteinizing hormone: Part I. Pharmacokinetics after intravenous administration to healthy female volunteers and comparison with urinary human luteinizing hormone. *Fertil Steril* 1998; 69: 189–94.
74. Weissman, Lurie S, Zalel Y et al. Human chorionic gonadotropin; pharmacokinetics of subcutaneous administration. *Gynecol Endocrinol* 1996; 10: 273–6.
75. Diczfalusy E, Harlin J. Clinical–pharmacological studies on human menopausal gonadotrophin. *Hum Reprod* 1988; 3: 21–7.
76. Braunstein GD, Bloch SK, Rasor JL, Winikoff J. Characterization of antihuman chorionic gonadotropin serum antibody appearing after ovulation induction. *J Clin Endocrinol Metab* 1983; 57: 1164–72.
77. Ludwig M, Doody KJ, Doody KM. Use of recombinant human chorionic gonadotropin in ovulation induction. *Fertil Steril* 2003; 79: 1051–9.
78. Kyle CV, Griffin J, Jarrett A, Odell WD. Inability to demonstrate an ultrashort loop feedback mechanism for luteinizing hormone in humans. *J Clin Endocrinol Metab* 1989; 69: 170–6.
79. Nader S, Berkowitz AS. Endogenous luteinizing hormone surges following administration of human chorionic gonadotropin: Further evidence for lack of loop feedback in humans. *J Assist Reprod Genet* 1992; 9: 124–7.
80. Demoulin A, Dubois M, Gerday C et al. Variations of luteinizing hormone serum concentrations after exogenous human chorionic gonadotropin administration during ovarian hyperstimulation. *Fertil Steril* 1991; 55: 797–804.
81. Golan A, Ron-El R, Herman A et al. Ovarian hyperstimulation syndrome: An update review. *Obstet Gynecol Surv* 1989; 44: 430–40.
82. Gidley-Baird AA, O'Neill C, Sinosich MJ et al. Failure of implantation in human *in vitro* fertilization and embryo transfer patients: The effects of altered progesterone/estrogen ratios in humans and mice. *Fertil Steril* 1986; 45: 69–74.
83. Forman R, Fries N, Testart J et al. Evidence for an adverse effect of elevated serum estradiol concentrations on embryo implantation. *Fertil Steril* 1988; 49: 118–22.
84. Tan SL, Balen A, el Hussein E et al. A prospective randomized study of the optimum timing of human chorionic gonadotropin administration after pituitary desensitization in *in vitro* fertilization. *Fertil Steril* 1992; 57: 1259–64.
85. Abdalla HI, Ah-Moye M, Brinsden P et al. The effect of the dose of human chorionic gonadotropin and the type of gonadotropin stimulation on oocyte recovery rates in an *in vitro* fertilization program. *Fertil Steril* 1987; 48: 958–63.
86. Lathi RB, Milki AA. Recombinant gonadotropins. *Curr Womens Health Rep* 2001; 1: 157–63.
87. The European Recombinant Human Chorionic Gonadotrophin Study Group. Induction of final follicular maturation and early luteinization in women undergoing ovulation induction for assisted reproduction treatment—Recombinant hCG versus urinary hCG. *Hum Reprod* 2000; 15: 1446–51.
88. Van Dorsselaer A, Carapito C, Delalande F et al. Detection of prion protein in urine-derived injectable fertility products by a targeted proteomic approach. *PLoS ONE* 2011; 6: e17815.
89. Aschheim S, Zondek B. *Klin Wochenschr* 1928; 7: 8–9.
90. Zondek B. *Klin Wochenschr* 1930; 9: 393–6.
91. Cole H, Hart GH. *Am J Physiol* 1931; 93: 57–68.
92. Stewart HL, Sano ME, Montgomery TL. *J Clin Endocrinol* 1948; 8: 175–88.
93. Kotz HL, Hermann W. A review of the endocrine induction of human ovulation. *Fertil Steril* 1961; 12: 375–94.
94. Knobil E, Kostyo J, Greep R. *Fed Proc* 1958; 17: 88.
95. Katzman PA, Godfrid M, Cain CK, Doisy EA. *J Biol Chem* 1943; 148: 501–7.
96. Donini P, Montezemolo R. Rassegna di Clinica, Terapia e Scienze Affini. *(A publication of the Biologic Laboratories of the Instituto Serono)* 1949; 48: 3–28.
97. Lunenfeld B, Sulimovici S, Rabau E, Eshkol A. L'Induction de l'ovulation dans les amenorhées hypophysaires par un traîtment de gonado trophines

urinaries menopausiques et de gonado trophines chroniques. *C R Soc Française de Gynecol* 1973; 5: 1–6.
98. Cook AS, Webster BW, Terranova PF, Keel BA. Variation in the biologic and biochemical characteristics of human menopausal gonadotropin. *Fertil Steril* 1988; 49: 704–12.
99. Howles CM, Loumaye E, Giroud D, Luyet G. Multiple follicular development and ovarian steroidogenesis following subcutaneous administration of a highly purified urinary FSH preparation in pituitary desensitized women undergoing IVF: A multicentre European phase III study. *Hum Reprod* 1994; 9: 424–30.
100. Giudice E, Crisci C, Eshkol A, Papoian R. Composition of commercial gonadotrophin preparations extracted from human post-menopausal urine: Characterization of non-gonadotrophin proteins. *Hum Reprod* 1994; 9: 2291–9.
101. Li TC, Hindle JE. Adverse local reaction to intramuscular injections of urinary-derived gonadotrophins. *Hum Reprod* 1993; 8: 1835–6.
102. Al-Inany HG, bou-Setta AM, Aboulghar MA et al. Highly purified hMG achieves better pregnancy rates in IVF cycles but not ICSI cycles compared with recombinant FSH: A meta-analysis. *Gynecol Endocrinol* 2009; 25: 372–8.
103. Kuwabara Y, Mine K, Katayama A et al. Proteomic analyses of recombinant human follicle-stimulating hormone and urinary-derived gonadotropin preparations. *J Reprod Med* 2009; 54: 459–66.
104. Steelman SL, Pohley FM. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. *Endocrinology* 1953; 53: 604–16.
105. le Cotonnec JY, Porchet HC, Beltrami V et al. Clinical pharmacology of recombinant human follicle- stimulating hormone (FSH). I. Comparative pharmacokinetics with urinary human FSH. *Fertil Steril* 1994; 61: 669–78.
106. Schoemaker J, Wentz AC, Jones GS et al. Stimulation of follicular growth with "pure" FSH in patients with anovulation and elevated LH levels. *Obstet Gynecol* 1978; 51: 270–7.
107. Raj SG, Berger MJ, Grimes EM, Taymor ML. The use of gonadotropins for the induction of ovulation in women with polycystic ovarian disease. *Fertil Steril* 1977; 28: 1280–4.
108. McFaul PB, Traub AI, Thompson W. Treatment of clomiphene citrate-resistant polycystic ovarian syndrome with pure follicle-stimulating hormone or human menopausal gonadotropin. *Fertil Steril* 1990; 53: 792–7.
109. Jacobson A, Marshall JR. Ovulatory response rate with human menopausal gonadotropins of varying FSH–LH ratios. *Fertil Steril* 1969; 20: 171–5.
110. Louwerens B. The clinical significance of the FSH–LH ratio in gonadotropin preparations of human origin. A review. *Acta Obstet Gynecol Scand* 1969; 48(Suppl 1): 31–40.
111. Anderson RE, Cragun JM, Chang RJ et al. A pharmacodynamic comparison of human urinary follicle-stimulating hormone and human menopausal gonadotropin in normal women and polycystic ovary syndrome. *Fertil Steril* 1989; 52: 216–20.
112. le Cotonnec JY, Porchet HC, Beltrami V, Howles C. Comparative pharmacokinetics of two urinary human follicle stimulating hormone preparations in healthy female and male volunteers. *Hum Reprod* 1993; 8: 1604–11.
113. Driebergen R, Baer G. Quantification of follicle stimulating hormone (follitropin alfa): Is the *in vivo* bioassay still relevant in the recombinant age? *Curr Med Res Opin* 2003; 19: 1–6.
114. Bassett RM, Driebergen R. Continued improvements in the quality and consistency of follitropin alfa, recombinant human FSH. *Reprod Biomed Online* 2005; 10: 169–77.
115. Hermentin P, Witzel R, Kanzy EJ et al. The hypothetical N-glycan charge: A number that characterizes protein glycosylation. *Glycobiology* 1996; 6: 217–30.
116. Gervais A, Hammel YA, Pelloux S et al. Glycosylation of human recombinant gonadotropins: Characterization and batch-to-batch consistency. *Glycobiology* 2003; 13: 179–89.
117. Hugues JN, Barlow DH, Rosenwaks Z et al. Improvement in consistency of response to ovarian stimulation with recombinant human follicle stimulating hormone resulting from a new method for calibrating the therapeutic preparation. *Reprod Biomed Online* 2003; 6: 185–90.
118. Balasch J, Fabregues F, Penarrubia J et al. Outcome from consecutive assisted reproduction cycles in patients treated with recombinant follitropin alfa filled-by-bioassay and those treated with recombinant follitropin alfa filled-by-mass. *Reprod Biomed Online* 2004; 8: 408–13.
119. Lass A, McVeigh E, UK Gonal-f FbM PMS Group. Routine use of r-hFSH follitropin alfa filled-bymass for follicular development for IVF: A large multicentre observational study in the UK. *Reprod Biomed Online* 2004; 9: 604–10.
120. Martinez G, Sanguineti F, Sepulveda J et al. A comparison between follitropin alpha filled by mass and follitropin alpha filled by bioassay in the same egg donors. *Reprod Biomed Online* 2007; 14: 26–8.
121. Carizza C, Alam V, Yeko T et al. Gonal-F® filled by mass in ovulation induction. *Hum Reprod Suppl* 2003; 18: O117.
122. Wikland M, Hugues JN, Howles C. Improving the consistency of ovarian stimulation: Follitropin alfa filled-by-mass. *Reprod Biomed Online* 2006; 12: 663–8.
123. Wolzt M, Gouya G, Sator M, Hemetsberger T, Irps C, Rettenbacher M, Vcelar B. Comparison of pharmacokinetic and safety profiles between Bemfola® and Gonal-f® after subcutaneous application. *Eur J Drug Metab Pharmacokinet* 2016; 41: 259–65.

124. Rettenbacher M, Andersen AN, Garcia-Velasco JA et al. A multi-centre phase 3 study comparing efficacy and safety of Bemfola® versus Gonal-f® in women undergoing ovarian stimulation for IVF. *Reprod Biomed Online* 2015; 30: 504–13.
125. Orvieto R, Seifer DB. Biosimilar FSH preparations: Are they identical twins or just siblings? *Reprod Biol Endocrinol* Jun 14, 2016; 14(1): 32.
126. Schiestl M, Stangler T, Torella C, Cepeljnik T, Toll H, Grau R. Acceptable changes in quality attributes of glysolylated biopharmaceuticals. *Nature Biotechnology* 2011; 29: 310–2.
127. Horsman G, Talbot JA, McLoughlin JD, Lambert A, Robertson WR. A biological, immunological and physico-chemical comparison of the current clinical batches of the recombinant FSH preparations Gonal-F and Puregon. *Hum Reprod* 2000; 15: 1898–902.
128. Tulppala M, Aho M, Tuuri T et al. Comparison of two recombinant follicle-stimulating hormone preparations in *in-vitro* fertilization: A randomized clinical study. *Hum Reprod* 1999; 14: 2709–15.
129. Harlin J, Csemiczky G, Wramsby H, Fried G. Recombinant follicle stimulating hormone in *in-vitro* fertilization treatment-clinical experience with follitropin alpha and follitropin beta. *Hum Reprod* 2000; 15: 239–44.
130. Orvieto R, Nahum R, Rabinson J, Ashkenazi J, Anteby EY, Meltcer S. Follitropin-alpha (Gonal-F) versus follitropin-beta (Puregon) in controlled ovarian hyperstimulation for *in vitro* fertilization: Is there any difference? *Fertil Steril* 2009; 91(4 Suppl): 1522–5.
131. Olsson H, Sandstrom R, Grundemar L. Different pharmacokinetic and pharmacodynamic properties of recombinant follicle-stimulating hormone (rFSH) derived from a human cell line compared with rFSH from a non-human cell line. *J Clin Pharmacol* 2014; 54: 1299–307.
132. Arce JC, Nyboe Andersen A, Fernandez-Sanchez M et al. Ovarian response to recombinant human follicle- stimulating hormone: A randomized, antimu€llerian hormone-stratified, dose-response trial in women undergoing *in vitro* fertilization/ intracytoplas- mic sperm injection. *Fertil Steril* 2014; 102: 1633–40.e5.
133. Nyboe Andersen A, Nelson SM, Fauser BC, García-Velasco JA, Klein BM, Arce JC; ESTHER-1 study group. Individualized versus conventional ovarian stimulation for in vitro fertilization: A multicenter, randomized, controlled, assessor-blinded, phase 3 noninferiority trial. *Fertil Steril* 2017 Feb; 107(2): 387–96.
134. Bouloux PM, Handelsman DJ, Jockenhovel F et al. First human exposure to FSH–CPT in hypogonadotrophic hypogonadal males. *Hum Reprod* 2001; 16: 1592–7.
135. Duijkers IJ, Klipping C, Boerrigter PJ et al. Single dose pharmacokinetics and effects on follicular growth and serum hormones of a long acting recombinant FSH preparation (FSH–CTP) in healthy pituitary-suppressed females. *Hum Reprod* 2002; 17: 1987–93.
136. Beckers NG, Macklon NS, Devroey P et al. First live birth after ovarian stimulation using a chimeric long-acting human recombinant follicle-stimulating hormone (FSH) agonist (recFSH–CTP) for *in vitro* fertilization. *Fertil Steril* 2003; 79: 621–3.
137. Balen AH, Mulders AG, Fauser BC et al. Pharmacodynamics of a single low dose of long-gacting recombinant follicle-stimulating hormone (FSH–carboxy terminal peptide, corifollitropin alfa) in women with World Health Organization group II anovulatory infertility. *J Clin Endocrinol Metab* 2004; 89: 6297–304.
138. Devroey P, Fauser BC, Platteau P et al. Induction of multiple follicular development by a single dose of long-acting recombinant follicle-stimulating hormone (FSH–CTP, corifollitropin alfa) for controlled ovarian stimulation before *in vitro* fertilization. *J Clin Endocrinol Metab* 2004; 89: 2062–70.
139. Corifollitropin Alfa Dose-finding Study Group. A randomized dose–response trial of a single injection of corifollitropin alfa to sustain multifollicular growth during controlled ovarian stimulation. *Hum Reprod* 2008; 23: 2484–92.
140. Corifollitropin alfa Ensure Study Group. Corifollitropin alfa for ovarian stimulation in IVF: A randomized trial in lower-body-weight women. *Reprod Biomed Online* 2010; 21: 66–76.
141. Devroey P, Boostanfar R, Koper NP et al. A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol. *Hum Reprod* 2009; 24: 3063–72.
142. Norman RJ, Zegers-Hochschild F, Salle BS et al. Repeated ovarian stimulation with corifollitropin alfa in patients in a GnRH antagonist protocol: No concern for immunogenicity. *Hum Reprod* 2011; 26: 2200–8.
143. Santjohanser C. Letter to the editor "Long-acting-FSH (FSH–CTP) in reproductive medicine". *Gynakologische Endokrinologie* 2009; 3: 183.
144. Kolibianakis EM, Venetis CA, Bosdou JK, Zepiridis L, Chatzimeletiou K, Makedos A, Masouridou S, Triantafillidis S, Mitsoli A, Tarlatzis BC. Corifollitropin alfa compared with follitropin beta in poor responders undergoing ICSI: A randomized controlled trial. *Hum Reprod* 2015; 30: 432–40.
145. Polyzos NP, Camus M, Llacer J, Pantos K, Tournaye H. Corifollitropin α followed by menotropin for poor ovarian responders' trial (COMPORT): A protocol of a multicentre randomised trial. *BMJ Open* 2013; 3: e002938.
146. Polyzos NP, De Vos M, Corona R, Vloeberghs V, Ortega-Hrepich C, Stoop D, Tournaye H. Addition of highly purified HMG after corifollitropin alfa in antagonist-treated poor ovarian responders: A pilot study. *Hum Reprod* 2013; 28: 1254–60.

147. Polyzos NP, Devos M, Humaidan P, Stoop D, Ortega-Hrepich C, Devroey P, Tournaye H. Corifollitropin alfa followed by rFSH in a GnRH antagonist protocol for poor ovarian responder patients: An observational pilot study. *Fertil Steril* 2013; 99: 422–6.
148. Boostanfar R, Shapiro B, Levy M, Rosenwaks Z, Witjes H, Stegmann BJ, Elbers J, Gordon K, Mannaerts B, Pursue investigators. Large, comparative, randomized double-blind trial confirming noninferiority of pregnancy rates for corifollitropin alfa compared with recombinant follicle-stimulating hormone in a gonadotropin-releasing hormone antagonist controlled ovarian stimulation protocol in older patients undergoing *in vitro* fertilization. *Fertil Steril* 2015; 104: 94–103.e1.
149. Polyzos NP, Corona R, Van De Vijver A, Blockeel C, Drakopoulos P, Vloeberghs V, De Vos M, Camus M, Humaidan P, Tournaye H. Corifollitropin alfa followed by hpHMG in GnRH agonist protocols. Two prospective feasibility studies in poor ovarian responders. *Gynecol Endocrinol* 2015; 31: 885–90.
150. Pouwer AW, Farquhar C, Kremer JA. Long-acting FSH versus daily FSH for women undergoing assisted reproduction. *Cochrane Database Syst Rev* 2015; 7: CD009577.
151. van Wely M, Kwan I, Burt AL et al. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. *Cochrane Database Syst Rev* 2011; 2: CD005354.
152. Al-Inany H, Aboulghar M, Mansour R, Serour G. Meta-analysis of recombinant versus urinary-derived FSH: An update. *Hum Reprod* 2003; 18: 305–13.
153. European and Israeli Study Group on Highly Purified Menotropin versus Recombinant Follicle-Stimulating Hormone. Efficacy and safety of highly purified menotropin versus recombinant follicle-stimulating hormone in *in vitro* fertilization/intracytoplasmic sperm injection cycles: A randomized, comparative trial. *Fertil Steril* 2002; 78: 520–8.
154. Gordon UD, Harrison RF, Fawzy M et al. A randomized prospective assessor-blind evaluation of luteinizing hormone dosage and *in vitro* fertilization outcome. *Fertil Steril* 2001; 75: 324–31.
155. Ng EH, Lau EY, Yeung WS, Ho PC. HMG is as good as recombinant human FSH in terms of oocyte and embryo quality: A prospective randomized trial. *Hum Reprod* 2001; 16: 319–25.
156. Westergaard LG, Erb K, Laursen SB et al. Human menopausal gonadotropin versus recombinant follicle-stimulating hormone in normogonadotropic women down-regulated with a gonadotropin-releasing hormone agonist who were undergoing *in vitro* fertilization and intracytoplasmic sperm injection: A prospective randomized study. *Fertil Steril* 2001; 76: 543–9.
157. van Wely M, Westergaard LG, Bossuyt PMM, van der Veen F. Effectiveness of human menopausal gonadotropin versus recombinant follicle stimulating hormone for controlled ovarian hyperstimulation in assisted reproductive cycles. A meta-analysis. *Fertil Steril* 2003; 80: 1086–93.
158. van Wely M, Westergaard LG, Bossuyt PM, van der Veen F. Human menopausal gonadotropin versus recombinant follicle stimulation hormone for ovarian stimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2003; 1: CD003973.
159. Al-Inany H, Aboulghar MA, Mansour RT, Serour GI. Ovulation induction in the new millennium: Recombinant follicle-stimulating hormone versus human menopausal gonadotropin. *Gynecol Endocrinol* 2005; 20: 161–9.
160. Al-Inany HG, bou-Setta AM, Aboulghar MA et al. Efficacy and safety of human menopausal gonadotrophins versus recombinant FSH: A meta-analysis. *Reprod Biomed Online* 2008; 16: 81–8.
161. Coomarasamy A, Afnan M, Cheema D et al. Urinary hMG versus recombinant FSH for controlled ovarian hyperstimulation following an agonist long down-regulation protocol in IVF or ICSI treatment: A systematic review and meta-analysis. *Hum Reprod* 2008; 23: 310–5.
162. Lehert P, Schertz JC, Ezcurra D. Recombinant human follicle-stimulating hormone produces more oocytes with a lower total dose per cycle in assisted reproductive technologies compared with highly purified human menopausal gonadotrophin: A meta-analysis. *Reprod Biol Endocrinol* 2010; 8: 112.
163. Sunkara SK, Rittenberg V, Raine-Fenning N et al. Association between the number of eggs and live birth in IVF treatment: An analysis of 400 135 treatment cycles. *Hum Reprod* 2011; 26: 1768–74.
164. Nardo LG, Fleming R, Howles CM et al. Conventional ovarian stimulation no longer exists: Welcome to the age of individualized ovarian stimulation. *Reprod Biomed Online* 2011; 23: 141–8.
165. Olivennes F, Howles CM, Borini A et al. Individualizing FSH dose for assisted reproduction using a novel algorithm: The CONSORT study. *Reprod Biomed Online* 2009; 18: 195–204.
166. Howles CM, Saunders H, Alam V, Engrand P. Predictive factors and a corresponding treatment algorithm for controlled ovarian stimulation in patients treated with recombinant human follicle stimulating hormone (follitropin alfa) during assisted reproduction technology (ART) procedures. An analysis of 1378 patients. *Curr Med Res Opin* 2006; 22: 907–18.
167. Klinkert ER, Broekmans FJ, Looman CW et al. The antral follicle count is a better marker than basal follicle-stimulating hormone for the selection of older patients with acceptable pregnancy prospects after *in vitro* fertilization. *Fertil Steril* 2005; 83: 811–4.

168. Popovic-Todorovic B, Loft A, Lindhard A et al. A prospective study of predictive factors of ovarian response in "standard" IVF/ICSI patients treated with recombinant FSH. A suggestion for a recombinant FSH dosage normogram. *Hum Reprod* 2003; 18: 781–7.
169. Broer SL, Mol B, Dolleman M et al. The role of anti-Mullerian hormone assessment in assisted reproductive technology outcome. *Curr Opin Obstet Gynecol* 2010; 22: 193–201.
170. Broer SL, Dolleman M, Opmeer BC et al. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. *Hum Reprod Update* 2011; 17: 46–54.
171. Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: Prediction of live birth and extremes of response in stimulated cycles—Implications for individualization of therapy. *Hum Reprod* 2007; 22: 2414–21.
172. Nelson SM, Yates RW, Lyall H et al. Anti-Mullerian hormone-based approach to controlled ovarian stimulation for assisted conception. *Hum Reprod* 2009; 24: 867–75.
173. Barad DH, Weghofer A, Gleicher N. Utility of age-specific serum anti-Mullerian hormone concentrations. *Reprod Biomed Online* 2011; 22: 284–91.
174. Nelson SM, Messow MC, Wallace AM et al. Nomogram for the decline in serum antimullerian hormone: A population study of 9,601 infertility patients. *Fertil Steril* 2011; 95: 736–41.
175. Nelson SM, Messow MC, McConnachie A et al. External validation of nomogram for the decline in serum anti-Mullerian hormone in women: A population study of 15,834 infertility patients. *Reprod Biomed Online* 2011; 23: 204–6.
176. Howles CM, Alam V, Tredway D et al. Factors related to successful ovulation induction in patients with WHO group II anovulatory infertility. *Reprod Biomed Online* 2010; 20: 182–90.
177. Imani B, Eijkemans MJ, Faessen GH et al. Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: An approach to increase safety and efficiency. *Fertil Steril* 2002; 77: 83–90.
178. van Wely M, Fauser BC, Laven JS et al. Validation of a prediction model for the follicle-stimulating hormone response dose in women with polycystic ovary syndrome. *Fertil Steril* 2006; 86: 1710–5.
179. Mulders AG, Laven JS, Eijkemans MJ et al. Patient predictors for outcome of gonadotrophin ovulation induction in women with normogonadotrophic anovulatory infertility: A meta-analysis. *Hum Reprod Update* 2003; 9: 429–49.
180. Kobayashi M, Nakano R, Ooshima A. Immunohistochemical localization of pituitary gonadotrophins and gonadal steroids confirms the "two-cell, two-gonadotrophin" hypothesis of steroidogenesis in the human ovary. *J Endocrinol* 1990; 126: 483–8.
181. European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: A dose-finding study. *J Clin Endocrinol Metab* 1998; 83: 1507–14.
182. Bosch E. Recombinant human FSH and recombinant human LH in a 2:1 ratio combination: A new tool for ovulation induction. *Expert Rev Obstet Gynecol* 2009; 4: 491–8.
183. Kolibianakis EM, Kalogeropoulou L, Griesinger G et al. Among patients treated with FSH and GnRH analogues for *in vitro* fertilization, is the addition of recombinant LH associated with the probability of live birth? A systematic review and meta-analysis. *Hum Reprod Update* 2007; 13: 445–52.
184. Mochtar MH, van der Veen F, Ziech M, van Wely M. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2007; 2: CD005070.
185. Howles CM. Luteininzing hormone supplementation in ART. In: *How to Improve Your ART Success Rates*. Kovacs G (ed.). London, UK: Cambridge University Press, 2011, pp. 99–104.
186. Bosch E, Labarta E, Simón C et al. The impact of luteinizing hormone supplementation in gonadotropin releasing hormone antagonist cycles. An age adjusted randomized trial. *Fertil Steril* 2008; 90: S41.
187. Humaidan P, Bungum M, Bungum L, Yding Andersen C. Effects of recombinant LH supplementation in women undergoing assisted reproduction with GnRH agonist down-regulation and stimulation with recombinant FSH: An opening study. *Reprod Biomed Online* 2004; 8: 635–43.
188. Marrs R, Meldrum D, Muasher S et al. Randomized trial to compare the effect of recombinant human FSH (follitropin alfa) with or without recombinant human LH in women undergoing assisted reproduction treatment. *Reprod Biomed Online* 2004; 8: 175–82.
189. Matorras R, Prieto B, Exposito A et al. Mid-follicular LH supplementation in women aged 35–39 years undergoing ICSI cycles: A randomized controlled study. *Reprod Biomed Online* 2009; 19: 879–87.
190. Fabregues F, Creus M, Penarrubia J et al. Effects of recombinant human luteinizing hormone supplementation on ovarian stimulation and the implantation rate in down-regulated women of advanced reproductive age. *Fertil Steril* 2006; 85: 925–31.
191. Nilsson C, Pettersson K, Millar RP et al. Worldwide frequency of a common genetic variant of luteinizing hormone: An international collaborative research. International Collaborative Research Group. *Fertil Steril* 1997; 67: 998–1004.
192. de Castro F, Moron FJ, Montoro L et al. Human controlled ovarian hyperstimulation outcome is a polygenic trait. *Pharmacogenetics* 2004; 14: 285–93.

193. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: Implications for human reproduction. *Hum Reprod Update* 2002; 8: 413–21.
194. Lisi F, Rinaldi L, Fishel S et al. Use of recombinant FSH and recombinant LH in multiple follicular stimulation for IVF: A preliminary study. *Reprod Biomed Online* 2001; 3: 190–4.
195. De Placido G, Alviggi C, Mollo A et al. Effects of recombinant LH (rLH) supplementation during controlled ovarian hyperstimulation (COH) in normogonadotrophic women with an initial inadequate response to recombinant FSH (rFSH) after pituitary downregulation. *Clin Endocrinol (Oxf)* 2004; 60: 637–43.
196. Ferraretti AP, Gianaroli L, Magli MC et al. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. *Fertil Steril* 2004; 82: 1521–6.
197. Carmel PW, Araki S, Ferin M. Pituitary stalk portal blood collection in rhesus monkeys: Evidence for pulsatile release of gonadotropin-releasing hormone (GnRH). *Endocrinology* 1976; 99: 243–8.
198. Schally AV, Arimura A, Kastin AJ et al. Gonadotropin-releasing hormone: One polypeptide regulates secretion of luteinizing and folliclestimulating hormones. *Science* 1971; 173: 1036–8.
199. Amoss M, Burgus R, Blackwell R et al. Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. *Biochem Biophys Res Commun* 1971; 44: 205–10.
200. Backstrom CT, McNeilly AS, Leask RM, Baird DT. Pulsatile secretion of LH, FSH, prolactin, oestradiol and progesterone during the human menstrual cycle. *Clin Endocrinol (Oxf)* 1982; 17: 29–42.
201. Reame N, Sauder SE, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion during the human menstrual cycle: Evidence for altered frequency of gonadotropin-releasing hormone secretion. *J Clin Endocrinol Metab* 1984; 59: 328–37.
202. Belchetz PE, Plant TM, Nakai Y et al. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. *Science* 1978; 202: 631–3.
203. Suarez-Quian CA, Wynn PC, Catt KJ. Receptor mediated endocytosis of GnRH analogs: Differential processing of gold-labeled agonist and antagonist derivatives. *J Steroid Biochem* 1986; 24: 183–92.
204. Schvartz I, Hazum E. Internalization and recycling of receptor-bound gonadotropin-releasing hormone agonist in pituitary gonadotropes. *J Biol Chem* 1987; 262: 17046–50.
205. Karten MJ, Rivier JE. Gonadotropin-releasing hormone analog design. Structure–function studies toward the development of agonists and antagonists: Rationale and perspective. *Endocrinol Rev* 1986; 7: 44–66.
206. Coy DH, Labrie F, Savary M et al. LH-releasing activity of potent LH-RH analogs *in vitro*. *Biochem Biophys Res Commun* 1975; 67: 576–82.
207. Lemay A, Metha AE, Tolis G et al. Gonadotropins and estradiol responses to single intranasal or subcutaneous administration of a luteinizing hormone-releasing hormone agonist in the early follicular phase. *Fertil Steril* 1983; 39: 668–73.
208. Fogelman I. Gonadotropin-releasing hormone agonists and the skeleton. *Fertil Steril* 1992; 57: 715–24.
209. Sopelak VM, Hodgen GD. Infusion of gonadotropin releasing hormone agonist during pregnancy: Maternal and fetal responses in primates. *Am J Obstet Gynecol* 1987; 156: 755–60.
210. Brogden RN, Buckley MM, Ward A. Buserelin. A review of its pharmacodynamic and pharmacokinetic properties, and clinical profile. *Drugs* 1990; 39: 399–437.
211. Golan A, Ron-El R, Herman A et al. Fetal outcome following inadvertent administration of long-acting DTRP6 GnRH microcapsules during pregnancy: A case report. *Hum Reprod* 1990; 5: 123–4.
212. Dicker D, Goldman JA, Vagman I et al. Pregnancy outcome following early exposure to maternal luteinizing-hormone-releasing hormone agonist (buserelin). *Hum Reprod* 1989; 4: 250–1.
213. Weissman A, Shoham Z. Favourable pregnancy outcome after administration of a long-acting gonadotrophin-releasing hormone agonist in the mid-luteal phase. *Hum Reprod* 1993; 8: 496–7.
214. Ron-El R, Golan A, Herman A et al. Midluteal gonadotropin-releasing hormone analog administration in early pregnancy. *Fertil Steril* 1990; 53: 572–4.
215. Lahat E, Raziel A, Friedler S et al. Long-term followup of children born after inadvertent administration of a gonadotrophin-releasing hormone agonist in early pregnancy. *Hum Reprod* 1999; 14: 2656–60.
216. Bajusz S, Kovacs M, Gazdag M et al. Highly potent antagonists of luteinizing hormone-releasing hormone free of edematogenic effects. *Proc Natl Acad Sci USA* 1988; 85: 1637–41.
217. Ljungqvist A, Feng DM, Hook W et al. Antide and related antagonists of luteinizing hormone release with long action and oral activity. *Proc Natl Acad Sci USA* 1988; 85: 8236–40.
218. Rivier J, Porter J, Hoeger C et al. Gonadotropin-releasing hormone antagonists with N omega-triazolylornithine, -lysine, or -p-aminophenylalanine residues at positions 5 and 6. *J Med Chem* 1992; 35: 4270–8.
219. Nestor JJ, Jr., Tahilramani R, Ho TL et al. Potent gonadotropin releasing hormone antagonists with low histamine-releasing activity. *J Med Chem* 1992; 35: 3942–8.
220. Garnick MB, Campion M. Abarelix depot, a GnRH antagonist, v. LHRH superagonists in prostate cancer: Differential effects on follicle-stimulating hormone. Abarelix Depot Study Group. *Mol Urol* 2000; 4: 275–7.

221. Cook T, Sheridan WP. Development of GnRH antagonists for prostate cancer: New approaches to treatment. *Oncologist* 2000; 5: 162–8.
222. Deghenghi R, Boutignon F, Wuthrich P, Lenaerts V. Antarelix (EP 24332) a novel water soluble LHRH antagonist. *Biomed Pharmacother* 1993; 47: 107–10.
223. Felberbaum R, Diedrich K. Ovarian stimulation for *in vitro* fertilization/intracytoplasmic sperm injection with gonadotrophins and gonadotrophinreleasing hormone analogues: Agonists and antagonists. *Hum Reprod* 1999; 14(Suppl 1): 207–21.
224. Bajusz S, Csernus VJ, Janaky T et al. New antagonists of LHRH. II. Inhibition and potentiation of LHRH by closely related analogues. *Int J Pept Protein Res* 1988; 32: 425–35.
225. Diedrich K, Diedrich C, Santos E et al. Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist cetrorelix during ovarian stimulation. *Hum Reprod* 1994; 9: 788–91.
226. Felberbaum RE, Albano C, Ludwig M et al. Ovarian stimulation for assisted reproduction with hMG and concomitant midcycle administration of the GnRH antagonist cetrorelix according to the multiple dose protocol: A prospective uncontrolled phase III study. *Hum Reprod* 2000; 15: 1015–20.
227. Borm G, Mannaerts B. Treatment with the gonadotrophin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: Results of a controlled, randomized, multicentre trial. The European Orgalutran Study Group. *Hum Reprod* 2000; 15: 1490–8.
228. The European Middle East Orgalutran Study Group. Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation. *Hum Reprod* 2001; 16: 644–51.
229. Fluker M, Grifo J, Leader A et al. Efficacy and safety of ganirelix acetate versus leuprolide acetate in women undergoing controlled ovarian hyperstimulation. *Fertil Steril* 2001; 75: 38–45.
230. Mannaerts B, Gordon K. Embryo implantation and GnRH antagonists: GnRH antagonists do not activate the GnRH receptor. *Hum Reprod* 2000; 15: 1882–3.
231. Demirel LC, Weiss JM, Polack S et al. Effect of the gonadotropin-releasing hormone antagonist ganirelix on cyclic adenosine monophosphate accumulation of human granulosa–lutein cells. *Fertil Steril* 2000; 74: 1001–7.
232. Ortmann O, Weiss JM, Diedrich K. Embryo implantation and GnRH antagonists: Ovarian actions of GnRH antagonists. *Hum Reprod* 2001; 16: 608–11.
233. Raga F, Casan EM, Kruessel J et al. The role of gonadotropin-releasing hormone in murine preimplantation embryonic development. *Endocrinology* 1999; 140: 3705–12.
234. The Ganirelix Dose-finding Study Group. A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). *Hum Reprod* 1998; 13: 3023–31.
235. Rackow BW, Kliman HJ, Taylor HS. GnRH antagonists may affect endometrial receptivity. *Fertil Steril* 2008; 89: 1234–9.
236. Prapas N, Prapas Y, Panagiotidis Y et al. GnRH agonist versus GnRH antagonist in oocyte donation cycles: A prospective randomized study. *Hum Reprod* 2005; 20: 1516–20.
237. Saucedo de la Llata E, Moraga Sanchez MR, Batiza RV et al. Comparison of GnRH agonists and antagonists in an ovular donation program. *Ginecol Obstet Mex* 2004; 72: 53–6.
238. Vlahos NF, Bankowski BJ, Zacur HA et al. An oocyte donation protocol using the GnRH antagonist ganirelix acetate, does not compromise embryo quality and is associated with high pregnancy rates. *Arch Gynecol Obstet* 2005; 272: 1–6.
239. Devroey P, Aboulghar M, Garcia-Velasco J et al. Improving the patient's experience of IVF/ICSI: A proposal for an ovarian stimulation protocol with GnRH antagonist co-treatment. *Hum Reprod* 2009; 24: 764–74.
240. Reissmann T, Felberbaum R, Diedrich K et al. Development and applications of luteinizing hormone-releasing hormone antagonists in the treatment of infertility: An overview. *Hum Reprod* 1995; 10: 1974–81.
241. Tur-Kaspa I, Ezcurra D. GnRH antagonist, cetrorelix, for pituitary suppression in modern, patient-friendly assisted reproductive technology. *Expert Opin Drug Metab Toxicol* 2009; 5: 1323–36.
242. Al-Inany HG, Youssef MA, Aboulghar M et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011; 5: CD001750.
243. Youssef MA, Van d V, Al-Inany HG et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist assisted reproductive technology cycles. *Cochrane Database Syst Rev* 2011; 1: CD008046.
244. Griesinger G, Schultz L, Bauer T et al. Ovarian hyperstimulation syndrome prevention by gonadotropin-releasing hormone agonist triggering of final oocyte maturation in a gonadotropin-releasing hormone antagonist protocol in combination with a "freeze-all" strategy: A prospective multicentric study. *Fertil Steril* 2011; 95: 2029–33, 2033.e1.

The role of follicle-stimulating hormone and luteinizing hormone in ovarian stimulation

40

Current concepts

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INTRODUCTION

Current practice of controlled ovarian stimulation (COS) for *in vitro* fertilization (IVF) has the possibility of using different protocols depending on the choice of the gonadotrophin-releasing hormone analog (GnRHa) and the diverse gonadotropin preparations (1). With regard to the latter, a major debate continues regarding using pure follicle-stimulating hormone (FSH)-alone regimens or administering some kind of luteinizing hormone (LH) activity-containing preparations.

While the physiological role of LH during the follicular phase of a natural cycle is unquestionable (2,3), its impact during a COS cycle on outcome and the need for adding it as a supplement remain controversial. A number of studies have analyzed this topic, but the conclusions are still confusing: although there is evidence supporting that there is no benefit of LH activity supplementation in an unselected population (4), it is also stated that it might be useful in some particular populations, especially in poor responders and older patients (5).

The present chapter is a mini-review on the role of LH activity administration in COS for IVF/intracytoplasmic sperm injection, in which the ultimate action of LH when administered and its impact on ovarian response and cycle outcome are analyzed.

BASIC PHYSIOLOGICAL BACKGROUND

The initial steps of follicular maturation are independent of gonadotropin action (6). However, from the early antral follicular stage, follicles become sensitive to the action of gonadotropins. FSH is required to start the development from antral follicles, and this first period is FSH dependent, while LH promotes androgen secretion by theca cells and is implicated in processes related to ovulation itself: follicular dominance, complete maturation (which depends on the follicle transfer of FSH dependency to LH dependency) (7), ovulation, and support of the corpus luteum (8).

The amount of LH necessary to induce a response in the follicle varies from a minimum ("LH threshold") to a maximum ("LH ceiling") (9). This amount has not been determined, but it has been suggested that less than 1% of follicular LH receptors need to be occupied in order to produce a steroidogenic response (10).

Of 1000 recruited follicles per cycle, only one will be dominant and the others will suffer atresia (11). The presence of FSH and LH is vital in this complex process.

LH receptors are located in the membranes of theca cells, granulosa cells, interstitial cells, and luteal cells, but also in cells of different tissues, including the endometrium, cervix, and tubal epithelium (12). These receptors have high affinity and selectivity to bind their respective glycoproteins, and their expression is induced by FSH (13).

Levels of LH vary during the cycle in response to pulsate liberation of GnRH. Acid forms of the gonadotropin are more common in the follicular phase, whereas the alkaline forms are more common in the luteal phase (14). In the absence of LH, ovarian follicle growth is arrested when the FSH levels decline in the mid or late follicular phase. The expression of LH receptors in granulosa cells allows the larger follicles to grow and to develop dominance over smaller follicles (15).

The main functions of LH in the ovarian cycle include promoting steroid synthesis in granulosa cells acting in synergy with FSH, offering androgens as a substrate to estradiol (E2) production, inducing the maturation of the oocyte up to the metaphase II state, inducing the production of proteases, and playing a special role in the ovulation. LH can also induce atresia of medium follicles when its concentration is greater than the "LH ceiling," and finally it acts as an inductor of luteinization, namely the change that takes place in the structure and function of granulosa cells to produce progesterone and E2 during the luteal phase (16).

HYPOGONADOTROPIC PATIENTS

The need for LH in the follicular phase is clearly demonstrated in hypogonadotropic patients. Hypogonadotropic hypogonadism (HH) is a rare reproductive function disorder characterized by the absence or decreased function of gonads due to a lack of effective hypothalamic–pituitary activity (17). It results in arrested or attenuated gonadal function, and individuals with HH do not have the necessary threshold levels of endogenous LH required to achieve optimal follicular development and steroidogenesis after administration of FSH alone. Patients with absence of endogenous gonadotropins are excellent models for studying the effects of LH. An open, randomized, dose-finding, multicenter study was designed with the aim of evaluating the efficacy

of lutropin-α addition during follitropin-α stimulation, and of identifying the minimal effective dose in the treatment of women with HH. Thirty-eight women with HH and a mean age of 28.7 years received two daily subcutaneous injections of lutropin-α (0, 25, 75, or 225 IU) and follitropin-α (150 IU).

Analyses confirmed the strong influence of the recombinant LH (rLH) dose on E2 secretion, resulting in very different endometrial growth in the treatment groups. No pregnancies occurred in the 0- or 25-IU dose groups. In the 75- and 225-IU dose groups, pregnancy occurred in 16.6% and 11.1% of patients, respectively. Although the individual requirement of rLH varied, a daily dose of 75 IU rLH was effective in the majority of patients (18).

USE OF LH IN COS FOR IVF

In most cases, COS for IVF is performed under conditions of pituitary suppression to prevent LH surge and spontaneous ovulation through the use of GnRHas, either agonists or antagonists. Therefore, most patients reach very low concentrations of serum LH, similar to those observed in hypogonadotropic patients. The administration of LH activity in COS induces several differences in the synthesis of follicular steroids, which may have an impact on oocyte maturation and competence.

To analyze the impacts of adding different amounts of rLH in COS on serum and follicular hormonal profiles, oocyte and embryo quality, and cycle outcomes, our group performed a randomized controlled trial in which 30 pure and altruistic normovulatiory oocyte donors aged 18–35 years, undergoing COS under pituitary down-regulation with a nafarelin long protocol, were allocated by computer-generated randomization to three groups (19). Group A received 300 IU of recombinant FSH (rFSH) for starting COS. Group 2 received 225 IU of rFSH and 75 IU of rLH. Group 3 received 150 IU of rFSH and 150 IU of rLH. The initial protocol was maintained for two days. Then, serum E2 was determined and the rFSH dose adjusted, while rLH was continued with the same dose until the end of COS. When four or more follicles reached 18 mm in diameter, human chorionic gonadotropin (hCG) was administered and oocyte retrieval scheduled for 36 hours later.

On the day of hCG administration, serum E2, progesterone (P), androstenedione (A), testosterone (T), dehydroepiandrosterone sulfate (DHEAS), FSH, LH, and hCG were determined. The first two follicles of each ovary were aspirated individually, and E2, P, A, T, DHEAS, and LH were determined for each follicular fluid sample. Oocytes obtained from each follicle were labeled for classification and follow-up of the resulting embryo, if any.

The results of this study showed that, interestingly, no differences were observed among groups for any of the serum hormone determinations except for FSH levels, which were significantly higher in group A, as expected (Table 40.1). Figure 40.1 shows hormonal levels in follicular fluid. As can be observed, there was a dose-dependent increase of follicular fluid E2, A, and T according to LH dose. Metaphase I oocytes were obtained from follicles that had significantly lower E2 concentrations and higher T and A levels. On the other hand, oocytes that showed multiple anomalies were recovered from follicles with significantly higher LH levels. Oocytes with perivitelline space anomalies were obtained from follicles that showed significantly higher T, A, and DHEAS concentrations.

In summary, women who received high rLH amounts during COS produced follicles with higher androgens and E2 production. Defects of intrafollicular E2 are related to metaphase I oocytes, while excess of LH and androgens is related to oocyte anomalies.

The findings of steroids in follicular fluid are consistent with those observed in the MERIT study (20), in which patients who received highly purified human menopausal gonadotropin (hMG) for stimulation showed higher concentrations of E2, A, and T than those who were stimulated with rFSH. Therefore, the action of LH may be helpful for patients with low serum androgen levels.

It has been shown that serum androgens decline steeply with age, with a decrease from menarche to menopause that ranges from 49% for free T to 77% for DHEAS, despite constant levels of serum hormone binding globulin (21). Moreover, it has been demonstrated that while the synthesis of E2 in response to rFSH stimulation is preserved in older women, there is a significant decrease in the

Table 40.1 Serum hormone concentrations the day of human chorionic gonadotropin observation according to different amounts of recombinant follicle-stimulating hormone and recombinant luteinizing hormone

	FSH 300 IU	FSH/LH 225/75 IU	FSH/LH 150/150 IU	p-value
E2 (pg/mL)	2662 ± 1239	2208 ± 852	2700 ± 1339	NS
P4 (ng/mL)	1.1 ± 0.7	0.6 ± 0.3	0.6 ± 0.5	NS
FSH (mIU/mL)	13.4 ± 4.5 (a)	8.6 ± 4.1 (b)	7.5 ± 1.3 (b)	0.009 (a > b)
LH (mIU/mL)	2.0 ± 1.9	1.6 ± 1.5	2.2 ± 1.8	NS
Te (ng/mL)	0.6 ± 0.2	0.5 ± 0.2	0.8 ± 0.3	NS
Δ4 (ng/mL)	2.7 ± 0.7	2.4 ± 0.4	2.9 ± 1.1	NS
DHEAS (μg/dL)	206 ± 57	190 ± 142	192 ± 78	NS

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; E2, estradiol; DHEAS, dehydroepiandrosterone sulfate; NS, non-significant; P4, progesterone; Te, testosterone; Δ4, androstenodione.

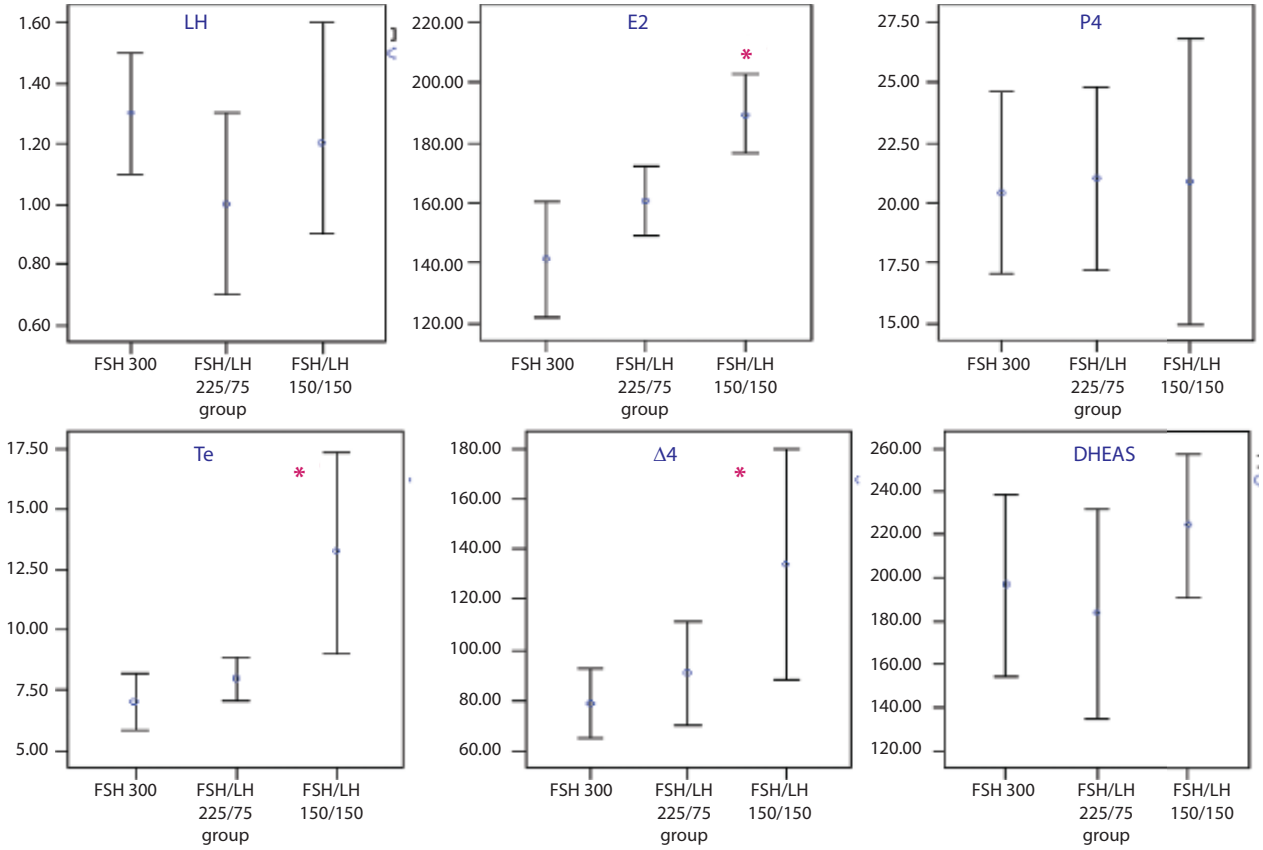

Figure 40.1 Follicular fluid hormonal determinations on the day of human chorionic gonadotropin observation. *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone (mIU/mL); E2, estradiol (pg/mL); DHEAS, dehydroepiandrosterone sulfate (mcg/dL); P4, progesterone (ng/mL); Te, testosterone (ng/mL); Δ4, androstenodione (ng/mL). *p $<$ 0.05.

synthesis of A in older women when rFSH alone is given for stimulation (22).

Indeed, in a prospective randomized study, we observed that in patients with basal T below the mean (0.45 ng/mL), the ongoing pregnancy rate was better when LH was associated with rFSH in COS for IVF, compared to rFSH alone in a GnRH agonist long protocol (23). On the other hand, no differences were observed when both protocols were compared in women with T above the mean (Table 40.2). No other differences were observed with respect to other serum androgen levels. Taken together, this supports a potential benefit of LH administration in older women, for whom basal androgens and their synthesis in response to rFSH are diminished.

Normogonadotropic patients

This group includes the majority of patients that undergo ovarian stimulation for IVF. Studies published until now show that no benefit is obtained by combining LH and FSH in ovarian stimulation for IVF in normogonadotropic patients when using GnRHas (5). This is especially true for an unselected population (4).

Table 40.2 Ongoing pregnancy per started cycle according to basal androgen levels

	FSH (95% CI)	FSH + LH (95% CI)	RR (95% CI)	p-value
Te ≤0.45 ng/mL	33.1 (25.4–41.7)	44.4 (36.1–53.2)	1.34 (0.98–1.85)	0.06
Te >0.45 ng/mL	50.0 (37.5–62.5)	40.0 (28.6–52.6)	0.80 (0.53–1.20)	0.28
DHEAS ≤156 μg/L	32.4 (24.3–41.7)	38.2 (29.6–47.5)	1.18 (0.82–1.69)	0.37
DHEAS >156 μg/L	47.3 (36.3–58.5)	43.4% (32.9–54.6)	0.92 (0.65–1.30)	0.63
Δ4 ≤1.90 ng/mL	39.1 (30.5–48.4)	46.0 (37.1–55.2)	1.18 (0.87–1.60)	0.30
Δ4 >1.90 ng/mL	40.3 (29.7–51.8)	47.9 (36.9–59.2)	1.19 (0.82–1.72)	0.35

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; RR, relative risk; CI, confidence interval; DHEAS, dehydroepiandrosterone sulfate ; Te, testosterone; Δ4, androstenodione.

Advanced reproductive age women

The potential benefit of LH administration in patients of advanced reproductive age (i.e., >35 years) has been recently evaluated in a systematic review and meta-analysis (24). In this study, it is clearly shown that LH administration leads to significantly better implantation and clinical pregnancy rates that rFSH-alone stimulation. Moreover, it is demonstrated that while rFSH leads to a higher oocyte yield, there are no differences in terms of metaphase II oocytes, and the fertilization rate is better in patients receiving LH.

These were also our findings in an age-adjusted randomized controlled trial performed in normogonadotropic patients following COS in a GnRH antagonist protocol (25). It was observed that while results were virtually the same in both stimulation groups (rFSH vs. rFSH + rLH) in patients aged up to 35 years, the implantation rate was significantly higher in women receiving rFSH and rLH in the 36–39 years of age group, with a clinically relevant increase in ongoing pregnancy rate.

Interestingly, serum progesterone levels at the end of stimulation were significantly higher in the rFSH group at all ages. This could be related to better endometrial receptivity when LH is given.

Recently, a similar randomized controlled trial has been published (26). In it, patients aged 35 years or older were stimulated under a GnRH antagonist protocol and randomized to receive either rFSH alone across the cycle or to add 75 IU of rLH form day 6 of stimulation. In this study, no benefits of rLH administration were observed.

These findings could, at first glance, be contrary to those published by our group (25). Nevertheless, an analysis in detail of the differences between both studies allows us to draw interesting and complementary conclusions about the possible role of LH in the treatment of this particular population (27). Although the patients included in our study were of better prognosis (age limit 39 years and only first IVF cycles), the methodological differences that may explain the inconsistency of the results are the use of a contraceptive pill (CP) during the cycle prior to stimulation and the substitution of 75 IU of rFSH per day with 75 IU of rLH in the study group.

These differences are reflected in the ovarian response, in the synthesis of E2 and P, and in the follicular development and oocyte yield. Although in our study hormonal determinations before starting stimulation are not available, is very likely that after one cycle of CP, all values (E2, FSH, LH, P, and T) were lower than in the present study. This would explain the greater difficulty in response for the group receiving rFSH alone at the beginning of stimulation, due to excessive ovarian suppression. In this scenario, LH administration helps with better steroidogenesis due to greater androgen synthesis as a substrate for their later aromatization to estrogens. This may also explain why in IVF cycles stimulated with rFSH alone and a GnRH antagonist, the administration of a CP during the previous cycle is associated with a lower pregnancy rate (28).

The substitution of 75 IU of rFSH with 75 IU of rLH from the beginning of stimulation may also explain the lower P levels on the day of hCG observation. Through its action at the theca layer, LH enhances the conversion of pregnenolone into androstenediol and A, while FSH enhances its conversion into progesterone in the granulosa cells. This progesterone cannot be converted into androgens in the human being (29), so if its production is excessive, it is delivered into circulation (30). In fact, in a multivariate analysis of more than 4000 cycles, we observed that a P increase at the end of stimulation is significantly related to the daily dose of FSH, but not of LH (31).

So, the impact of LH on ovarian stimulation is more patent when its administration is started at the beginning of the cycle. In the Köning study, LH is given from the sixth day of stimulation, when follicular recruitment is already completed (26). As a consequence, only a modest increase in E2 and T levels is observed, but this is probably too late to have an impact on the final response and cycle outcome. Indeed, no differences in terms of follicular response and oocyte yield are observed, while in our study, patients who received LH obtained fewer overall oocytes, but more metaphase II oocytes, reflecting a selective role of LH in ovarian response (25). This, together with lower P levels on the day of hCG observation, may explain the better outcome of these patients when rLH is administered from stimulation day 1.

Poor responders

Other authors have investigated the role of LH supplementation in patients who were hyporesponsive to ovarian stimulation with rFSH alone. These patients previously required very high doses of FSH (>3500 IU) or showed a plateau of follicular growth and E2 production when stimulated with FSH alone. These studies have shown that the addition of rLH during ovarian stimulation, when ovarian response to rFSH alone is adequate, leads to a better outcome than if the dose of FSH is increased (32–35). The reason why some patients show this type of ovarian response may not be due merely to a low ovarian reserve. It has recently been suggested that the presence of a common LH polymorphism may explain the need for abnormally high amounts of rFSH for ovarian stimulation in IVF (36). Although other studies have reported conflicting results (37,38), overall, the meta-analysis from the Cochrane Database shows a clear benefit of LH administration in these types of patients (5).

Patients with high levels of LH

Polycystic ovary syndrome (PCOS) is a common endocrine disorder associated with obesity, hyperinsulinemia, elevated levels of androgens and LH, follicular atresia, and anovulation (39). Furthermore, in PCOS, inappropriate pituitary gonadotropin secretion is generally characterized by higher mean LH serum concentrations, greater LH pulse frequency, and enhanced LH response to GnRH with respect to those of normal women (40). LH acts on theca

cells, increasing the secretion of androgens that induce atresia of non-dominant follicles (41). Excessive LH secretion could be responsible for the abnormal follicle dynamics of PCOS patients, and may hasten late follicular-phase meiotic maturation (42).

While many studies which exclude PCOS have focused on the differences obtained with FSH compared to hMG (43), very few have been published about ovarian stimulation using gonadotropins in PCOS patients with LH activity. No differences were found between outcomes in PCOS patients stimulated with rFSH versus those stimulated with hMG. Indeed, similar oocyte maturation and fertilization rates were achieved in both groups (44). In a recent review about ovarian stimulation in women with PCOS, no significant difference was demonstrated between FSH and hMG in terms of pregnancy rate. However, given the potential advantages in terms of purity and a reduction in the risk of ovarian hyperstimulation syndrome (OHSS), highly purified FSH or rFSH are likely to be widely adopted in the future (45).

In a study of 20 patients with PCOS, 10 received hMG and 10 were stimulated with FSH, with a reduction of DHEAS synthesis being observed in the former group. These findings suggest that, in PCOS patients, exogenous hMG induces a different steroid synthesis pattern than pure FSH, possibly by reduction of the δ5 steroid synthesis pathway in the adrenals and/or in the ovary (46).

There is a fear surrounding the use of gonadotropins with LH activity in PCOS patients because of the risk of OHSS, but no prospective study has yet demonstrated that the use of LH increases this risk in said patients.

Insulin seems to modulate LH levels, as has been recently reported in a study showing a clear alteration of LH levels as a direct result of insulin infusion (47). Drugs that exert an action on insulin resistance in PCOS patients have been extensively described, particularly metformin, but this topic is beyond the scope of this review.

In summary, on the basis of the available evidence, it is not possible to confirm the benefits and the harmful effects of gonadotropins on LH in PCOS patients. A well-designed study is required to answer the question of whether LH is necessary in women with PCOS.

CONCLUSION

The treatment of infertility involves ovarian stimulation, which often calls for the use of gonadotropins. Both FSH and LH form part of the therapeutic arsenal employed to achieve multiple follicular development. The need to develop protocols that improve the possibility of infertile patients becoming parents is a major challenge to both clinicians and pharmaceutical companies. In the next few years, it will be crucial to clearly define the differences in ovarian response, oocyte–embryo quality, endometrial receptivity, and cycle outcomes between patients undergoing IVF–embryo transfer with a combination of rFSH and rLH and those receiving the more established rFSH and hMG protocols.

Studies have provided sufficient evidence to support the proposed dose of 75 IU of lutropin-α in the combined product, and in general terms, a starting dose of 75 IU would appear to be appropriate.

Studies reveal that while young, normovulatory patients do not benefit from the use of rLH, there is a specific population in which better results are achieved when rLH is combined with rFSH. Although a clear definition of such patients is still lacking, the available data suggest that some women over 35 years of age with LH polymorphism may benefit from rLH administration.

Also, a supplementation of rLH to rFSH in a 1:2 ratio has been shown to lower the risk of suffering an increase of P levels at the end of stimulation (48).

Finally, it seems that LH can provide a means of selecting larger follicles and curtailing smaller, less mature follicles, and it can be used to rescue the luteal phase in patients in whom ovulation induction is performed with a-GnRH, a strategy that is used to prevent OHSS (49).

REFERENCES

1. Bosch E, Ezcurra D. Individualised controlled ovarian stimulation (iCOS): Maximising success rates for assisted reproductive technology patients. *Reprod Biol Endocrinol* 2011; 9: 82.
2. Balasch J, Miró F, Burzaco I, Casamitjana R, Civico S, Ballesca JL, Puerto B, vanrell JA. The role of luteinizing hormone in human follicle development and oocyte fertility: Evidence from IVF in a woman with long-standing hypogonadism and using recombinant human follicle stimulating hormone. *Hum Reprod* 1995; 10: 1678–83.
3. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: A dose-finding study. The European Recombinant Human LH Study Group. *J Clin Endocrinol Metab* 1998; 83: 1507–14.
4. Kolibianakis EM, Kalogeropoulou L, Griesinger G, Papanikolaou EG, Papadimas J, Bontis J, Tarlatzis BC. Among patients treated with FSH and GnRH analogues for *in vitro* fertilization, is the addition of recombinant LH associated with the probability of live birth? A systematic review and meta-analysis. *Hum Reprod Update* 2007; 13: 445–52.
5. Mochtar MH, Van der Veen, Ziech M, van Wely M. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2007; 2: CD005070.
6. Lamminen T, Jokinen P, Jiang M, Pakarinen P, Simonsen H, Huhtaniemi I. Human FSHβ subunit gene is highly conserved. *Mol Hum Reprod* 2005; 11: 601–5.
7. Fauser BC, Van Heusden AM. Manipulation of human ovarian function: Physiological concepts and clinical consequences. *Endocr Rev* 1997; 18: 71–106.

8. Brailly S, Gougeon A, Milgrom E, Bomsel HO, Papiernik E. Androgens and progestins in the human ovarian follicle: Differences in the evolution of preovulatory, healthy nonovulatory, and atretic follicles. *J Clin Endocrinol Metab* 1981; 53: 128–34.
9. Loumaye E. Ovarian stimulation: Is exogenous LH necessary in all patients? *Gynecol Obstet Fertil* 2002; 30: 890–5.
10. Chappel SC, Howles C. Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum Reprod* 1991; 6: 1206–12.
11. Fritz MA, Speroff L. (Eds.). The ovary—Embryology and development. In: *Clinical Endocrinology and Infertility*. Philadelphia, PA: Lippincott, Williams and Wilkins, 2011, pp. 105–20.
12. Kornyei JL, Lei ZM, Rao CHV. Human myometrial smooth muscle cells are novel targets of direct regulation by human chorionic gonadotropin. *Biol Reprod* 1993; 49: 1149–57.
13. Nordhoff V, Gromoll J, Simoni M. Constitutively active mutations of G protein-coupled receptors: The case of human luteinizing hormone and follicle-stimulating receptors. *Arch Med Res* 1999; 30: 501–9.
14. Wide L, Bakos O. More basic forms of both human follicle stimulating hormone and luteinizing hormone in serum at mid-cycle compared with follicular or luteal phase. *J Clin Endocrinol Metab* 1993; 76: 885–9.
15. Campbell BK, Dobson H, Baird DT, Scaramuzzi RJ. Examination of the relative role of FSH and LH in the mechanism of ovulatory follicle selection in sheep. *J. Reprod Fertil* 1999; 117: 355–67.
16. Eppig JJ. Oocyte control of ovarian follicular development and function in mammals. *Reproduction* 2001; 122: 829–38.
17. Reame NE, Sauder ED, Case GD, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion in women with hypothalamic amenorrhea: Evidence that reduced frequency of gonadotropin releasing hormone secretion is the mechanism of persistent anovulation. *J Clin Endocrinol Metab* 1985; 61: 851–8.
18. Bosch E. Recombinant human follicular stimulating hormone and recombinant human luteinizing hormone in a 2:1 ratio combination. Pharmacological characteristics and clinical applications. *Expert Opin Biol Ther* 2010; 10: 1001–9.
19. Bosch Pau E, Albert C, Zuzuarregui JL, Remohí J, Pellicer A. Impact of different amounts of LH in controlled ovarian hyperstimulation oocyte donation cycles. *Fertil Steril* 2006; 86(Suppl 2): S425.
20. Smitz J, Andersen AN, Devroey P, Arce JC, MERIT Group. Endocrine profile in serum and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients. *Hum Reprod* 2007; 22: 676–87.
21. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: Changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab* 2005; 90: 3847–53.
22. Welt CK, Jimenez Y, Sluss PM, Smith PC, Hall JE. Control of estradiol secretion in reproductive ageing. *Hum Reprod* 2006; 21: 2189–93.
23. Bosch E, Labarta E, Vidal C, Giles J, Bellver J, Zuzuarregui JL, Pellicer A. The relationship between serum androgen levels and the need of LH administration during controlled ovarian stimulation for *in vitro* fertilization: An explorative study. *Hum Reprod* 2011; 26(Suppl 1): i26, O: 064.
24. Hill MJ, Levens ED, Levy G, Ryan ME, Csokmay JM, DeCherney AH, Whitcomb BW. The use of recombinant luteinizing hormone in patients undergoing assisted reproductive techniques with advanced reproductive age: A systematic review and meta-analysis. *Fertil Steril* 2012; 97: 1108–14.
25. Bosch E, Labarta E, Crespo J, Simón C, Remohí J, Pellicer A. Impact of luteinizing hormone administration on gonadotropin-releasing hormone antagonist cycles: An age-adjusted analysis. *Fertil Steril* 2011; 95: 1031–6.
26. König TE, van der Houwen LE, Overbeek A et al. Recombinant LH supplementation to a standard GnRH antagonist protocol in women of 35 years or older undergoing IVF/ICSI: A randomized controlled multicentre study. *Hum Reprod* 2013; 28: 2804–12.
27. Bosch E. Comment on "Recombinant LH supplementation to a standard GnRH antagonist protocol in women of 35 years old or older undergoing IVF/ICSI: A randomized controlled multicentre study". *Hum Reprod* 2014; 29: 636–7.
28. Griesinger G, Kolibianakis EM, Venetis C, Diedrich K, Tarlatzis B. Oral contraceptive pretreatment significantly reduces ongoing pregnancy likelihood in gonadotropin-releasing hormone antagonist cycles: An updated meta-analysis. *Fertil Steril* 2010; 94: 2382–4.
29. Yding Andersen C, Bungum L, Nyboe Andersen A, Humaidan P. Preovulatory progesterone concentration associates significantly to follicle number and LH concentration but not to pregnancy rate. *Reprod Biomed Online* 2011; 23: 187–95.
30. Fleming R, Jenkins J. The source and implications of progesterone rise during the follicular phase of assisted reproduction cycles. *Reprod Biomed Online* 2010; 21: 446–9.
31. Bosch E, Labarta E, Crespo J, Simón C, Remohí J, Jenkins J, Pellicer A. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for *in vitro* fertilization: Analysis of over 4000 cycles. *Hum Reprod* 2010; 25: 2092–100.
32. Lisi F, Rinaldi L, Fishel S, Lisi R, Pepe GP, Picconeri MG. Use of recombinant follicle-stimulating hormone (Gonal F) and a recombinant luteinizing

hormone (Luveris) in multiple follicular stimulation in patients with a suboptimal response to *in vitro* fertilization. *Fertil Steril* 2003; 79: 1037–8.
33. Ferraretti AP, Gianaroli L, Magli MC, D'angelo A, Farfalli V, Montanaro N. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. *Fertil Steril* 2004; 82: 1521–6.
34. De Placido G, Alviggi C, Perino A, Strina I, Lisi F, Fasolino A, Italian Collaborative Group on Recombinant Human Luteinizing Hormone. Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. *Hum Reprod* 2005; 20: 390–6.
35. Ruvolo G, Bosco L, Pane A, Morici G, Cittadini E, Roccheri MC. Lower apoptosis rate in human cumulus cells after administration of recombinant luteinizing hormone to women undergoing ovarian stimulation for *in vitro* fertilization procedures. *Fertil Steril* 2007; 87: 542–6.
36. Alviggi C, Clarizia R, Pettersson K, Mollo A, Humaidan P, Strina I, Coppola M, Ranieri A, D'Uva M, De Placido G. Suboptimal response to GnRHa long protocol is associated with a common LH polymorphism. *Reprod Biomed Online* 2009; 18: 9–14.
37. Chung K, Krey L, Katz J, Noyes N. Evaluating the role of exogenous luteinizing hormone in poor responders undergoing *in vitro* fertilization with gonadotropin-releasing hormone antagonist. *Fertil Steril* 2005; 84: 313–8.
38. Barrenetxea G, Agirregoikoa JA, Jiménez MR, de Larruzea AL, Ganzabal T, Carbonero K. Ovarian response and pregnancy outcome in poor-responder women: A randomized controlled trial on the effect of luteinizing hormone supplementation on *in vitro* fertilization cycles. *Fertil Steril* 2008; 89: 546–53.
39. Vignesh JP, Mohan V. Polycystic ovary syndrome: A component of metabolic syndrome? *J Postgrad Med* 2007; 53:128–34.
40. The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; 19: 41–7.
41. Uilenbroek JT, Woutersen PJ, van-der Schoot P. Atresia of preovulatory follicles: Gonadotropin binding and steroidogenic activity. *Biol Reprod* 1980; 23: 219–29.
42. Filicori M, Cognigni G, Samara A, Melappioni S, Perri T, Cantelli B, Parmegiani L, Pelusi G, DeAloysio D. The use of LH activity to drive folliculogenesis: Exploring uncharted territories in ovulation induction. *Hum Reprod Update* 2002; 8: 543–57.
43. Coomarasamy A, Afnan M, Cheema D, van der Veen F, Bossuyt PMM, van Wely M. Urinary hMG versus recombinant FSH for controlled ovarian hyperstimulation following an agonist long down-regulation protocol in IVF or ICSI treatment: A systematic review and meta-analysis. *Hum Reprod* 2008; 23: 310–5.
44. Teissier MP, Chable H, Paulhac S, Aubard Y. Recombinant human follicle stimulating hormone versus human menopausal gonadotrophin induction: Effects in mature follicle endocrinology. *Hum Reprod* 1999; 14: 2236–41.
45. Weiss NS, Nahuis M, Bayram N, Mol BWJ, Van der Veen F, van Wely M. Gonadotrophins for ovulation induction in women with polycystic ovarian syndrome. *Cochrane Database Syst Rev* 2015; 9: CD010290.
46. Turkmen S, Backstrom T, Idil M. Reduction of dehydroepiandrosterone sulphate synthesis in women with polycystic ovary syndrome by human menopausal gonadotropin but not purified urinary follicle stimulating hormone: A comparative pilot study. *Gynecol Endocrinol* 2004; 19: 69–78.
47. Lawson MA, Jain S, Sun S, Patel K, Malcolm PJ, Chang RJ. Evidence for insulin suppression of baseline luteinizing hormone in women with polycystic ovarian syndrome and normal women. *J Clin Endocrinol Metab* 2008; 93: 2089–96.
48. Werner MD, Forman EJ, Hong KH, Franasiak JM, Molinaro TA, Scott RT, Jr. Defining the "sweet spot" for administered luteinizinghormone-to-follicle-stimulating hormone gonadotropin ratios during ovarianstimulation to protect against a clinically significant late follicular increase in progesterone: An analysis of 10,280 first *in vitro* fertilization cycles. *Fertil Steril* 2014; 102: 1312–7.
49. Papanikolaou EG, Verpoest W, Fatemi H, Tarlatzis B, Devroey P, Tournaye H. A novel method of luteal supplementation with recombinant luteinizing hormone when a gonadotropin-releasing hormone agonist is used instead of human chorionic gonadotropin for ovulation triggering: A randomized prospective proof of concept study. *Fertil Steril* 2011; 95: 1174–7.

Endocrine characteristics of assisted reproduction technology cycles

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BULENT URMAN, BARIS ATA, and HAKAN YARALI

INTRODUCTION

Ovarian stimulation for assisted reproduction technology (ART) cycles aims to provide multiple pre-ovulatory follicles for oocyte collection. There are three main components of a conventional ART stimulation cycle: (i) induction of multi-follicular growth with exogenous gonadotropins; (ii) prevention of endogenous luteinizing hormone (LH) surge by using gonadotropin-releasing hormone (GnRH) analogs; and (iii) inducing an endogenous LH surge or mimicking it with exogenous human chorionic gonadotropin (hCG) for oocyte maturation. In this chapter, we will briefly review endocrinologic aspects of each of these components.

INDUCTION OF MULTI-FOLLICULAR GROWTH WITH EXOGENOUS GONADOTROPINS

A finite number of primordial follicles exist in the ovaries of reproductive aged women. A cohort of these primordial follicles starts growing in a random and continuous fashion, in a process called "primary recruitment." Primordial follicle growth occurs until the antral stage independent of gonadotropin stimulation. For further growth, follicle-stimulating hormone (FSH) is required. In the absence of adequate FSH supply, as happens before puberty, antral follicles undergo atresia before reaching the pre-ovulatory stage. The FSH threshold is the minimum level of FSH required for continuing follicle growth beyond the antral stage. Importantly, follicles at different stages of growth have different FSH thresholds, a fact that precludes defining it with a single serum FSH level.

In a natural menstrual cycle, endogenous FSH production increases following the demise of the corpus luteum and the resultant fall in progesterone, estradiol, and inhibin A levels. Increasing FSH levels exceed the threshold and enable the antral follicles, which have gained FSH responsiveness through expression of FSH receptors on the granulosa cells, to continue growth, a process that is called "secondary" or "cyclic, gonadotropin-dependent recruitment." It is estimated that 10 antral follicles per ovary are thus recruited during the luteo-follicular transition in a healthy young woman (1).

Growing antral follicles produce increasing amounts of estradiol and inhibin-B, which exert negative feedback on the hypothalamus and pituitary, leading to a decline in pituitary FSH production to levels below the threshold. While the antral follicles, which are still dependent on FSH for growth, undergo atresia, the dominant follicle that has started expressing LH receptors on its granulosa cells can continue its growth independent of FSH stimulation. The period of FSH supply over the threshold is named the "FSH window" (Figure 41.1).

The rationale of ovarian stimulation for ART is to increase the number of follicles reaching the pre-ovulatory stage, a process that requires extension of the FSH window. This is achieved either by exogenous FSH administration or by anti-estrogenic agents that block the negative feedback mechanisms (i.e., selective estrogen receptor modulators or aromatase inhibitors).

In conventional ART cycles, exogenous FSH administration is started in the early follicular phase, a period where endogenous FSH levels falls below the threshold for the already existing antral follicles. This enables the growth of a group of antral follicles up to the pre-ovulatory stage. Follicular response to FSH stimulation is monitored by ultrasound examination of the ovaries. Serum estradiol measurements also provide a rough estimate of follicular growth during ovarian stimulation. While serum estradiol levels $<$100 pg/mL on the sixth day of FSH stimulation suggest an inadequate follicular response, levels $>$500 pg/mL are a sign of overstimulation. The course of serum estrogen levels reflects follicular growth throughout stimulation. Declining estradiol levels prior to triggering of oocyte maturation are associated with decreased pregnancy rates. Inhibin-B is another product of the granulosa cells of early antral follicles, and its serum levels can also be used as a marker of follicular growth. Indeed, earlier studies demonstrated an association between serum inhibin-B levels between the fourth and sixth days of FSH stimulation and the number of mature oocytes collected. However, the additional value of measuring inhibin-B levels over ultrasound and serum estradiol monitoring is questionable, so this is not routinely practiced. Serum FSH levels are not informative with regard to follicular growth. The most likely reason for this is the limiting factor for follicular response being the number of available antral follicles rather than FSH level per se, provided that FSH is above the threshold. Moreover, the threshold varies for individual follicles, and there is a significant overlap in serum FSH levels between anovulatory women who responded with or without follicular growth to exogenous FSH stimulation (2).

PREVENTION OF ENDOGENOUS LH SURGE AND FOLLICLE RUPTURE

Multi-follicular growth induced with exogenous FSH stimulation risks a premature LH surge, which can lead

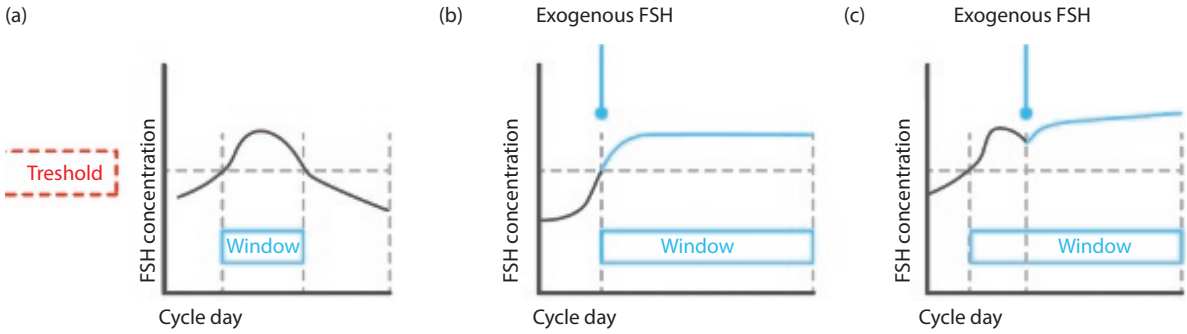

Figure 41.1 The FSH window corresponds to the period during which FSH levels are above the threshold levels for continuing antral follicle growth. (a) Natural menstrual cycle; (b) long GnRH agonist protocol; (c) GnRH antagonist cycle. *Abbreviation*: FSH, follicle-stimulating hormone.

to rupture of follicles before oocyte collection. This is prevented by blocking GnRH action on the pituitary gonadotrophs. There are two means of pituitary suppression: the first involves pituitary desensitization by a prolonged exposure to exogenous GnRH (i.e., GnRH agonist administration starting from the mid-luteal phase of the preceding cycle or simultaneously with gonadotropin injections—the long luteal GnRH agonist, and the short GnRH agonist protocols, respectively). The second involves daily administration of a GnRH antagonist when an endogenous LH surge is likely to occur (i.e., in the late follicular phase). GnRH antagonists compete with endogenous GnRH at the pituitary receptor level and provide rapid blockage of GnRH activity.

GnRH agonist injections initially lead to the release of FSH and LH from the pituitary (i.e., a flare effect), but they eventually provide a hypogonadotropic state (i.e., severely suppressed endogenous FSH and LH production). This is due to internalization of GnRH receptors on the gonadotrophs following prolonged exposure to GnRH. By contrast, in GnRH antagonist protocols, endogenous gonadotropin production remains unaltered until the initiation of GnRH antagonist in the late follicular phase. Thus, overall FSH consumption is lower in GnRH antagonist cycles than in GnRH agonist cycles.

THE ROLE OF LH

The two-cell, two-gonadotropin theory suggests that ovarian steroidogenesis is the result of actions of FSH and LH on granulosa and theca cells, respectively, through receptors specific to each gonadotropin. LH stimulates conversion of cholesterol to androstenedione in theca cells. Androstenedione diffuses into the granulosa cells, where, under FSH influence, it is aromatized to estrogens. Thus, LH action is necessary for the production of estradiol. In studies conducted on hypogonadal subjects, stimulation by only FSH promotes follicle development but cannot induce steroidogenesis (3).

The concept of a therapeutic LH window that has been introduced by Balasch and Fabregues states that below a certain threshold of LH, follicular maturation is impaired due to inadequate theca cell androgen synthesis and reduced aromatization of androgens to estrogens, resulting in incomplete oocyte maturation (4). If serum LH level is kept in an ideal range, optimal follicular growth and development leads to full oocyte maturation. GnRH analogs used during ovarian stimulation create an LH-deficient environment that may, in theory, be detrimental to follicle growth and maturity (5). Abnormally high levels of LH, on the other hand, result in LH receptor down-regulation and impaired granulosa cell proliferation, causing follicular atresia of the subordinate follicles and premature luteinization of the dominant follicle (4). LH may also play a role in the deselection of subordinate follicles. Preclinical evidence shows that developing follicles have specific requirements for exposure to LH beyond which normal maturation ceases (6). This finding gave rise to the concept of an "LH ceiling," meaning that each follicle would have an upper limit of stimulation.

Recent observations suggest that LH may also act on the granulosa cells through its own receptors. Therefore, it appears that LH regulates both granulosa and theca cells. FSH and LH induce the local production of the soluble molecule inhibin-B and growth factors. Among these, insulin-like growth factor (IGF)-I and -II, which are expressed by both granulosa and theca cells throughout folliculogenesis, are important in promoting follicular maturation (7). These findings may explain the observation that FSH activity can be totally substituted by LH once granulosa cells express adequate amounts of LH receptors (8).

Besides its role in follicle growth and maturation, the secretion of LH may, in theory, be beneficial in reducing the exposure of the growing follicles and the endometrium to a subtle increase in progesterone concentrations. The relevance of late follicular-phase progesterone concentration will be discussed further later. It may be concluded that LH is necessary for optimal follicular growth, steroid environment, and implantation. However, whether too much LH is detrimental for follicular growth, retrieval of good-quality mature oocytes, and embryo implantation is still a matter of debate.

Despite these theoretical concerns, findings from clinical trials of LH supplementation of ovarian stimulation are conflicting. Currently, three groups of commercially

available gonadotropin preparations contain LH activity: (i) urinary human menopausal gonadotropins (hMGs), in which 95% of the LH activity is derived from hCG; (ii) LH glycoprotein produced by recombinant technology; and (iii) a combination of recombinant FSH (rFSH) and LH glycoproteins in a fixed ratio of 2:1.

Retrospective evaluation of large randomized controlled trials (RCTs) comparing rFSH with hMG or corifollitropin-α, an extended-action FSH molecule, failed to show any association between endogenous LH levels and ART outcomes (9,10). It appears that low endogenous LH levels associated with the long luteal GnRH agonist protocols do not decrease the probability of a successful ART outcome.

Meta-analyses comparing rFSH with hMG show similar clinical pregnancy rates, with rFSH yielding a higher number of oocytes despite using lower doses of gonadotropin (11). It is generally concluded that ample evidence exists for the equivalence of rFSH and HMG regarding clinical outcomes of ART cycles (12). However, given the higher oocyte yield in the rFSH group, more RCTs are required in order to compare the cumulative pregnancy rates including fresh and frozen–thawed embryo transfers from one stimulation cycle. Despite the fact that rFSH stimulates more follicles, resulting in higher peak estradiol levels, and is associated with a higher number of retrieved oocytes, it appears that the incidence of ovarian hyperstimulation syndrome is similar to that in women stimulated with urinary gonadotropins (13). There also does not appear to be a difference in pregnancy rates of frozen–thawed embryo transfer cycles that were previously treated with rFSH or hMG (14).

The addition of rLH to rFSH was evaluated in several studies. A meta-analysis by Kolibianakis et al. summarized the available evidence from seven RCTs including a total of 701 women and found no difference in live birth rates with or without the addition of rLH to rFSH (15).

The endocrine profiles of ART cycles stimulated with rFSH and rLH versus hMG were compared in a prospective study involving oocyte donors (16). On the sixth day of stimulation and on the day of triggering, serum steroid hormone levels were slightly but not significantly higher in the rFSH group compared with the hMG group. No statistically significant differences were observed for intrafollicular levels of steroid hormones between the two protocols; ongoing pregnancy rates were also similar (46.1% vs. 46.1%). It appears that the endocrine profile of the controlled ovarian stimulation cycle is not affected by the source of LH activity.

In conclusion, there is inadequate evidence to prove that routine LH administration is associated with an improvement in ART outcome, including implantation and pregnancy rates (17). However, there is some evidence suggesting a beneficial effect of LH in subsets of patients, namely older women and women who have a diminished ovarian reserve (18).

Given the above-mentioned uncertainties, routine monitoring of serum LH levels seems unwarranted during stimulation cycles, neither to confirm pituitary down-regulation in the long GnRH agonist protocol nor to determine an endogenous LH surge during GnRH antagonist cycles. Plasma LH levels rapidly decline after GnRH antagonist administration, and the relevance of an LH surge without an accompanying increase in progesterone level is controversial. Therefore, the detection of an isolated LH surge in GnRH antagonist stimulation cycles is unlikely to alter management of the cycle.

PROGESTERONE DURING OVARIAN STIMULATION FOR ART

Progesterone is synthesized mainly by the ovary and, to a much lesser extent, by the adrenal gland. Both the granulosa and theca cells synthesize progesterone (Figure 41.2). However, C17 hydroxylase activity is only present in theca cells and hence progesterone produced by the granulosa cells diffuses into the theca cells to be hydroxylated; alternatively, progesterone produced by granulosa cells may acquire access to the circulation if produced in excess amounts (Figure 41.2). Progesterone in theca cells is metabolized into androgens and subsequently aromatized to estrogens back in the granulosa cells.

Throughout the follicular phase, with LH stimulation, the synthesis and metabolization (hydroxylation) of progesterone in the theca cell compartment is in balance. Similarly, throughout the follicular phase, FSH, via 3β-hydroxysteroid dehydrogenase, stimulates synthesis of progesterone in the granulosa cells. The effect of LH on granulosa cells is cycle-stage specific since granulosa cells gradually acquire LH receptors with follicular growth until the time of ovulation (19). Following LH receptor expression, the stimulatory effect of LH on progesterone production in the granulosa cells is three-fold stronger compared with FSH (20,21). All of the above clearly indicate that progesterone is not only an intermediate product in steroid hormone biosynthesis, but also an important secretory product of granulosa cells in the late follicular phase. There is a physiological role for pre-ovulatory serum progesterone increase in the natural cycle, which is to facilitate the positive feedback of estrogen during an LH surge acting at a hypothalamo-pituitary level.

Early follicular-phase progesterone levels

Menstrual bleeding follows the demise of the corpus luteum, which is the source of progesterone. Serum progesterone levels are <1 ng/mL until the start of an LH surge in a natural cycle. In the long GnRH agonist protocol, corpus luteum can be rescued by the initial flare effect of GnRH agonist on LH secretion. Increased progesterone levels accompanied by the presence of an ovarian cyst on the starting day of gonadotropin injections suggests the presence of an active corpus luteum. This would require either extending down-regulation with the GnRH agonist or delaying gonadotropin start until after the demise of the corpus luteum, and aspiration of the cyst prior to gonadotropin injections. However, routine measurement of serum progesterone levels to confirm pituitary down-regulation is not required if the ultrasound scan shows a thin endometrium in the absence of an ovarian cyst of >10 mm.

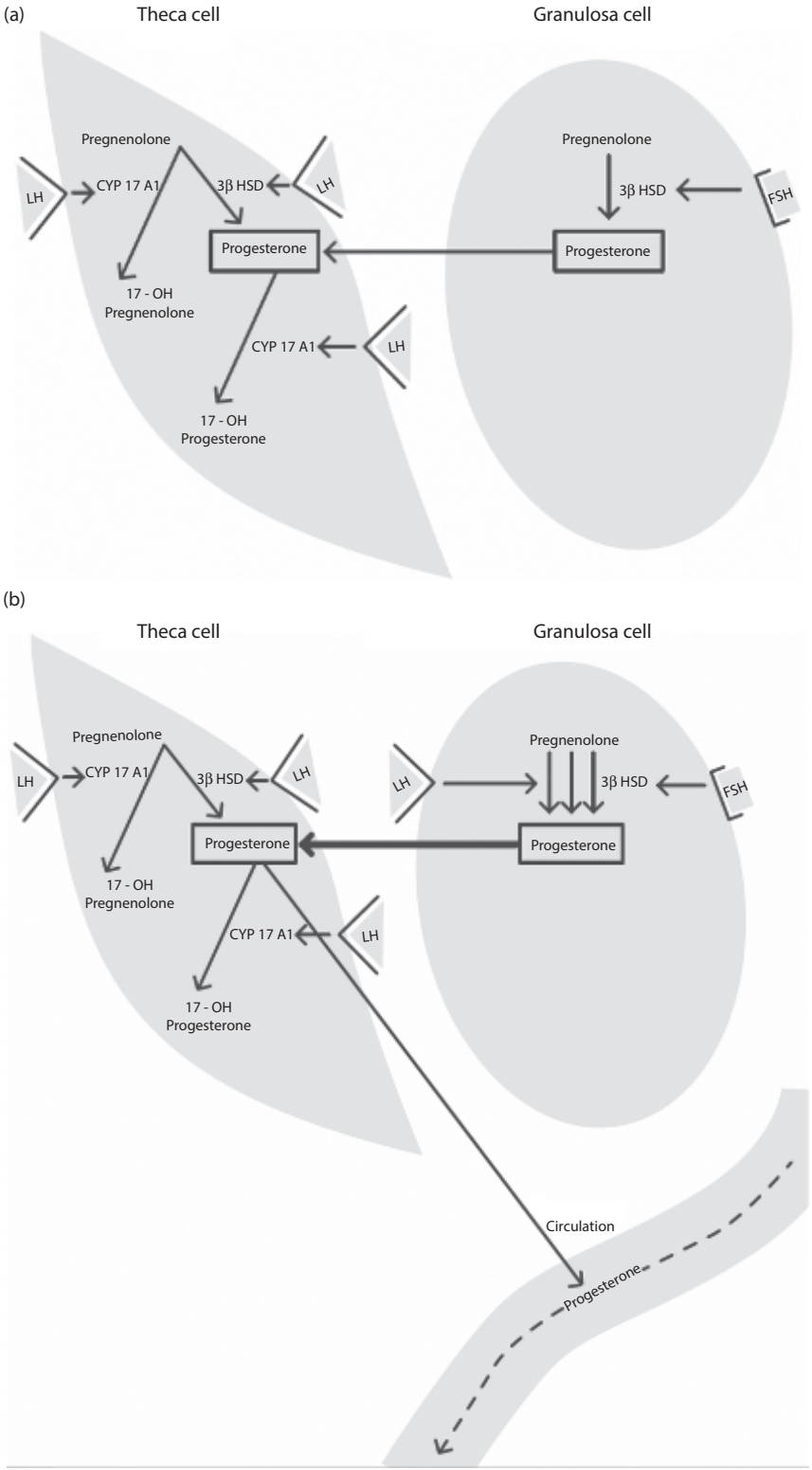

Figure 41.2 Progesterone synthesis and metabolism during the follicular phase. CYP 17 A1 (C17 hydroxylase) activity is only present in theca cells and hence progesterone produced by the granulosa cells diffuses into the theca cells to be hydroxylated. (a) During the early follicular phase, LH acts only on theca cells to stimulate 3β-hydroxysteroid dehydrogenase to convert pregnenolone to progesterone and CYP 17 A1 (C17 hydroxylase) to convert progesterone to 17-hydroxylated progesterone. (b) During the late follicular phase, LH receptors are heavily expressed on granulosa cells; the stimulatory effect of LH on progesterone production in the granulosa cells is three-fold stronger compared with FSH. Progesterone produced by granulosa cells may acquire access to the circulatory blood if produced in excess amounts. *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Incomplete luteolysis is the most likely reason for high progesterone levels early in the cycle. A serum progesterone level above 1.5 ng/mL on the second day of a spontaneous menstrual cycle has been reported in 4%–13% of women who were due to start ovarian stimulation in a GnRH antagonist cycle (22–24). Studies have consistently shown significantly decreased pregnancy rates in women with elevated early follicular-phase progesterone levels. However, given the low incidence of elevated progesterone on the second day of the cycle and the absence of a proven intervention to restore pregnancy rates, routine screening of serum progesterone levels before commencing stimulation is not recommended in GnRH antagonist cycles.

Late follicular-phase serum progesterone levels

There has been great interest in the impact of late follicular serum progesterone elevation on *in vitro* fertilization (IVF) outcomes since the first report by Schoolcraft et al. in 1991 (25). Elevated late follicular serum progesterone levels have been reported in 2%–35% of ART cycles (26,27). Even though conclusive data are lacking, high circulating late follicular progesterone levels may be detrimental to pregnancy outcomes (26,27). In this context, the type of progesterone assay used (28), the threshold for progesterone elevation (27), and the magnitude of ovarian response (hyper-response, normal response, and poor response) (29,30) should be taken into account.

The term "premature luteinization" should be avoided since serum LH levels are not necessarily elevated in all patients with high late follicular serum progesterone levels. The risk of late follicular progesterone elevation is strongly correlated with the intensity of ovarian stimulation; namely, FSH dose consumption (30,31), serum estradiol concentration, and number of oocytes retrieved (31–33). These observations clearly indicate that it is the granulosa cell "mass" that dictates the risk of premature progesterone elevation in hyper-responder patients. However, serum progesterone increases can also be noted in poor ovarian responders via an uncertain mechanism, although excessive FSH stimulation may still be the cause in such cases.

The type of GnRH analog employed may impact the risk of progesterone elevation. Serum progesterone on the trigger day is higher in GnRH agonist co-treated cycles compared with GnRH antagonist cycles (26,31,34). This is mainly due to approximately one to two more retrieved oocytes and a higher endogenous LH concentration during the last few days of stimulation in GnRH agonist co-treated cycles (34).

The impact of LH/hCG-containing products on late follicular progesterone elevation is controversial. In the MERIT trial (GnRH agonist; rFSH vs. highly purified [HP]-hMG; starting dose of FSH: 225 IU/day), significantly higher serum progesterone levels were noted on the day of hCG administration in the rFSH arm compared with the HP-hMG arm (1.07 ± 0.5 vs. 0.82 ± 0.41 ng/mL, respectively, $p < 0.01$) (35). However, such an increase might be more likely to be due to greater ovarian response in the rFSH arm (~2 oocytes; 11.8 ± 5.7 vs. 10.0 ± 5.4, $p < 0.01$), rather than the preparation itself. In concordance with this explanation, no significant difference in serum progesterone levels was noted between the rFSH and HP-hMG arms in the MEGASET trial (GnRH antagonist; rFSH vs. HP-hMG; starting dose of FSH: 150 IU/day), despite collection of significantly more oocytes in the rFSH arm (10.7 ± 5.8 vs. 9.1 ± 5.2, $p < 0.01$) (36). A recent Danish study also refuted the assumption that hCG/LH activity decreased the risk of late follicular progesterone elevation (37); patients were treated with a fixed dose of rFSH 150 IU/day and, starting on the first day of stimulation, they were randomized to a daily hCG co-treatment dosing of 0, 50, 100, or 150 IU. A dose-dependent increase in serum progesterone was noted with increasing daily hCG dosing (37).

TRIGGERING OF FINAL OOCYTE MATURATION

In the natural cycle, the mid-cycle LH surge induces the release of the oocyte and follicle rupture. However, in stimulated ART cycles, the LH surge is deliberately suppressed by GnRH analogs in order to prevent follicle rupture before oocyte retrieval. In the early days of ART, mimicking LH activity with hCG became the norm for two reasons: firstly, rLH was unavailable; and secondly, the GnRH agonist protocols prevented the induction of a spontaneous LH surge. Varying dosages of hCG between 2500 and 10,000 IU are used for triggering oocyte maturation. The plasma half-life of hCG is almost 10-fold longer than that of LH (38). Thus, it not only induces oocyte maturation and release, but also provides a sustained luteotropic effect. The implications of this sustained luteotropic effect for luteal-phase endocrinology are discussed in the next section. Even though it is possible to trigger oocyte maturation with the currently available rLH, very high dosages are required to this end, and this is not used in clinical practice (39,40).

Introduction of GnRH antagonists enabled induction of an endogenous LH surge with a single administration of GnRH agonist. Similarly to the natural cycle, an endogenous FSH surge accompanies the GnRH agonist-induced LH surge. Whether this simultaneous FSH surge confers other benefits is currently controversial (41). As compared to hCG triggering, the GnRH agonist-induced LH surge provides similar numbers of oocytes collected, fertilization rates, and embryo quality (41). However, the LH surge induced with GnRH agonists lasts than the LH surge in the natural cycle. This results in rapid luteolysis and impairs ongoing pregnancy rates when the luteal phase is supported only with progesterone. Luteal-phase support following GnRH agonist triggering is mentioned in another chapter and will not be further discussed in detail.

LUTEAL PHASE FOLLOWING OVARIAN STIMULATION FOR ART

Progesterone, the main product of the corpus luteum, is indispensable for successful implantation and maintenance of early pregnancy. LH concentrations during the

luteal phase of a spontaneous cycle range between 4 and 10 IU/L. This range of LH concentration suffices to produce a mid-luteal peak of progesterone production, which coincides with the time of implantation. A circulating mid-luteal progesterone level exceeding 10 ng/mL is generally considered to reflect ovulation and a normally functioning corpus luteum in a spontaneous cycle (42).

The luteal phase in stimulated ART cycles is defective. Both the profile and duration of endogenous progesterone production in ART cycles are different as compared with the natural cycle. Firstly, the profile is different; following the hCG trigger, there is a boost of progesterone production from multiple corpora lutea in the early luteal phase, attaining peak levels exceeding 50 ng/mL on the day of embryo transfer (Figure 41.3) (43). This is clearly different from the natural cycle in which serum progesterone levels peak (~10 ng/mL) in the mid-luteal phase, coinciding with the window of implantation. Exposure to supra-physiological progesterone early in the luteal phase can cause endometrial advancement and impair endometrial receptivity. Secondly, the luteal phase lasts in non-supplemented ART cycles due to premature luteolysis secondary to circulating supra-physiologic estrogen and progesterone levels inhibiting endogenous LH secretion (Figure 41.3) (44). Inhibition of endogenous LH release combined with the significantly shorter half-life of LH compared with hCG result in a severely defective luteal phase when final oocyte maturation is triggered by a GnRH agonist; hence, modified luteal-phase support strategies should be employed in such cycles if fresh embryo transfer is to be performed (41).

Mid-luteal serum progesterone levels of 25–30 ng/mL are required for sustained implantation in ART cycles (45). In a study of 341 patients undergoing ART, patients with viable pregnancies had significantly higher mean progesterone levels during the preimplantation and postimplantation periods compared to those of non-pregnant cycles or abortions (46). On the day of implantation, 73.2% of viable pregnancies, 41.7% of clinical abortions, and 20% of preclinical abortions had a progesterone concentration of 30 ng/mL (47). Similarly, a significant association was noted between clinical pregnancy rate and luteal serum progesterone levels in a study of 544 women undergoing IVF (48). These observations are in concordance with the initial studies in which a mid-luteal serum progesterone concentration of 10 ng/mL was found to be the lower limit of conception in a natural cycle, whereas a three-fold increase (~30 ng/mL) was required in stimulated cycles with gonadotropins (42).

However, the optimum serum progesterone levels for maximizing implantation rates in frozen embryo transfer cycles with different protocols may be different.

ANDROGENS

Androgens are produced by the theca cells and serve as a substrate for estrogen biosynthesis. Androgen receptor (AR) expression was identified in human follicles. Androgens may exert a direct autocrine and paracrine effect in regulating follicular function (49). Androgens also up-regulate their receptors and augment FSH receptors in granulosa cells (50). Studies in primates show that testosterone treatment increases FSH receptors in granulosa cells, stimulates early stages of follicle growth, and increases the numbers of preantral and antral follicles (51). Similarly, there is a strong correlation between follicular fluid testosterone levels and FSH receptor expression in

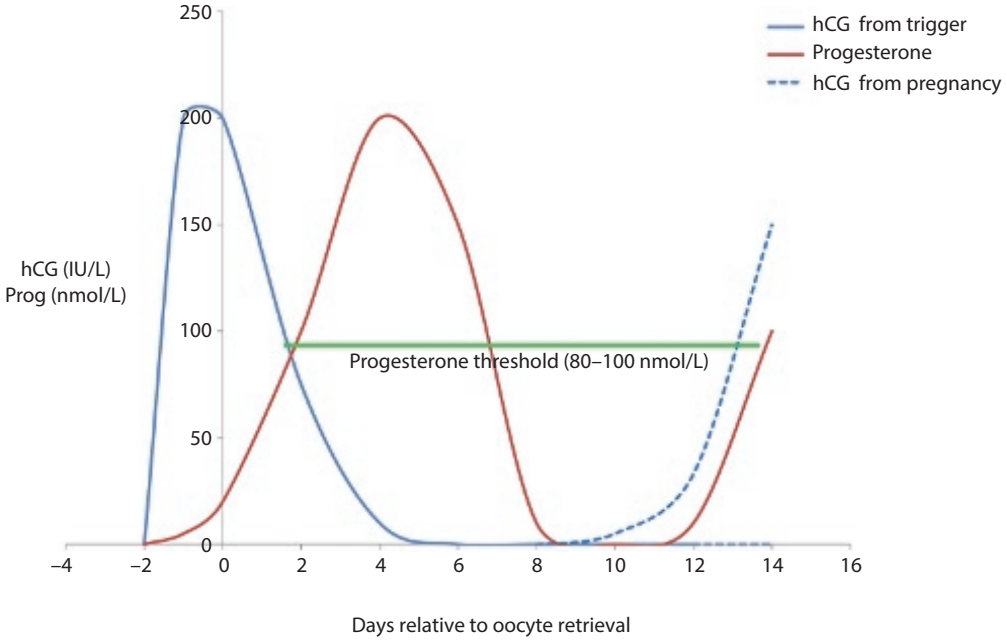

Figure 41.3 Circulating hCG and progesterone levels from hCG administration until early pregnancy during an *in vitro* fertilization cycle (1 nmol/L progesterone equals 0.31 ng/mL). *Abbreviation*: hCG, human chorionic gonadotropin.

granulosa cells from the small (3–9 mm) antral follicles of humans (50). Androgen excess has been shown to stimulate the early stages of follicular growth and increase the number of preantral and antral follicles. AR mRNA was not detected in primordial follicles, but was detected from the transitional stage onward. The number of AR-positive follicles increases at each progressive growth stage, suggesting a role for androgens in promoting early follicle growth (52). Testosterone increases the response of antral follicles to stimulation and its effects are mediated or potentiated by IGF-I (53). Increased circulating levels of insulin and IGF-I and exogenous testosterone and increased local ovarian testosterone concentrations due to aromatase inhibition or exogenous LH/hCG are all associated with an increased ovarian response to gonadotropins. These theoretical possibilities led to treatment strategies aimed at increasing circulating or local androgens in poor responders.

While oral administration of dehydroepiandrosterone sulfate does not seem beneficial, moderate-quality evidence supports the use of transdermal testosterone application prior to ovarian stimulation for increasing oocyte yield and live birth rates in women with decreased ovarian reserve (54). However, more trials are required investigating the outcome of controlled ovarian stimulation in poor responders, particularly regarding different androgen preparations. At present, there is no indication to monitor serum androgen levels during ovarian stimulation.

UNCONVENTIONAL OVARIAN STIMULATION

Classical dogma dictates the initiation of stimulation in the early follicular phase. The rationale is the simultaneous stimulation of a synchronous cohort of antral follicles recruited during the luteo-follicular transition. Interestingly, there is increasing evidence to indicate that multiple waves of antral follicles develop during one menstrual cycle, challenging the concept of a single recruitment episode during the follicular phase (55). Among different theories of follicular recruitment, the wave theory forms the basis of ovarian stimulation during the luteal phase. While the dominant follicle formed in the final wave of the inter-ovulatory interval reaches ovulation, the preceding waves are anovulatory (55). However, a dominant follicle may also be selected during the anovulatory waves that precede and follow the ovulatory wave in some women. This has led to starting ovarian stimulation at any time during the menstrual cycle; this is called "random start ovarian stimulation" (56).

Initially, random start stimulation was used for fertility preservation in women with cancer (57,58). More recently, encouraging results have been reported with luteal-phase start ovarian stimulation in women with normal or poor ovarian reserve (59,60). As the endometrium is out of phase following luteal-phase stimulation, embryo freezing is usually recommended, followed by a frozen embryo transfer in a subsequent cycle (61,62). An alternative strategy that has been tried in poor responders is to stimulate the ovaries twice during a single menstrual cycle. The initial stimulation is commenced in the follicular phase and another cycle of stimulation is initiated after egg collection (59).

Some follicles recruited at the start of the luteo-follicular transition may have already reached the pre-ovulatory stage early in the follicular phase. Thus, it is also possible to collect mature oocytes early in the follicular phase that are capable of leading to a live birth (63).

It may be concluded that ovarian stimulation may be undertaken with unconventional means that challenge the current dogma of universal follicular-phase stimulation. More studies are needed before such strategies become common practice in reproductive endocrinology.

CONCLUSIONS

The endocrine profile of a stimulated ART cycle is different from that of the natural cycle during both the follicular and luteal phases. Overshooting the FSH threshold is clearly mandatory for stimulation of multi-follicular growth. However, serum FSH values are not useful for predicting the extent of multi-follicular growth, thus routine monitoring of it is unnecessary. Despite the clear requirement for some LH activity for proper follicle growth, an LH threshold has not been determined, and evidence to support routine monitoring of serum LH levels during stimulation is missing. Serum estradiol levels are higher in ART cycles than in the natural cycle, and this reflects the extent of multi-follicular growth. Even though different patterns of estradiol during stimulation can be related to treatment outcome (e.g., predicting pregnancy or the occurrence of ovarian hyperstimulation syndrome), currently available evidence does not demonstrate a clear advantage of monitoring serum estradiol levels over ultrasound-only monitoring of the ART cycle (64). If the ultrasound examination prior to commencement of gonadotropins fails to confirm pituitary suppression in the long luteal GnRH agonist protocol, serum levels of LH, estradiol, and progesterone can be measured, together or separately, for confirmation. While the low incidence of progesterone elevation at the start of the GnRH antagonist cycle precludes routine measurement of progesterone levels at this stage, elevated progesterone levels during the late follicular phase seem to have implications for treatment outcome and can thus be informative for clinical decision making. Some experts advocate monitoring luteal-phase serum progesterone levels for tailoring luteal support protocols in the presence of low progesterone levels. However, more information is required before implementing luteal-phase progesterone monitoring into routine practice.

REFERENCES

1. Pache TD, Wladimiroff JW, de Jong FH, Hop WC, Fauser BC. Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. *Fertil Steril* 1990; 54: 638–42.
2. van Weissenbruch MM, Schoemaker HC, Drexhage HA, Schoemaker J. Pharmaco-dynamics of human menopausal gonadotrophin (HMG) and follicle-stimulating hormone (FSH). The importance of the

FSH concentration in initiating follicular growth in polycystic ovary-like disease. *Hum Reprod* 1993; 8: 813–21.
3. Loumaye E, Engrand P, Shoham Z, Hillier SG, Baird DT. Clinical evidence for an LH "ceiling" effect induced by administration of recombinant human LH during the late follicular phase of stimulated cycles in World Health Organization type I and type II anovulation. *Hum Reprod* 2003; 18: 314–22.
4. Balasch J, Fabregues F. Is luteinizing hormone needed for optimal ovulation induction? *Curr Opin Obstet Gynecol* 2002; 14: 265–74.
5. Shoham Z. The clinical therapeutic window for luteinizing hormone in controlled ovarian stimulation. *Fertil Steril* 2002; 77: 1170–7.
6. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. *Hum Reprod* 1994; 9: 188–91.
7. Filicori M, Cognigni GE, Pocognoli P, Ciampaglia W, Bernardi S. Current concepts and novel applications of LH activity in ovarian stimulation. *Trends Endocrinol Metab* 2003; 14: 267–73.
8. Filicori M, Cognigni GE, Tabarelli C et al. Stimulation and growth of antral ovarian follicles by selective LH activity administration in women. *J Clin Endocrinol Metab* 2002; 87: 1156–61.
9. Griesinger G, Shapiro DB. Luteinizing hormone add-back: Is it needed in controlled ovarian stimulation, and if so, when? *J Reprod Med* 2011; 56: 279–300.
10. Griesinger G, Shapiro DB, Kolibianakis EM, Witjes H, Mannaerts BM. No association between endogenous LH and pregnancy in a GnRH antagonist protocol: Part II, recombinant FSH. *Reprod Biomed Online* 2011; 23: 457–65.
11. Lehert P, Kolibianakis EM, Venetis CA et al. Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: Systematic review and meta-analysis. *Reprod Biol Endocrinol* 2014; 12: 17.
12. van Wely M, Kwan I, Burt AL et al. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. A Cochrane review. *Hum Reprod Update* 2012; 18: 111.
13. van Wely M, Kwan I, Burt AL et al. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. *Cochrane Database Syst Rev* 2011; 2: CD005354.
14. Al-Inany HG, van Gelder P. Effect of urinary versus recombinant FSH on clinical outcomes after frozen–thawed embryo transfers: A systematic review. *Reprod Biomed Online* 2010; 21: 151–8.
15. Kolibianakis EM, Kalogeropoulou L, Griesinger G et al. Among patients treated with FSH and GnRH analogues for *in vitro* fertilization, is the addition of recombinant LH associated with the probability of live birth? A systematic review and meta-analysis. *Hum Reprod Update* 2007; 13: 445–52.
16. Requena A, Cruz M, Ruiz FJ, Garcia-Velasco JA. Endocrine profile following stimulation with recombinant follicle stimulating hormone and luteinizing hormone versus highly purified human menopausal gonadotropin. *Reprod Biol Endocrinol* 2014; 12: 10.
17. Alviggi C, Clarizia R, Mollo A, Ranieri A, De Placido G. Outlook: Who needs LH in ovarian stimulation? *Reprod Biomed Online* 2006; 12: 599–607.
18. Wong PC, Qiao J, Ho C et al. Current opinion on use of luteinizing hormone supplementation in assisted reproduction therapy: An Asian perspective. *Reprod Biomed Online* 2011; 23: 81–90.
19. Jeppesen JV, Kristensen SG, Nielsen ME et al. LH-receptor gene expression in human granulosa and cumulus cells from antral and preovulatory follicles. *J Clin Endocrinol Metab* 2012; 97: E1524–31.
20. Lindeberg M, Carlstrom K, Ritvos O, Hovatta O. Gonadotrophin stimulation of non-luteinized granulosa cells increases steroid production and the expression of enzymes involved in estrogen and progesterone synthesis. *Hum Reprod* 2007; 22: 401–6.
21. Yong EL, Baird DT, Yates R, Reichert LE, Jr., Hillier SG. Hormonal regulation of the growth and steroidogenic function of human granulosa cells. *J Clin Endocrinol Metab* 1992; 74: 842–9.
22. Hamdine O, Macklon NS, Eijkemans MJ et al. Elevated early follicular progesterone levels and *in vitro* fertilization outcomes: A prospective intervention study and meta-analysis. *Fertil Steril* 2014; 102: 448–54 e1.
23. Kolibianakis EM, Zikopoulos K, Smitz J et al. Elevated progesterone at initiation of stimulation is associated with a lower ongoing pregnancy rate after IVF using GnRH antagonists. *Hum Reprod* 2004; 19: 1525–9.
24. Christophe B, Miriam B, De Vos M, Greta V, Paul D. Administration of GnRH antagonists in case of elevated progesterone at initiation of the cycle: A prospective cohort study. *Curr Pharm Biotechnol* 2011; 12: 423–8.
25. Schoolcraft W, Sinton E, Schlenker T, Huynh D, Hamilton F, Meldrum DR. Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. *Fertil Steril* 1991; 55: 563–6.
26. Papanikolaou EG, Pados G, Grimbizis G et al. GnRH-agonist versus GnRH-antagonist IVF cycles: Is the reproductive outcome affected by the incidence of progesterone elevation on the day of HCG triggering? A randomized prospective study. *Hum Reprod* 2012; 27: 1822–8.
27. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: A systematic review and meta-analysis of over 60 000 cycles. *Hum Reprod Update* 2013; 19: 433–57.
28. Sonigo C, Dray G, Roche C, Cedrin-Durnerin I, Hugues JN. Impact of high serum progesterone during the late follicular phase on IVF outcome. *Reprod Biomed Online* 2014; 29: 177–86.

29. Griesinger G, Mannaerts B, Andersen CY, Witjes H, Kolibianakis EM, Gordon K. Progesterone elevation does not compromise pregnancy rates in high responders: A pooled analysis of *in vitro* fertilization patients treated with recombinant follicle-stimulating hormone/gonadotropin-releasing hormone antagonist in six trials. *Fertil Steril* 2013; 100: 1622–8. e1–3.
30. Xu B, Li Z, Zhang H et al. Serum progesterone level effects on the outcome of *in vitro* fertilization in patients with different ovarian response: An analysis of more than 10,000 cycles. *Fertil Steril* 2012; 97: 1321–7.e1–4.
31. Bosch E, Labarta E, Crespo J et al. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for *in vitro* fertilization: Analysis of over 4000 cycles. *Hum Reprod* 2010; 25: 2092–100.
32. Fatemi HM, Doody K, Griesinger G, Witjes H, Mannaerts B. High ovarian response does not jeopardize ongoing pregnancy rates and increases cumulative pregnancy rates in a GnRH-antagonist protocol. *Hum Reprod* 2013; 28: 442–52.
33. Kyrou D, Al-Azemi M, Papanikolaou EG et al. The relationship of premature progesterone rise with serum estradiol levels and number of follicles in GnRH antagonist/recombinant FSH-stimulated cycles. *Eur J Obstet Gynecol Reprod Biol* 2012; 162: 165–8.
34. Hugues JN, Masse-Laroche E, Reboul-Marty J, Boiko O, Meynant C, Cedrin-Durnerin I. Impact of endogenous luteinizing hormone serum levels on progesterone elevation on the day of human chorionic gonadotropin administration. *Fertil Steril* 2011; 96: 600–4.
35. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: A randomized assessor-blind controlled trial. *Hum Reprod* 2006; 21: 3217–27.
36. Devroey P, Pellicer A, Nyboe Andersen A, Arce JC, Menopur in GnRH Antagonist Cycles with Single Embryo Transfer Trial Group. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer. *Fertil Steril* 2012; 97: 561–71.
37. Thuesen LL, Smitz J, Loft A, Nyboe Andersen A. Endocrine effects of hCG supplementation to recombinant FSH throughout controlled ovarian stimulation for IVF: A dose–response study. *Clin Endocrinol (Oxf)* 2013; 79: 708–15.
38. Kohler PO, Ross GT, Odell WD. Metabolic clearance and production rates of human luteinizing hormone in pre- and postmenopausal women. *J Clin Invest* 1968; 47: 38–47.
39. Manau D, Fabregues F, Arroyo V, Jimenez W, Vanrell JA, Balasch J. Hemodynamic changes induced by urinary human chorionic gonadotropin and recombinant luteinizing hormone used for inducing final follicular maturation and luteinization. *Fertil Steril* 2002; 78: 1261–7.
40. European Recombinant LH Study Group. Human recombinant luteinizing hormone is as effective as, but safer than, urinary human chorionic gonadotropin in inducing final follicular maturation and ovulation in *in vitro* fertilization procedures: Results of a multicenter double-blind study. *J Clin Endocrinol Metab* 2001; 86: 2607–18.
41. Turkgeldi E, Turkgeldi L, Seyhan A, Ata B. Gonadotropin-releasing hormone agonist triggering of oocyte maturation in assisted reproductive technology cycles. *J Turk Soc Obstet Gynecol* 2015: 12: 96–101.
42. Hull MG, Savage PE, Bromham DR, Ismail AA, Morris AF. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle ("ovulation") derived from treated and untreated conception cycles. *Fertil Steril* 1982; 37: 355–60.
43. Fauser BC, de Jong D, Olivennes F et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for *in vitro* fertilization. *J Clin Endocrinol Metab* 2002; 87: 709–15.
44. Beckers NG, Macklon NS, Eijkemans MJ et al. Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in *in vitro* fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. *J Clin Endocrinol Metab* 2003; 88: 4186–92.
45. Yding Andersen C, Vilbour Andersen K. Improving the luteal phase after ovarian stimulation: Reviewing new options. *Reprod Biomed Online* 2014; 28: 552–9.
46. Liu XR, Mu HQ, Shi Q, Xiao XQ, Qi HB. The optimal duration of progesterone supplementation in pregnant women after IVF/ICSI: A meta-analysis. *Reprod Biol Endocrinol* 2012; 10: 107.
47. Liu HC, Pyrgiotis E, Davis O, Rosenwaks Z. Active corpus luteum function at pre-, peri- and postimplantation is essential for a viable pregnancy. *Early Pregnancy* 1995; 1: 281–7.
48. Mitwally MF, Diamond MP, Abuzeid M. Vaginal micronized progesterone versus intramuscular progesterone for luteal support in women undergoing *in vitro* fertilization-embryo transfer. *Fertil Steril* 2010; 93: 554–69.
49. Suzuki T, Sasano H, Kimura N et al. Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: Relationship to expression of steroidogenic enzymes. *Hum Reprod* 1994; 9: 1589–95.

50. Nielsen ME, Rasmussen IA, Kristensen SG et al. In human granulosa cells from small antral follicles, androgen receptor mRNA and androgen levels in follicular fluid correlate with FSH receptor mRNA. *Mol Hum Reprod* 2011; 17: 63–70.
51. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab* 1999; 84: 2951–6.
52. Rice S, Ojha K, Whitehead S, Mason H. Stage-specific expression of androgen receptor, follicle-stimulating hormone receptor, and anti-Mullerian hormone type II receptor in single, isolated, human preantral follicles: Relevance to polycystic ovaries. *J Clin Endocrinol Metab* 2007; 92: 1034–40.
53. Meldrum DR, Chang RJ, Giudice LC, Balasch J, Barbieri RL. Role of decreased androgens in the ovarian response to stimulation in older women. *Fertil Steril* 2013; 99: 5–11.
54. Ata B, Seli E. Strategies for controlled ovarian stimulation in the setting of ovarian aging. *Semin Reprod Med* 2015; 33: 436–48.
55. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: A review. *Hum Reprod Update* 2012; 18: 73–91.
56. Sonmezer M, Turkcuoglu I, Coskun U, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. *Fertil Steril* 2011; 95: 2125 e9–11.
57. Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. *Curr Opin Obstet Gynecol* 2015; 27: 215–21.
58. Kim JH, Kim SK, Lee HJ et al. Efficacy of random-start controlled ovarian stimulation in cancer patients. *J Korean Med Sci* 2015; 30: 290–5.
59. Kuang Y, Chen Q, Hong Q et al. Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol). *Reprod Biomed Online* 2014; 29: 684–91.
60. Kuang Y, Hong Q, Chen Q et al. Luteal-phase ovarian stimulation is feasible for producing competent oocytes in women undergoing *in vitro* fertilization/intracytoplasmic sperm injection treatment, with optimal pregnancy outcomes in frozen-thawed embryo transfer cycles. *Fertil Steril* 2014; 101: 105–11.
61. Kansal Kalra S, Ratcliffe S, Gracia CR, Martino L, Coutifaris C, Barnhart KT. Randomized controlled pilot trial of luteal phase recombinant FSH stimulation in poor responders. *Reprod Biomed Online* 2008; 17: 745–50.
62. Rombauts L, Suikkari AM, MacLachlan V, Trounson AO, Healy DL. Recruitment of follicles by recombinant human follicle-stimulating hormone commencing in the luteal phase of the ovarian cycle. *Fertil Steril* 1998; 69: 665–9.
63. Hatirnaz S, Hatirnaz E, Ata B. Live birth following early follicular phase oocyte collection and vitrified-warmed embryo transfer 8 days later. *Reprod Biomed Online* 2015; 31: 819–22.
64. Kwan I, Bhattacharya S, Kang A, Woolner A. Monitoring of stimulated cycles in assisted reproduction (IVF and ICSI). *Cochrane Database Syst Rev* 2014; 8: CD005289.

The use of gonadotropin-releasing hormone agonists and the efficiency of *in vitro* fertilization

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INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is the primary hypothalamic regulator of reproductive function. The chemical structure of this compound was discovered in 1971 by a group of scientists in Andrew Schally's laboratory in New Orleans after they derived a small amount of GnRH from porcine hypothalami (1,2). Roger Guillemin then characterized and independently synthesized the hormone, and they both received the Nobel Prize for their achievements. GnRH is a decapeptide that is synthesized as part of a much larger precursor peptide, the GnRH-associated peptide. This peptide is composed of a sequence of 56 amino acids. The availability of the synthetic hormone for dynamic endocrine testing and receptor studies created new insights into the physiological role of GnRH in the hypothalamic–pituitary–gonadal axis (3).

GnRH is produced and released by a group of loosely connected neurons located in the medial basal hypothalamus, primarily within the arcuate nucleus, and in the preoptic area of the ventral hypothalamus. It is synthesized in the cell body, transported along the axons to the synapse, and released in a pulsatile fashion into the complex capillary net of the portal system of the pituitary gland (4). GnRH binds selectively to the highly specific receptors of the anterior pituitary gonadotropic cells and activates intracellular signaling pathways via the coupled G proteins, leading to the generation of several second messengers, including diacylglycerol and inositol-4,5-triphosphate. The former leads to activation of protein kinase C and the latter to the production of cyclic AMP and the release of calcium ions from intracellular pools (5–7). Both events result in secretion and synthesis of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). A pulsatile GnRH release from the hypothalamus to the pituitary is required to ensure gonadotropin secretion (8–10). In humans, the pulsatile release frequencies range from the shortest interpulse frequency of about 71 minutes in the late follicular phase to an interval of 216 minutes in the late luteal phase (11–13). High frequency (>3 pulses/hour) and continuous exposure of GnRH to the pituitary failed to produce normal LH and FSH release (14–16) due to pituitary receptor desensitization. This mechanism is still not clear; however, we know that post-receptor signaling is involved and true receptor loss (down-regulation) plays only an initial role in the process (17). The pulsatile release by the GnRH neurons is likely based on an ultrashort feedback loop with GnRH itself; this autocrine process could serve as a timing mechanism to control the frequency of neurosecretion. Several mechanisms, based on calcium and cyclic AMP signaling, have been proposed to account for the pulse secretion. Another role of intracellular signaling in pulsatile generation has been suggested by the marked inhibition of Gi protein activation by LH, human chorionic gonadotropin (hCG), muscarine, estradiol (E2), and GnRH levels (7,18,19).

After the discovery of the chemical structure of native GnRH type I, which proved to be the classic reproductive neuroendocrine factor, many were synthetically produced. Most were able to elicit a massive FSH and LH release from the pituitary and were therefore called GnRH agonists. However, under continuous administration of a GnRH agonist, both the synthesis and the subsequent release of LH, and to a lesser extent of FSH, became blocked (Figure 42.1). Other analogs by competitive receptor binding caused an immediate fall in pituitary gonadotropin secretion and were designated GnRH antagonists. In contrast to the agonistic compounds, the introduction of the GnRH antagonists into clinical practice has been hampered for a long time by problems concerning solubility and direct allergy-like side effects due to histamine release (20,21). Recently, these problems have been resolved, leading to the third-generation GnRH antagonists. Currently, two such drugs are on the market and many others are under investigation (22). The GnRH agonists have gained a wide range of clinical applications (23). The main goal of using GnRH agonists is the achievement of suppression of the pituitary–ovarian (or testicular) axis for a limited or even an extended period of time.

STRUCTURAL MODIFICATIONS

The elucidation of the structure, function, and metabolic pathways of native GnRH has prompted an intensive effort by research laboratories and the pharmaceutical industry to synthesize potent and longer-acting agonists and antagonists (24). Over the past three decades, thousands of analogs of GnRH have been synthesized. Only seven of the agonistic analogs of GnRH have been approved and are in clinical use. The first major step in increasing the potency of GnRH was made with substitutions of glycine number 10 at the C terminus. Although 90% of the biologic activity is lost by the splicing of glycine number 10, most of it is restored with the attachment of NH_2-ethylamide to the proline at position 9, leading to nonapeptides (25). The second

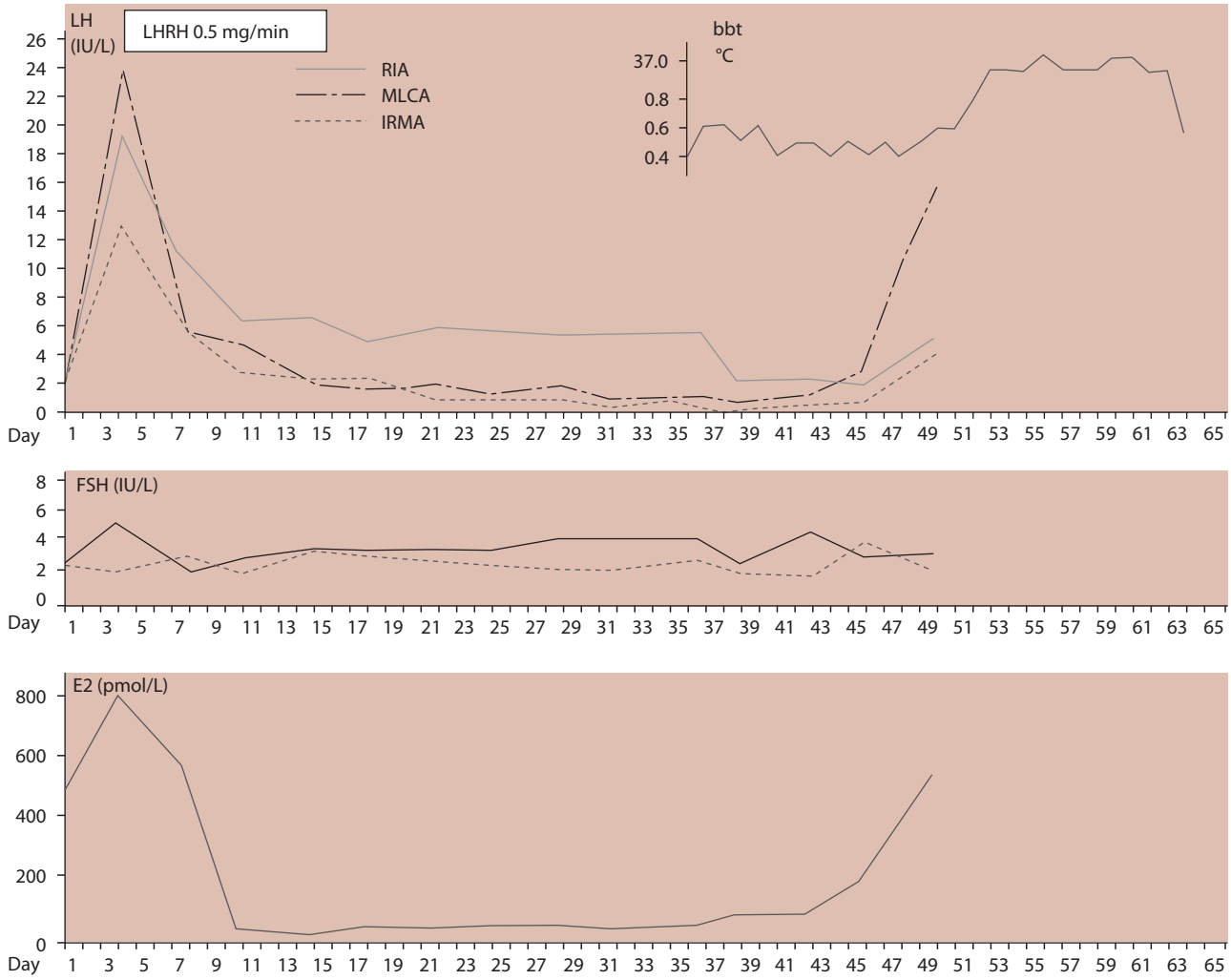

Figure 42.1 Hormone levels for FSH, LH, and E2 in a patient with continuous intravenous infusion of 0.5 mg/minute LHRH. LH was measured with three different assays and FSH with two different assays. *Abbreviations*: RIA, radioimmunoassay; MLCA, Magic Lite chemiluminescence assay; IRMA, immunoradiometric assay; bbt, basal body temperature; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E2, estradiol; LHRH, luteinizing hormone-releasing hormone. (Courtesy of Prof. J. Schoemaker.)

major modification was the replacement of the glycine at position 6 by D-amino acids, which slows down enzymatic degradation. The combination of these two modifications was found to have synergistic biologic activity and proved to exhibit a higher receptor binding affinity. The affinity can be increased further by the introduction of larger, hydrophobic, and more lipophilic D-amino acids at position number 6. The increased lipophilic content is also associated with a prolonged half-life, which may be attributed to reduced renal excretion through increased plasma protein binding, or fat tissue storage of non-ionized, fat-soluble compounds (25). For details about the structure, see Table 42.1.

CLINICAL APPLICATIONS

The original goal for the development of agonistic analogs of GnRH was that they would eventually be used for the treatment of anovulation. However, soon after the elucidation of the structure of GnRH, the "paradoxical" ability of agonistic analogs to inhibit reproductive function in experimental animals was demonstrated (26). The most important clinical applications of the potent GnRH agonists were derived from their capacity to cause rapid desensitization of the pituitary gland as a result of prolonged non-pulsatile administration, leading to a decrease in serum gonadotropin levels and subsequently inhibition of ovarian steroidogenesis and follicular growth. The potential for reversibly inducing a state of hypogonadotropic hypogonadism, which was also termed "medical gonadectomy" or "medical hypophysectomy," allowed for the relatively rapid and extensive introduction of GnRH agonists into clinical practice. For a variety of indications, complete abolition of gonadotropin secretion with subsequent suppression of gonadal steroids to the levels of castrated subjects was considered beneficial. This therapeutic approach has already had its efficacy and merits proven in the treatment of metastatic prostatic cancer, breast cancer, central precocious puberty, endometriosis (including adenomyosis), uterine fibroids, hirsutism, and other conditions (27,28).

Table 42.1 Amino acid sequence and substitution of the gonadotropin-releasing hormone (GnRH) agonists

Compound	Position 6	Position 10
Amino acid no	1 2 3 4 5 6 7 8 9	10
Native GnRH	Glu His Trp Ser Tyr Gly Leu Arg Pro	$GlyNH_2$
Nonapeptides		
Leuprolide (Lupron, Lucrin),	Leu	$N\text{-}Et\text{-}NH_2$
buserelin (Suprefact),	Ser(O'Bu)	$N\text{-}Et\text{-}NH_2$
goserelin (Zoladex), histrelin	Ser(O'Bu)	$AzaGlyNH_2$
(Supprelin), deslorelin	D-His(Bzl)	$AzaGlyNH_2$
(Ovuplant)	D-Tr	$N\text{-}Et\text{-}NH_2$
Decapeptides		
Nafarelin (Synarel),	2Nal	$GlyNH_2$
triptorelin (Decapeptyl)	Trp	$GlyNH_2$

Since the first report on the use of the combination of the GnRH agonist buserelin and gonadotropins for ovarian stimulation for *in vitro* fertilization (IVF) in 1984 (29), numerous studies have demonstrated the efficacy of this concept. Subsequently, the use of GnRH agonists has gained widespread popularity, and the vast majority of assisted reproduction technology (ART) programs use this approach for controlled ovarian stimulation (COS) IVF. The major advantage initially offered by the agonists was the efficient abolition of the spontaneous LH surge (30). The incidence of premature LH surges and subsequent luteinization in cycles with exogenous gonadotropin stimulation, without the use of a GnRH agonist, was observed by several investigators to range between 20% and 50%, leading to an increased cancellation rate (31). Moreover, a deleterious effect on both fertilization and pregnancy rates was noted (30,32). A meta-analysis of randomized controlled trials has shown that the use of GnRH agonists has not only reduced cancellation rates, but also increased the number of oocytes and embryos, allowing better selection (33), so that, on average, the outcome in terms of pregnancy rates was improved (34).

More recently, several studies have compared the use of GnRH agonists and GnRH antagonists in ovarian stimulation protocols. Some studies have cited several advantages of using GnRH antagonists, including shorter durations of treatment, a reduction in the dose requirement of gonadotropin, and a lower incidence of ovarian hyperstimulation syndrome (OHSS) (35). However, there is still ongoing debate about this issue, with many arguing that the GnRH agonist protocol leads to higher clinical pregnancy rates and should therefore be favored, particularly in good-prognosis patients who are not at high risk of severe OHSS (36).

A number of controversial issues remain concerning the use of GnRH agonists in assisted reproduction. The problems can be divided into the following four categories:

1. Which route of administration is the best?
2. Which agonist(s) should be used in ART?
3. What is the optimal dose?
4. What is the optimal scheme?

Which route of administration is the best?

Administration routes of GnRH agonists are intramuscular or subcutaneous depot injection, intranasal, or subcutaneous daily administration. Although there is an advantage for the patient in the single injection of the depot preparations, the duration of action is prolonged and rather unpredictable. The effect can last until the first weeks of pregnancy (37). Broekmans et al. showed that rapid induction of a hypogonadotropic and hypogonadal state is possible in regularly cycling women by administration of a single depot of triptorelin. However, suppression of pituitary and ovarian function appears to be continued until the eighth week after the injection (37). This is far longer than is actually needed. Devreker et al. found several negative effects of depot preparations, including a longer stimulation phase and consequently the need for more ampoules of stimulation medications, but more importantly they saw lower implantation and delivery rates (32.8% vs. 21.1% and 48.9% vs. 29.1%, respectively). Their conclusion was that since a long-acting GnRH agonist might interfere with the luteal phase and embryo development, short-acting GnRH agonists should be preferred in ART (38).

A meta-analysis comparing depot versus daily administration concluded that there is no clear difference in pregnancy rate. Furthermore, the use of depot GnRH analogs is associated with increased gonadotropin requirements and longer stimulation periods and should therefore not be used based on cost-effectiveness (39). Moreover, on a theoretical basis, it would be desirable to avoid any possible direct effect on the embryo, although several authors claim a normal outcome of pregnancy following inadvertent administration of a GnRH agonist during early pregnancy (40–45). Lahat et al. reported a high incidence of attention deficit hyperactivity disorder in long-term follow-up of children inadvertently exposed to GnRH agonists early in pregnancy (46).

Thus, although depot preparations seem attractive because of their ease of administration for the patient,

they should not be routinely used in IVF. One exception to this statement might be the prolonged use of GnRH analogs before IVF embryo transfer in patients with severe endometriosis, which is associated with higher ongoing pregnancy rates (47).

With the intranasal route the absorption of the GnRH agonist fluctuates inter- and intra-individually, giving an unpredictable desensitization level, but usually this is sufficient to prevent premature LH surges. For research or study purposes, daily subcutaneous injections are preferred because of their more stable effect. The clinician has to strike a balance between comfort for the patient and a more stable effect in selecting the intranasal versus the subcutaneous route of administration.

Which agonist(s) should be used in ART?

Table 42.1 lists seven different GnRH agonists, but only four are commonly used in IVF programs. An extensive search revealed only one article on the use of histrelin in IVF (48), while deslorelin has never been applied in human IVF. Except for its combination for the treatment of endometriosis, goserelin is not routinely used in ART, partly because it is only available as a depot preparation. Depot preparations also on the market for triptorelin and leuprolide, which are not to be used as first choices, as discussed earlier. Thirteen prospective randomized trials have compared different agonists with each other (49–60). The problem with those studies is that the optimal dosage has not been determined for any of the applied individual agonists, and therefore the ability of these articles to answer the question of which compound should be used is limited. All the agonists seem effective and the differences in the studies can be explained by dosage incompatibility. These studies make absolutely clear that proper dose-finding studies for the use of GnRH agonists in ART are still urgently needed. It is obvious that the dose required for the prevention of premature LH surges during controlled ovarian stimulation cycles in ART will be different from that required to treat carcinoma of the prostate, which requires complete chemical castration (see below).

What is the optimal dose?

Finding the right dose in the treatment of infertility disorders has been notoriously difficult. Because proper dose-finding studies for the use of gonadotropins were lacking, it took until the middle of the 1980s before an adequate treatment protocol, with a maximum of effect and a minimum of side effects, was introduced (61). There is only one prospective, randomized, double-blind, placebo-controlled dose-finding study performed in IVF for the GnRH agonist triptorelin. This study demonstrated that the dosage needed for the suppression of the LH surge is much smaller than the dosage needed for the treatment of a malignant disease, namely only 15%–50% (31). It is very likely that dose-finding studies for the other agonists will give similar results. As per the recent literature, such studies have not been performed.

What is the optimal scheme?

Many treatment schedules with the use of GnRH agonists in ART have been designed. The duration and initiation of agonist administration before the start of the actual ovarian stimulation varies widely. Initiation of the agonist treatment may be in either the early follicular or the mid-luteal phase of the preceding cycle. The cycle may be spontaneous or induced by progestogen and/or estrogen compounds. There is still much debate about the optimal GnRH agonist protocol. Tan published a review article in 1994 stating that the so-called long protocol was superior to the short and ultrashort protocols (62). Moreover, a major advantage of the long GnRH agonist protocol is its contribution to the planning of the ovum pick-up, since both the initiation of exogenous gonadotropins after pituitary desensitization and the administration of hCG can be delayed without any detrimental effect on IVF outcome (63,64). A meta-analysis comparing ultrashort, short, and long IVF protocols showed a higher number of oocytes retrieved and higher pregnancy rates in the long protocol, although more ampoules of gonadotropins were needed (65). In terms of gonadotropin suppression and numbers of retrieved oocytes, the mid-luteal phase of the preceding cycle is the optimal moment for the initiation of the GnRH agonist, in comparison to the follicular, early, or late luteal phases (66–68).

However, a problem with prospective randomized clinical studies is that certain groups of patients, such as poor responders (with or without elevated basal FSH) or patients with polycystic ovary syndrome (PCOS), are often excluded. There is a possibility that especially in the excluded groups other schemes are preferable. An unwanted side effect of starting the GnRH agonist in the luteal or follicular phase in the long protocol is the induction of the formation of functional cysts. Keltz et al. observed both a poor stimulation outcome and a reduction in pregnancy rates in a cycle with cyst formation (69). However, Feldberg et al. could not confirm this finding (70). Ovarian cyst formation was reduced when pretreatment with an oral contraceptive was applied (71). Damario et al. showed the beneficial effect of this strategy in high-responder patients with respect to cancellation rates and pregnancy rates (72). A long GnRH agonist protocol in combination with an oral contraceptive seems to be advantageous in the prevention of functional ovarian cysts and especially for the larger IVF centers for programming of IVF cycles. Another practical advantage of including an oral contraceptive is the fact that the coincidence of GnRH agonist use and early pregnancy is prevented.

The mean desensitization phase with an agonist in the long protocols is about three weeks. Several investigators have tried to shorten this long duration of administration, leading to the so-called "early cessation protocol" (73–76). Increased human menopausal gonadotropin/FSH requirements and cancellation rates were reported after early cessation in 137 normal IVF patients (76), but the opposite was found in a study that included 230 normally ovulating

IVF patients (73), although pregnancy rates were the same in both studies (76). The paradoxical drop of serum LH following early cessation that leads to significantly lower E2 levels on the day of hCG administration may have a deleterious effect on IVF outcome (73,76). The early discontinuation protocol may improve ovarian response based on a hypothetical effect on the ovary, and was therefore additionally tested in poor responders. Although the number of retrieved oocytes was significantly higher and the amount of required gonadotropins was reduced after early cessation in comparison to the long protocol, this new approach reported no further advantages in these patients in terms of pregnancy and implantation rates (74,75). In conclusion, the currently available data do not favor an "early cessation" protocol, but this approach might have some beneficial effects in poor responders.

To prevent any detrimental effect of the profound suppression of circulating serum gonadotropins after cessation of GnRH agonist therapy, the opposite regimens have recently been developed in which the GnRH agonist administration is continued during the luteal phase, the so-called "continuous-long protocol." In a large prospective randomized study (n = 319) comparing this continuous-long protocol versus the standard long protocol, higher implantation and pregnancy rates were found in the continuous-long protocol (77).

Since the use of a long protocol in poor responders has been found to result in reduced ovarian responses to hormonal stimulation, the short GnRH agonist protocol has been proposed as providing better stimulation for these patients. In the short or flare-up protocol, GnRH agonist therapy is started at cycle day 2 and gonadotropin treatment is started one day later. The immediate stimulatory action of the GnRH agonist serves as the initial stimulus for follicular recruitment (so-called "flare-up"). Adequate follicular maturation is on average reached in 12 days, which should allow enough time for sufficient pituitary desensitization to prevent any premature LH surges. The initial stimulatory effect of GnRH agonist on pituitary hormone levels may improve the ovarian response (78). On the other hand, this short protocol might increase gonadotropins in the early phase, which induces enhanced ovarian androgen release. This is associated with lower oocyte quality and reduced ongoing pregnancy rates compared to the long protocol (79). Nevertheless, experience to date shows that the short protocol has an important role in the treatment of poor responders (80). Other investigators even promoted an "ultrashort protocol" in "poor responders," in which the agonist is given over a period of three days in the early follicular phase. On the second day of agonist administration, stimulation with gonadotropin administration (high dosages) is started (81). Modifications to both the short (82,84) and the long (83) protocols have been made in order to improve the response to COS in poor responders.

In very high responders or in patients at risk of OHSS, gonadotropin was discontinued whilst continuing the GnRH agonist; this so-called "coasting" might prevent the development of severe OHSS (85,86). This strategy allows a delay of a variable number of days in administering the hCG injection until safe E2 levels are attained. However,

Table 42.2 Summary of advantages and disadvantages of the different gonadotropin-releasing hormone (GnRH) agonist protocols

GnRH agonist protocol	Route of administration	Administration days of cycle (CD)	Duration of administration	Advantages	Disadvantages
Ultrashort protocol	IN/SC	CD 2, 3–4, 5	3 days	Patient's comfort	Low PR
Short protocol	IN/SC	CD 2, 3 until day of hCG	8–12 days	Patient's comfort	No programming
Long follicular	IN/SC	CD 2 until day of hCG	28–35 days	Programming, good PR	Long duration of administration
Long luteal	IN/SC	CD 21 until day of hCG	21–28 days	Programming, good PR	Long duration of administration
Menstrual early cessation	IN/SC	CD 21 until menses	7–12 days	Inconclusive	Low estradiol levels
Follicular early cessation	IN/SC	CD 21 until stimulation day 6, 7	13–20 days	Inconclusive	Low estradiol levels
Long follicular (depot)	Depot	CD 2	Once	Patient's comfort	(Too) long duration of action
Long luteal (depot)	Depot	CD 21	Once	Patient's comfort	(Too) long duration of action
Ultralong	IN/SC/depot	CD 2 or 21	8–12 weeks, depot two or three times	Only for special cases	Side effects due to estrogen deficiency

Abbreviations: CD, cycle day; hCG, human chorionic gonadotropin; IN, intranasal; PR, pregnancy rate; SC, subcutaneous.

sufficient randomized controlled trials comparing coasting with no coasting are lacking (87). Only one prospective comparative trial in 60 IVF patients showed a similar incidence of moderate and severe OHSS whether coasting was applied or not (88).

The most important advantages and disadvantages of the different GnRH agonist protocols are summarized in Table 42.2.

After the clinical availability of GnRH antagonists, an additional indication for the use of GnRH agonists became of interest. GnRH analogs may be used as an alternative way for hCG to trigger the endogenous LH and FSH surges and subsequent final maturation of the oocytes and ovulation (89,90). Since hCG is believed to contribute to the occurrence of OHSS owing to its prolonged circulating half-life compared with native LH, this strategy seems to be an attractive alternative for preventing OHSS. In the early 1990s, it was already shown that single-dose GnRH agonists administrated in COS-IVF patients were able to induce an endogenous rise in both LH and FSH levels, leading to follicular maturation and pregnancy (91,92). Mean serum LH and FSH levels rose over 4–12 hours and were elevated for 24–34 hours after GnRH agonist, in comparison to approximately six days of elevated hCG levels after 5000 IU hCG administration. The capacity for a single administration of GnRH analog to trigger follicular rupture in anovulatory women or in preparation for intrauterine insemination (IUI) has been well established. This seems to induce lower OHSS rates with comparable or even improved results, despite short luteal phases, in comparison to hCG cycles (89,90,93). Interest in this approach was lost during the 1990s, because GnRH agonists were introduced in ovarian hyperstimulation protocols to prevent premature luteinization by pituitary desensitization, precluding stimulation of the endogenous LH surge. However, interest has returned following the introduction of GnRH antagonist protocols in which the pituitary responsiveness is preserved (94). This new concept of triggering final oocyte maturation after GnRH antagonist treatment by a single GnRH agonist injection was successfully tested in COS patients for IUI and in high responders for IVF (95). None of these patients developed OHSS. The efficacy and success of this new treatment regimen was established in a prospective multicenter trial in which 47 patients were randomized to receive either 0.2 mg triptorelin, 0.5 mg leuprorelin, or 10,000 IU hCG (96). The LH surges peaked at four hours after agonist administration and returned to baseline after 24 hours; the luteal-phase steroid levels were also closer to the physiologic range compared to the hCG groups. In terms of triggering the final stages of oocyte maturation, similar outcomes were observed in all groups, as demonstrated by the similar fertilization rates and oocyte quality (96).

A prospective randomized study in 105 stimulated IUI cycles treated with a GnRH antagonist in patients with clomiphene-resistant PCOS showed statistically significantly more clinical pregnancies after ovulation triggering by a GnRH agonist in comparison to hCG (28.2% vs. 17.0% per completed cycle, respectively) (97). Therefore, this new approach of ovulation triggering seems to be an attractive alternative to hCG in ART if administered in GnRH antagonist-treated cycles, with lower OHSS rates and similar or improved IVF outcomes (see Chapter 44).

CONCLUSIONS

GnRH agonists are widely used in IVF to control the endogenous LH surge and to achieve augmentation of multifollicular development. Disadvantages, such as the necessity for luteal support, increased total gonadotropin dose per treatment cycle, and consequently higher costs, appear to be outweighed by the observed increase in ability to control the cycle, the higher yield of good-quality oocytes and subsequently embryos, and the consequent improvement of pregnancy rates. The introduction of GnRH agonists in IVF is not an example of excellent research, since proper dose-finding studies are still awaited. Further research into finding the right dose and protocol could still improve the clinical benefits of the GnRH agonists. Initiatives to perform such studies are lacking. Daily administered short-acting preparations deserve preference to the depot formulations. Intranasal administration best fits a patient's comfort considerations, while the subcutaneous route may be advocated for research purposes. The long GnRH agonist protocols give the highest pregnancy rates in the normal responders. There is some evidence that the short flare-up protocol is the treatment of choice for patients with diminished ovarian reserve (poor responders). Dose reduction might be the key point in optimizing pregnancy rates. Finally, GnRH agonists can be used to induce final maturation and ovulation as an alternative to hCG in ART.

The efficiency of IVF

The use of ART procedures to treat infertile couples has significantly increased worldwide since its inception in the late 1970s. However, despite significant advancements in both clinical protocols for COS and in the embryology laboratory, the process of human reproduction has remained inefficient (98,99). By using the metric of number of live-born infants according to the number of embryos chosen for transfer, it has been demonstrated that over the years the majority of embryos produced during IVF cycles (about 85%) are wasted, since they fail to result in a live-born infant (100). Furthermore, when the metric of live-born infants is calculated according to the number of oocytes retrieved (oocyte to baby rate), it has been demonstrated that over the years only about 5%–6% of the total oocytes collected and used result in a live-born infant. One of the critical challenges in the field remains the ability to identify competent embryos that are capable of becoming a live-born infant. Women continue to be aggressively stimulated with high doses of gonadotropins with the goal of retrieving multiple oocytes to increase the number of embryos available for transfer. This approach, however, is associated with a number of risks, including OHSS, and increased cost due to the high doses of medications used. The use of GnRH agonists as a replacement for the hCG ovulation trigger has helped to significantly decrease the risk of OHSS.

By examining IVF efficiency according to age groups over the last decade (2004–2013), the embryo wastage rate decreased across all ages, but particularly in younger women (under 35 years of age), for whom this rate decreased from 76.1% in 2004 to 65.2% in 2013 ($p < 0.001$) (100). In the group of women over the age of 42 years, the embryo wastage rate only marginally decreased and remained relatively high from 2004 to 2013 (98.0% to 97.2%, respectively). In this age group, there was also the smallest, albeit still significant ($p < 0.001$), change in the mean number of embryos transferred (3.3 in 2004 to 2.8 in 2013). Further data analysis showed that the average number of embryos transferred per year, averaged across all age groups, positively correlated with the embryo wastage rate (Spearman coefficient = 0.988, $p < 0.001$). In other words, as the number of embryos transferred decreased, the percentage of embryos wasted also decreased without impacting the pregnancy rates. This pattern has been consistent since 1995 and is further proof that only a few embryos, if any, are competent for live birth per cohort in each ART cycle (101). In conclusion, the decrease in observed embryo wastage rate is not due to an improved oocyte or embryo biology, but merely to a reduction in the mean number of embryos transferred (i.e., a smaller denominator in the equation of total live births divided by total number of embryos transferred).

REFERENCES

1. Arimura A. The backstage story of the discovery of LHRH. *Endocrinology* 1991; 129: 1687–9.
2. Schally AV, Nair RM, Redding TW et al. Isolation of the luteinizing hormone and follicle-stimulating hormone-releasing hormone from porcine hypothalami. *J Biol Chem* 1971; 246: 7230–6.
3. Clayton RN, Catt KJ. Gonadotropin-releasing hormone receptors: Characterization, physiological regulation, and relationship to reproductive function. *Endocrinol Rev* 1981; 2: 186–209.
4. Carmel PW, Araki S, Ferin M. Pituitary stalk portal blood collection in rhesus monkeys: Evidence for pulsatile release of gonadotropin-releasing hormone (GnRH). *Endocrinology* 1976; 99: 243–8.
5. Stojilkovic SS, Reinhart J, Catt KJ. Gonadotropin-releasing hormone receptors: Structure and signal transduction pathways. *Endocrinol Rev* 1994; 15: 462–99.
6. Kaiser UB, Conn PM, Chin WW. Studies of gonadotropin-releasing hormone (GnRH) action using GnRH receptor-expressing pituitary cell lines. *Endocrinol Rev* 1997; 18: 46–70.
7. Catt KJ, Martinez-Fuentes AJ, Hu L et al. Recent insights into the regulation of pulsatile GnRH secretion (abstract O-004). *Gynecol Endocrinol* 2003; 17: 2.
8. Neill JD, Patton JM, Dailey RA et al. Luteinizing hormone releasing hormone (LHRH) in pituitary stalk blood of rhesus monkeys: Relationship to level of LH release. *Endocrinology* 1977; 101: 430–4.
9. Levine JE, Pau KY, Ramirez VD et al. Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology* 1982; 111: 1449–55.
10. Levine JE, Norman RL, Gliessman PM et al. *In vivo* gonadotropin-releasing hormone release and serum luteinizing hormone measurements in ovariectomized, estrogen-treated rhesus macaques. *Endocrinology* 1985; 117: 711–21.
11. Backstrom CT, McNeilly AS, Leask RM et al. Pulsatile secretion of LH, FSH, prolactin, oestradiol and progesterone during the human menstrual cycle. *Clin Endocrinol (Oxf)* 1982; 17: 29–42.
12. Reame N, Sauder SE, Kelch RP et al. Pulsatile gonadotropin secretion during the human menstrual cycle: Evidence for altered frequency of gonadotropin-releasing hormone secretion. *J Clin Endocrinol Metab* 1984; 59: 328–37.
13. Crowley WF, Jr., Filicori M, Spratt DI et al. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. *Recent Prog Horm Res* 1985; 41: 473–531.
14. Belchetz PE, Plant TM, Nakai Y et al. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science* 1978; 202: 631–3.
15. Gharib SD, Wierman ME, Shupnik MA et al. Molecular biology of the pituitary gonadotropins. *Endocrinol Rev* 1990; 11: 177–99.
16. Nillius SJ, Wide L. Variation in LH and FSH response to LH-releasing hormone during the menstrual cycle. *J Obstet Gynaecol Br Common* 1972; 79: 865–73.
17. Conn PM, Crowley WF, Jr. Gonadotropin-releasing hormone and its analogs. *Annu Rev Med* 1994; 45: 391–405.
18. Krsmanovic LZ, Martinez-Fuentes AJ, Arora KK et al. Local regulation of gonadotroph function by pituitary gonadotropin-releasing hormone. *Endocrinology* 2000; 141: 1187–95.
19. Merchenthaler I. Identification of estrogen receptor-B in the GnRH neurons of the rodent hypothalamus (abstract O-024). *Gynecol Endocrinol* 2003; 17: 12.
20. Reissmann T, Diedrich K, Comaru-Schally AM et al. Introduction of LHRH-antagonists into the treatment of gynaecological disorders. *Hum Reprod* 1994; 9: 769.
21. Gordon K, Hodgen GD. Will GnRH antagonists be worth the wait? *Reprod Med Rev* 1992; 1: 189–94.
22. Huirne JA, Lambalk CB. Gonadotropin-releasing-hormone-receptor antagonists. *Lancet* 2001; 358: 1793–803.
23. Andreyko JL, Marshall LA, Dumesic DA et al. Therapeutic uses of gonadotropin-releasing hormone analogs. *Obstet Gynecol Surv* 1987; 42: 1–21.
24. Nestor JJ Jr. Developments of agonistic LHRH analogs. In: *LHRH and its Analogs*. Vickery BH, Nestor JJ, Jr., Hafez ESE (eds). Lancaster, U.K.: MTP Press, 1984, pp. 3–15.
25. Karten MJ, Rivier JE. Gonadotropin-releasing hormone analog design. Structure–function studies

toward the development of agonists and antagonists: Rationale and perspective. *Endocrinol Rev* 1986; 7: 44–66.
26. Corbin A, Beattie CW. Post-coital contraceptive and uterotrophic effects of luteinizing hormone releasing hormone. *Endocrinol Res Commun* 1975; 2: 445–58.
27. Conn PM, Crowley WFJr. Gonadotropin-releasing hormone and its analogs. *N Engl J Med* 1991; 324: 93–103.
28. Klijn JGM. LHRH-agonist therapy in breast cancer (abstract O-016). *Gynecol Endocrinol* 2003; 17: 8.
29. Porter RN, Smith W, Craft IL et al. Induction of ovulation for *in vitro* fertilisation using buserelin and gonadotropins. *Lancet* 1984; 2: 1284–5.
30. Fleming R, Coutts JR. Induction of multiple follicular growth in normally menstruating women with endogenous gonadotropin suppression. *Fertil Steril* 1986; 45: 226–30.
31. Janssens RM, Lambalk CB, Vermeiden JP et al. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: A prospective, randomized, double-blind, placebo-controlled study. *Hum Reprod* 2000; 15: 2333–40.
32. Loumaye E. The control of endogenous secretion of LH by gonadotrophin-releasing hormone agonists during ovarian hyperstimulation for *in vitro* fertilization and embryo transfer. *Hum Reprod* 1990; 5: 357–76.
33. Templeton A, Morris JK. Reducing the risk of multiple births by transfer of two embryos after *in vitro* fertilization. *N Engl J Med* 1998; 339: 573–7.
34. Hughes EG, Fedorkow DM, Daya S et al. The routine use of gonadotropin-releasing hormone agonists prior to *in vitro* fertilization and gamete intrafallopian transfer: A meta-analysis of randomized controlled trials. *Fertil Steril* 1992; 58: 888–96.
35. Al-Inany HG, Youssef MAFM, Aboulghar M et al. Gonadotrophin releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011; 5: CD001750.
36. Orvieto R, Patrizio P. GnRH agonist versus GnRH antagonist in ovarian stimulation: An ongoing debate. *Reprod Biomed Online* 2013; 26: 4–8.
37. Broekmans FJ, Bernardus RE, Berkhout G et al. Pituitary and ovarian suppression after early follicular and mid-luteal administration of a LHRH agonist in a depot formulation: Decapeptyl CR. *Gynecol Endocrinol* 1992; 6: 153–61.
38. Devreker F, Govaerts I, Bertrand E et al. The long-acting gonadotropin-releasing hormone analogues impaired the implantation rate. *Fertil Steril* 1996; 65: 122–6.
39. Albuquerque LE, Saconato H, Maciel MC. Depot versus daily administration of gonadotrophin releasing hormone agonist protocols for pituitary desensitization in assisted reproduction cycles. *Cochrane Database Syst Rev* 2002; 3: CD002808.
40. Weissman A, Shoham Z. Favourable pregnancy outcome after administration of a long-acting gonadotrophin-releasing hormone agonist in the mid-luteal phase. *Hum Reprod* 1993; 8: 496–7.
41. Balasch J, Martinez F, Jove I et al. Inadvertent gonadotrophin-releasing hormone agonist (GnRHa) administration in the luteal phase may improve fecundity in *in vitro* fertilization patients. *Hum Reprod* 1993; 8: 1148–51.
42. Ron-El R, Lahat E, Golan A et al. Development of children born after ovarian superovulation induced by long-acting gonadotropin-releasing hormone agonist and menotropins, and by *in vitro* fertilization. *J Pediatr* 1994; 125: 734–7.
43. Cahill DJ, Fountain SA, Fox R et al. Outcome of inadvertent administration of a gonadotrophin-releasing hormone agonist (buserelin) in early pregnancy. *Hum Reprod* 1994; 9: 1243–6.
44. Gartner B, Moreno C, Marinaro A et al. Accidental exposure to daily long-acting gonadotrophin-releasing hormone analogue administration and pregnancy in an *in vitro* fertilization cycle. *Hum Reprod* 1997; 12: 2557–9.
45. Taskin O, Gokdeniz R, Atmaca R et al. Normal pregnancy outcome after inadvertent exposure to long-acting gonadotrophin-releasing hormone agonist in early pregnancy. *Hum Reprod* 1999; 14: 1368–71.
46. Lahat E, Raziel A, Friedler S et al. Long-term follow-up of children born after inadvertent administration of a gonadotrophin-releasing hormone agonist in early pregnancy. *Hum Reprod* 1999; 14: 2656–60.
47. Surrey ES, Silverberg KM, Surrey MW et al. Effect of prolonged gonadotropin-releasing hormone agonist therapy on the outcome of *in vitro* fertilization–embryo transfer in patients with endometriosis. *Fertil Steril* 2002; 78: 699–704.
48. de Ziegler D, Cedars MI, Randle D et al. Suppression of the ovary using a gonadotropin releasing-hormone agonist prior to stimulation for oocyte retrieval. *Fertil Steril* 1987; 48: 807–10.
49. Balasch J, Jove IC, Moreno V et al. The comparison of two gonadotropin-releasing hormone agonists in an *in vitro* fertilization program. *Fertil Steril* 1992; 58: 991–4.
50. Parinaud J, Oustry P, Perineau M et al. Randomized trial of three luteinizing hormone-releasing hormone analogues used for ovarian stimulation in an *in vitro* fertilization program. *Fertil Steril* 1992; 57: 1265–8.
51. Penzias AS, Shamma FN, Gutmann JN et al. Nafarelin versus leuprolide in ovulation induction for *in vitro* fertilization: A randomized clinical trial. *Obstet Gynecol* 1992; 79: 739–42.
52. Tapanainen J, Hovatta O, Juntunen K et al. Subcutaneous goserelin versus intranasal buserelin for pituitary down-regulation in patients undergoing IVF: A randomized comparative study. *Hum Reprod* 1993; 8: 2052–5.

53. Dantas ZN, Vicino M, Balmaceda JP et al. Comparison between nafarelin and leuprolide acetate for *in vitro* fertilization: Preliminary clinical study. *Fertil Steril* 1994; 61: 705–8.
54. Goldman JA, Dicker D, Feldberg D et al. A prospective randomized comparison of two gonadotrophin releasing hormone agonists, nafarelin acetate and buserelin acetate, in *in vitro* fertilization–embryo transfer. *Hum Reprod* 1994; 9: 226–8.
55. Tarlatzis BC, Grimbizis G, Pournaropoulos F et al. Evaluation of two gonadotropin-releasing hormone (GnRH) analogues (leuprolide and buserelin) in short and long protocols for assisted reproductive techniques. *J Assist Reprod Genet* 1994; 11: 85–91.
56. Lockwood GM, Pinkerton SM, Barlow DH. A prospective randomized single-blind comparative trial of nafarelin acetate with buserelin in long-protocol gonadotrophin-releasing hormone analogue controlled *in vitro* fertilization cycles. *Hum Reprod* 1995; 10: 293–8.
57. Tanos V, Friedler S, Shushan A et al. Comparison between nafarelin acetate and D-Trp6–LHRH for temporary pituitary suppression in *in vitro* fertilization (IVF) patients: A prospective crossover study. *J Assist Reprod Genet* 1995; 12: 715–19.
58. Oyesanya OA, Teo SK, Quah E et al. Pituitary down-regulation prior to *in vitro* fertilization and embryo transfer: A comparison between a single dose of Zoladex depot and multiple daily doses of Suprefact. *Hum Reprod* 1995; 10: 1042–4.
59. Avrech OM, Goldman GA, Pinkas H et al. Intranasal nafarelin versus buserelin (short protocol) for controlled ovarian hyperstimulation before *in vitro* fertilization: A prospective clinical trial. *Gynecol Endocrinol* 1996; 10: 165–70.
60. Corson SL, Gutmann JN, Batzer FR et al. A double-blind comparison of nafarelin and leuprolide acetate for down-regulation in IVF cycles. *Int J Fertil Menopausal Stud* 1996; 41: 446–9.
61. Polson DW, Mason HD, Saldahna MB et al. Ovulation of a single dominant follicle during treatment with low-dose pulsatile follicle stimulating hormone in women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 1987; 26: 205–12.
62. Tan SL. Luteinizing hormone-releasing hormone agonists for ovarian stimulation in assisted reproduction. *Curr Opin Obstet Gynecol* 1994; 6: 166–72.
63. Chang SY, Lee CL, Wang ML et al. No detrimental effects in delaying initiation of gonadotropin administration after pituitary desensitization with gonadotropin-releasing hormone agonist. *Fertil Steril* 1993; 59: 183–6.
64. Dimitry ES, Oskarsson T, Conaghan J et al. Beneficial effects of a 24 h delay in human chorionic gonadotrophin administration during *in vitro* fertilization treatment cycles. *Hum Reprod* 1991; 6: 944–6.
65. Daya S. Gonadotrophin-releasing hormone agonist protocols for pituitary desensitization in in vitro fertilization and gamete intrafallopian transfer cycles. *Cochrane Database Syst Rev* 2000; 2: CD001299.
66. Pellicer A, Simon C, Miro F et al. Ovarian response and outcome of *in vitro* fertilization in patients treated with gonadotrophin-releasing hormone analogues in different phases of the menstrual cycle. *Hum Reprod* 1989; 4: 285–9.
67. Kondaveeti-Gordon U, Harrison RF, Barry-Kinsella C et al. A randomized prospective study of early follicular or midluteal initiation of long protocol gonadotropin-releasing hormone in an *in vitro* fertilization program. *Fertil Steril* 1996; 66: 582–6.
68. San Roman GA, Surrey ES, Judd HL et al. A prospective randomized comparison of luteal phase versus concurrent follicular phase initiation of gonadotropin-releasing hormone agonist for *in vitro* fertilization. *Fertil Steril* 1992; 58: 744–9.
69. Keltz MD, Jones EE, Duleba AJ et al. Baseline cyst formation after luteal phase gonadotropin-releasing hormone agonist administration is linked to poor *in vitro* fertilization outcome. *Fertil Steril* 1995; 64: 568–72.
70. Feldberg D, Ashkenazi J, Dicker D et al. Ovarian cyst formation: A complication of gonadotropin-releasing hormone agonist therapy. *Fertil Steril* 1989; 51: 42–5.
71. Biljan MM, Mahutte NG, Dean N et al. Pretreatment with an oral contraceptive is effective in reducing the incidence of functional ovarian cyst formation during pituitary suppression by gonadotropin-releasing hormone analogues. *J Assist Reprod Genet* 1998; 15: 599–604.
72. Damario MA, Barmat L, Liu HC et al. Dual suppression with oral contraceptives and gonadotrophin releasing hormone agonists improves *in vitro* fertilization outcome in high responder patients. *Hum Reprod* 1997; 12: 2359–65.
73. Cedrin-Durnerin I, Bidart JM, Robert P et al. Consequences on gonadotrophin secretion of an early discontinuation of gonadotrophin-releasing hormone agonist administration in short-term protocol for *in vitro* fertilization. *Hum Reprod* 2000; 15: 1009–14.
74. Dirnfeld M, Fruchter O, Yshai D et al. Cessation of gonadotropin-releasing hormone analogue (GnRH-a) upon down-regulation versus conventional long GnRH-a protocol in poor responders undergoing *in vitro* fertilization. *Fertil Steril* 1999; 72: 406–11.
75. Garcia-Velasco JA, Isaza V, Requena A et al. High doses of gonadotrophins combined with stop versus non-stop protocol of GnRH analogue administration in low responder IVF patients: A prospective, randomized, controlled trial. *Hum Reprod* 2000; 15: 2292–6.
76. Fujii S, Sagara M, Kudo H et al. A prospective randomized comparison between long and

discontinuous-long protocols of gonadotropin-releasing hormone agonist for *in vitro* fertilization. *Fertil Steril* 1997; 67: 1166–8.
77. Fujii S, Sato S, Fukui A et al. Continuous administration of gonadotrophin-releasing hormone agonist during the luteal phase in IVF. *Hum Reprod* 2001; 16: 1671–5.
78. Padilla SL, Dugan K, Maruschak V et al. Use of the flare-up protocol with high dose human follicle stimulating hormone and human menopausal gonadotropins for *in vitro* fertilization in poor responders. *Fertil Steril* 1996; 65: 796–9.
79. Loumaye E, Coen G, Pampfer S et al. Use of a gonadotropin-releasing hormone agonist during ovarian stimulation leads to significant concentrations of peptide in follicular fluids. *Fertil Steril* 1989; 52: 256–63.
80. Fasouliotis SJ, Simon A, Laufer N. Evaluation and treatment of low responders in assisted reproductive technique: A challenge to meet. *J Assist Reprod Genet* 2000; 17: 357–73.
81. Acharya U, Irvine S, Hamilton M et al. Prospective study of short and ultrashort regimens of gonadotropin-releasing hormone agonist in an *in vitro* fertilization program. *Fertil Steril* 1992; 58: 1169–73.
82. Scott RT, Navot D. Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonist during ovulation induction for *in vitro* fertilization. *Fertil Steril* 1994; 61: 880–5.
83. Feldberg D, Farhi J, Ashkenazi J et al. Minidose gonadotropin-releasing hormone agonist is the treatment of choice in poor responders with high follicle-stimulating hormone levels. *Fertil Steril* 1994; 62: 343–6.
84. Surrey ES, Bower J, Hill DM et al. Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing *in vitro* fertilization. *Fertil Steril* 1998; 69: 419–24.
85. Sher G, Zouves C, Feinman M et al. "Prolonged coasting": An effective method for preventing severe ovarian hyperstimulation syndrome in patients undergoing *in vitro* fertilization. *Hum Reprod* 1995; 10: 3107–9.
86. Fluker MR, Hooper WM, Yuzpe AA. Withholding gonadotropins ("coasting") to minimize the risk of ovarian hyperstimulation during superovulation and *in vitro* fertilization-embryo transfer cycles. *Fertil Steril* 1999; 71: 294–301.
87. D'Angelo A, Amso N. "Coasting" (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev* 2002; 6: CD002811.
88. Egbase PE, Sharhan MA, Grudzinskas JG. Early unilateral follicular aspiration compared with coasting for the prevention of severe ovarian hyperstimulation syndrome: A prospective randomized study. *Hum Reprod* 1999; 14: 1421–5.
89. Emperaire JC, Ruffie A. Triggering ovulation with endogenous luteinizing hormone may prevent the ovarian hyperstimulation syndrome. *Hum Reprod* 1991; 6: 506–10.
90. Lanzone A, Fulghesu AM, Villa P et al. Gonadotropin-releasing hormone agonist versus human chorionic gonadotropin as a trigger of ovulation in polycystic ovarian disease gonadotropin hyperstimulated cycles. *Fertil Steril* 1994; 62: 35–41.
91. Gonen Y, Balakier H, Powell W et al. Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for *in vitro* fertilization. *J Clin Endocrinol Metab* 1990; 71: 918–22.
92. Itskovitz J, Boldes R, Levron J et al. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. *Fertil Steril* 1991; 56: 213–20.
93. Romeu A, Monzo A, Peiro T et al. Endogenous LH surge versus hCG as ovulation trigger after low-dose highly purified FSH in IUI: A comparison of 761 cycles. *J Assist Reprod Genet* 1997; 14: 518–24.
94. Olivennes F, Fanchin R, Bouchard P et al. Triggering of ovulation by a gonadotropin-releasing hormone (GnRH) agonist in patients pretreated with a GnRH antagonist. *Fertil Steril* 1996; 66: 151–3.
95. Itskovitz-Eldor J, Kol S, Mannaerts B. Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: Preliminary report: Short communication. *Hum Reprod* 2000; 15: 1965–8.
96. Fauser BC, de Jong D, Olivennes F et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for *in vitro* fertilization. *J Clin Endocrinol Metab* 2002; 87: 709–15.
97. Egbase PE, Grudzinskas JG, Al Sharhan M, Ashkenazi L. hCG or GnRH agonist to trigger ovulation in GnRH antagonist-treated intrauterine insemination cycles: A prospective randomized study. *Hum Reprod* 2002; 17: 2-O-006.
98. Patrizio P, Bianchi V, Lalioti MD, Gerasimova T, Sakkas D. High rate of biological loss in assisted reproduction: It is in the seed, not in the soil. *Reprod Biomed Online* 2007; 14: 92–5.
99. Bromer JG, Sakkas D, Siano LJ, Benadiva CA, Patrizio P. Reproductive efficiency of women over the age of 40 and the low risk of multiple pregnancies. *Reprod Biomed Online* 2009; 19(Suppl 4): 4316.
100. Ghazal S and Patrizio P. Embryo wastage rates remain high in assisted reproductive technology (ART): A look at the trends from 2004-2013 in the United States. *JARG* 2017; 34 (2): 159–66.
101. Kovalevsky G, Patrizio P. High rates of embryo wastage with use of assisted reproductive technology: A look at the trends between 1995 and 2001 in the United States. *Fertil Steril* 2005; 84: 325–30.

Gonadotropin-releasing hormone antagonists in ovarian stimulation for *in vitro* fertilization

43

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INTRODUCTION

Although the first baby born after *in vitro* fertilization (IVF) was conceived in a non-stimulated cycle (1), it was soon accepted that the role of IVF, as an efficient therapeutic modality for subfertile couples, could only be served through multifollicular development, achieved with the use of gonadotropins (2). Gonadotropin use, however, was frequently associated with premature luteinizing hormone (LH) surge prior to oocyte retrieval, which led to cycle cancellation in approximately one out of five women (3,4).

The problem of the premature LH surge was managed by the introduction of gonadotropin-releasing hormone (GnRH) agonists in ovarian stimulation (5), thanks to the pioneering work of Schally et al. in 1971 (6). Both GnRH agonists and antagonists were available in the early 1980s for suppression of endogenous LH secretion. GnRH antagonists, however, could not be used for this purpose due to the associated allergic reactions provoked by their administration (7), leaving GnRH agonists as the only available choice.

Following pituitary down-regulation by GnRH agonists and avoidance of a premature LH surge, unhindered use of gonadotropins in ovarian stimulation led to the collection of more oocytes and to an increase in the number of good-quality embryos available for transfer (8). This was associated with an increase in pregnancy rates compared to cycles where no suppression of a premature LH surge was performed, as shown by one of the first meta-analyses in reproductive medicine (9).

The use of GnRH agonists became universal through the 1980s, 1990s, and until the early 2000s, characterizing IVF throughout this period as the GnRH agonist era (10). However, there is probably not much doubt that if GnRH antagonist use had not been associated with allergic reactions, they would have been adopted as the analog of choice instead of GnRH agonists. This is mainly due to the fact that GnRH antagonist action starts immediately after their administration as opposed to the lengthy down-regulation period required with GnRH agonists. In addition, GnRH agonist use is associated with estrogen deprivation symptoms during the down-regulation period, the occurrence of ovarian cysts at initiation of stimulation, and ovarian hyperstimulation syndrome (OHSS) following human chorionic gonadotropin (hCG) administration.

For the above reasons, the introduction in the early 2000s of the third generation of GnRH antagonists, which lacked histamine release problems and thus did not lead to allergic reactions (11,12), was perceived by the scientific community as a great opportunity to simplify and optimize ovarian stimulation.

GnRH AGONISTS VERSUS GnRH ANTAGONISTS

The introduction of GnRH antagonists was followed by an initial period of debate regarding their comparative efficacy with GnRH agonists. This was fueled by several conflicting meta-analyses in favor (13) or against (14,15) their use.

The latest meta-analysis by the Cochrane group comparing GnRH agonists with GnRH antagonists (16) suggested that a move away from the standard GnRH agonist long protocol to a GnRH antagonist protocol was justified, heralding the end of the GnRH agonist era. This was based on the significantly increased safety of GnRH antagonists compared with GnRH agonists, combined with their equal effectiveness regarding the probability of live birth (16).

GnRH antagonists make ovarian stimulation more patient friendly, requiring fewer days of treatment compared to GnRH agonists (13). In addition, they constitute a more rational way to inhibit a premature LH rise compared to GnRH agonists, which need to be administered for this purpose approximately three weeks before the LH rise is likely to occur.

Based on data from the German Registry, GnRH antagonist usage increased with time from 14.1% in 2000 to 61.4% in 2013, gradually replacing GnRH agonists as the analog of choice for suppressing the premature LH rise (Figure 43.1) (17).

TYPE, SCHEME, DOSE, AND TIMING OF GnRH ANTAGONIST ADMINISTRATION

Two types of third-generation GnRH antagonists have been developed: ganirelix (Organon, Oss, The Netherlands) (18) and cetrorelix (ASTA-Medica, Frankfurt, Germany) (19).

GnRH antagonists can be administered according to a daily dose scheme (20) or a single-dose scheme (21), with the latter inhibiting the premature LH rise for four days. The single-dose scheme can be combined with the daily dose scheme, if necessary.

On the basis of dose-finding studies, the optimal dose for the daily dose GnRH antagonist scheme is 0.25 mg for both cetrorelix and ganirelix (22,23) and 3 mg for the

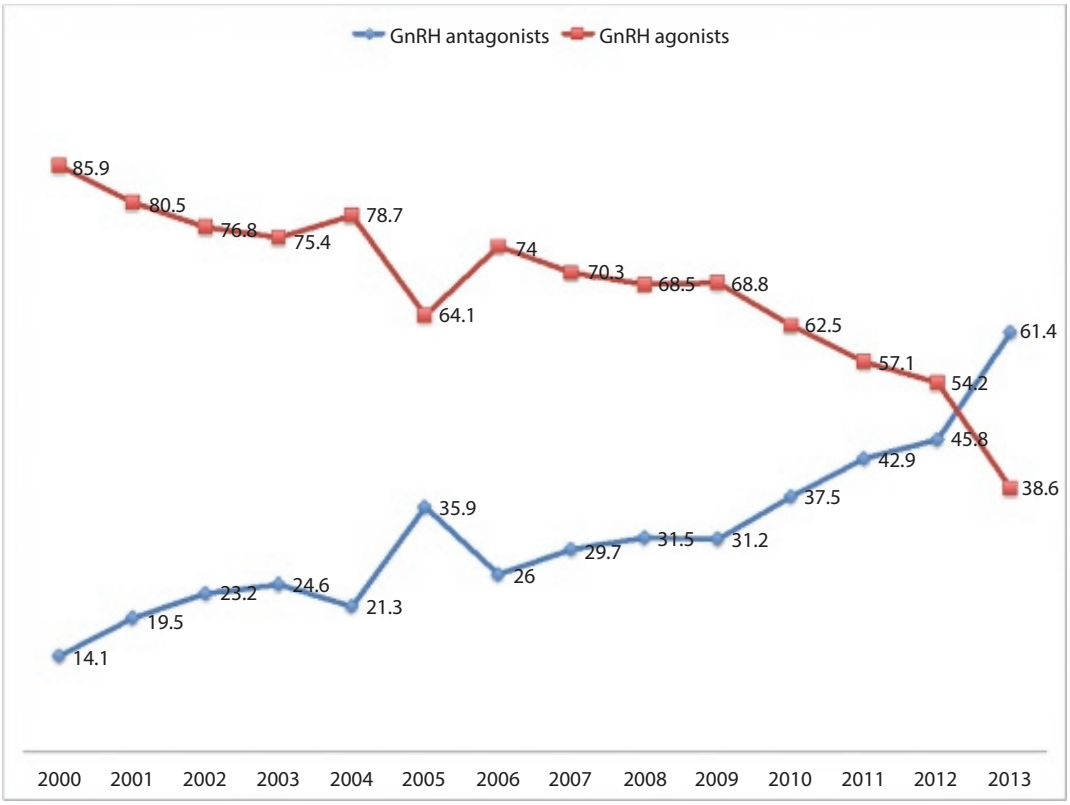

Figure 43.1 Proportion of cycles performed with either GnRH agonists or antagonists based on data published by the Deutsches IVF Register (D.I.R.) (2000–2013). *Abbreviation*: GnRH, gonadotropin-releasing hormone.

single-dose scheme (cetrorelix) (24). Only two comparative trials between the single-dose and the daily dose GnRH antagonist schemes (25,26) have been published to date, showing no difference in the probability of clinical pregnancy (rate difference = −2% in favor of the daily dose protocol; 95% confidence interval [CI]: −16% to +11%). Nevertheless, the majority of GnRH antagonist cycles performed today follow the daily dose scheme (20).

GnRH antagonists can be initiated in either a fixed or a flexible protocol. In the fixed protocol, antagonist initiation occurs on a certain stimulation day on which it is assumed that the LH rise becomes imminent in the majority of patients. In the early introductory GnRH antagonist studies, the LH rise was thought to become imminent on day 6 of stimulation (27), while in the more recent studies (23), this is believed to occur on day 5 of stimulation (28).

In a flexible GnRH antagonist protocol, the antagonist is administered only after certain endocrine and/or sonographic criteria indicating a risk for a LH rise are present. These criteria have differed between studies (29).

Apparently, fixed GnRH antagonist initiation is a simpler protocol that requires less monitoring compared to the flexible one. However, flexible GnRH antagonist administration might avoid unnecessary GnRH antagonist administration in those patients in whom the absence of follicular development on day 5 renders a LH rise unlikely.

Four randomized controlled trials (RCTs) have been published comparing fixed versus flexible GnRH antagonist administration in patients undergoing IVF (29–32). A stratified analysis of these RCTs suggests that no significant difference appears to exist in clinical pregnancy rates (risk ratio = 0.85, 95% CI: 0.65–1.11) (Figure 43.2). However, it is important to note that in the RCTs on fixed versus flexible protocols, only a fraction of the patients randomized to the flexible approach indeed had a later initiation of GnRH antagonists, and accordingly, the true effect of delayed GnRH antagonist initiation has not been precisely determined in these trials.

PROGRAMMING THE INITIATION OF A GnRH ANTAGONIST CYCLE

In a GnRH antagonist cycle, initiation of gonadotropin stimulation is dependent on the occurrence of menstruation. In contrast, in a long luteal GnRH agonist protocol, initiation of stimulation is more flexible, since it occurs 10–15 days following menstruation, when down-regulation is confirmed. However, if deemed necessary, it can be postponed for a number of days. In both GnRH agonist and antagonist cycles, knowing the type and length of patients' cycles makes it feasible to avoid the concomitant initiation of an excessive number of IVF trials that can increase a center's workload beyond what is considered manageable.

On the other hand, there have been efforts to program the initiation of an IVF cycle in order to prevent the occurrence of oocyte retrievals on Sundays or on weekends. This is a challenging task for both GnRH agonist and GnRH

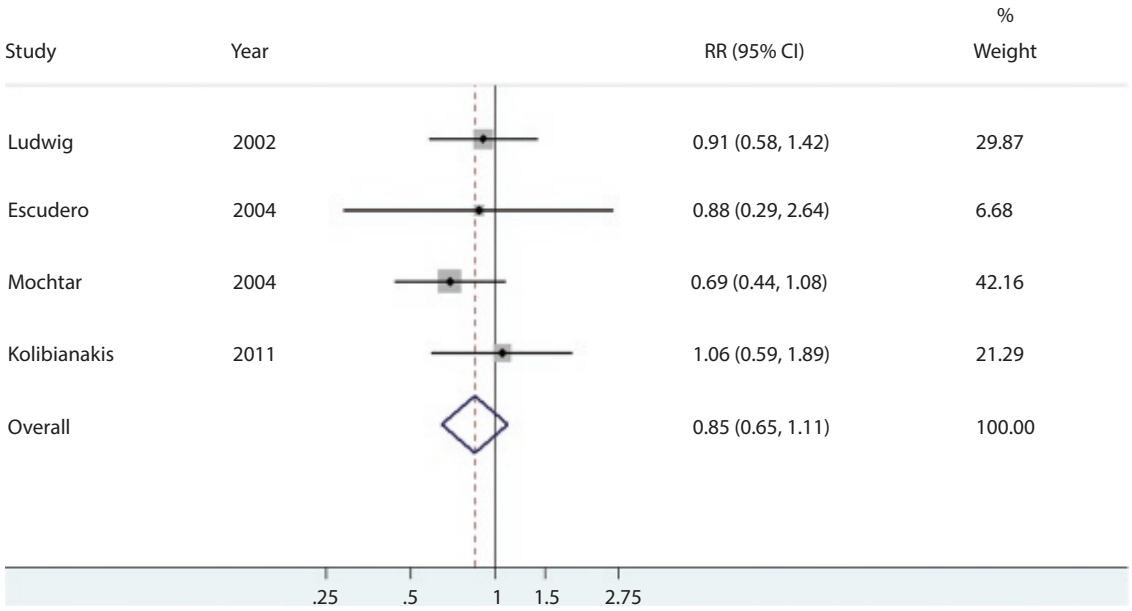

Figure 43.2 Meta-analysis of randomized controlled trials comparing fixed versus flexible gonadotropin-releasing hormone antagonist protocols. *Abbreviation*: CI, confidence interval; RR, Risk Ratio.

antagonist cycles, since duration of stimulation is characterized by a significant inter- and even intra-individual variation (27,33,34). In GnRH antagonist cycles, sex steroid pretreatment has been used for this purpose in the form of oral contraceptive pill (OCP) pretreatment for 14–28 days before initiation of stimulation (35). However, this strategy has been associated with a decreased probability of ongoing pregnancy (relative risk: 0.80, 95% CI: 0.66–0.97; $p = 0.02$) (36), a finding that remained remarkably robust in multiple sensitivity analyses (37). Moreover, OCP pretreatment in GnRH antagonist cycles increases duration of stimulation (weighted mean difference [WMD]: +1.35 days, 95% CI: +0.62 to +2.07 days; $p < 0.01$) and gonadotropin consumption (WMD: +360 IU, 95% CI: +158 to +563 IU; $p < 0.01$) (36), which are known advantages of GnRH antagonists over GnRH agonists (13).

Alternative ways that have been proposed to avoid weekend oocyte retrievals in GnRH antagonist cycles include delaying the day of starting gonadotropin stimulation from day 2 to day 3 of the cycle and/or postponing hCG administration by one day (38). It should be noted, however, that postponing hCG administration for two or more days as soon as three or more follicles of ≥17 mm are present at ultrasound has been associated with a significantly decreased probability of pregnancy in GnRH antagonist cycles (39).

GONADOTROPHIN STIMULATION IN A GnRH ANTAGONIST CYCLE

FSH starting dose

The optimal FSH starting dose is usually selected in IVF, based on the patient's body mass index, age, ovarian reserve (as assessed by antral follicle count and/or anti-Mullerian hormone) (40) and previous response to stimulation. However, efforts have also been made to determine it objectively (41,42). A starting dose of 150–200 IU is generally considered appropriate for a typical patient.

Two studies have been performed in GnRH antagonist cycles to determine whether a higher (200 IU or 225 IU) than the "standard" (150 IU) dose would increase the probability of pregnancy (43,44). The theoretical therapeutic principle behind using a higher dose of FSH lies in the effect of generating more oocytes and thus more embryos from which to choose for preferential transfer to the uterus.

Although the combined sample size available for analysis in these studies was small, it does not appear that pregnancy rates are increased by using a higher than the "standard" dose of FSH (odds ratio for clinical pregnancy: 0.81, 95% CI: 0.51–1.28). Notably, this finding only alludes to one "fresh transfer" and does not take into account differences between high and standard doses in terms of availability of surplus frozen embryos for later replacement in patients after high-dosed stimulation.

Initiation of gonadotropin stimulation

In GnRH antagonist cycles, FSH stimulation can start either on day 2 or day 3 of the cycle (45,46), without affecting the chance of pregnancy (47). A later initiation of FSH stimulation on day 5 is also possible in the so-called "mild stimulation protocols" (48), the target of which is increased safety and decreased drug consumption (49,50).

Increasing the FSH dose at antagonist initiation

Increasing the FSH dose at GnRH antagonist initiation has so far been evaluated in two RCTs, which did not show a beneficial effect on the probability of clinical pregnancy (odds ratio for clinical pregnancy: 1.03, 95% CI: 0.58–1.81) (51,52).

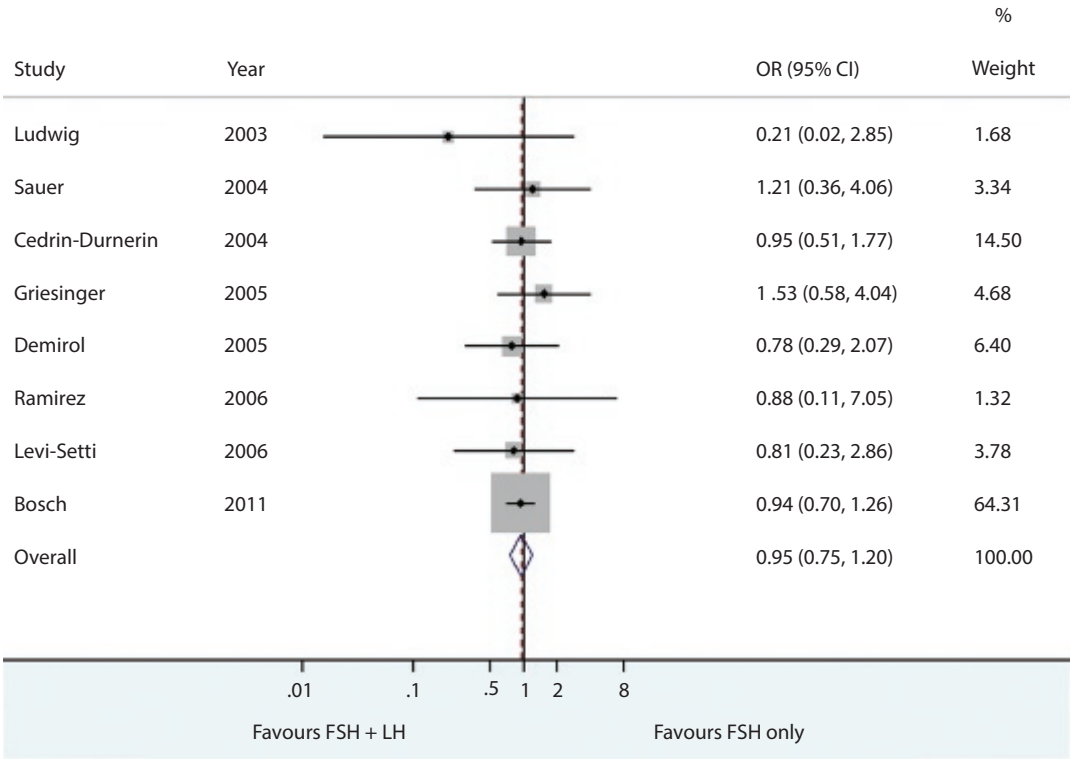

Figure 43.3 Addition of recombinant LH to recombinant FSH in gonadotropin-releasing hormone antagonist cycles. *Abbreviations*: CI, confidence interval; FSH, follicle-stimulating hormone; LH, luteinizing hormone; OR, odds ratio. (Based on Lehert P et al. *Reprod Biol Endocrinol* 2014; 12: 17.)

Addition of LH to FSH

Addition of LH to FSH in GnRH antagonist cycles has been evaluated in numerous RCTs and summarized in several meta-analyses (53–56). Based on the latest meta-analysis (54), LH addition does not appear to be beneficial in terms of pregnancy rate in GnRH antagonist cycles (Figure 43.3).

Long acting FSH

Corifollitropin-α, produced by the fusion of recombinant FSH and the C-terminal peptide of the β-subunit of hCG, is characterized by a slower absorption and a longer half-life than daily recombinant FSH and has been licensed for use in GnRH antagonist cycles. Corifollitropin-α replaces seven days of standard recombinant FSH injections and achieves similar efficacy and safety (57), offering increased patient friendliness during ovarian stimulation for IVF (58).

ENDOCRINE ASSOCIATIONS IN A GnRH ANTAGONIST CYCLE

Elevated serum progesterone, defined as progesterone >1.5 ng/mL, at initiation of stimulation in a spontaneous cycle following a natural luteal phase is a rather infrequent event in the general population (~5% of patients). If, in those patients, initiation of stimulation is postponed for one or two days, progesterone levels will normalize in the majority of cases (80%). However, pregnancy rates in this group are expected to be significantly lower compared with patients with normal progesterone levels at initiation of stimulation (59,60). On the other hand, administration of GnRH antagonist for three consecutive days in patients with elevated progesterone on day 2 of the cycle has been shown to result in acceptable pregnancy rates compared to those achieved in patients with normal progesterone levels prior to gonadotropin initiation (61).

Elevated progesterone levels on the day of triggering final oocyte maturation have been associated with a significantly decreased probability of pregnancy (risk ratio: 0.76; 95% CI: 0.60–0.97) (62). If progesterone elevation occurs, it is worth considering freezing all embryos and performing the transfer in a subsequent cycle (63).

Low endogenous LH levels during ovarian stimulation with GnRH antagonists for pregnancy achievement should not raise concern and cannot serve as a rationale for LH addition to FSH. This was shown initially by Kolibianakis et al. in 2006 (64) and was subsequently confirmed in a large, individual patient data meta-analysis (65). The odds ratios (95% CIs) for ongoing pregnancy for patients with LH levels less than the 25th centile and those with levels greater than the 25th centile on day 8 of stimulation as well as on the day of hCG administration were 0.96 (0.75–1.22) and 0.96 (0.76–1.21), respectively.

TRIGGERING OF FINAL OOCYTE MATURATION IN A GnRH ANTAGONIST CYCLE

Although the incidence of severe OHSS is significantly decreased in GnRH antagonist as compared to GnRH

agonist cycles (16), OHSS can still occur. This is especially true in high-responder patients or those treated with excessive doses of gonadotropins and is invariably associated with administration of hCG for triggering final oocyte maturation.

Thus, the unique option of replacing hCG with GnRH agonists in GnRH antagonist cycles represents one of the most important safety aspects of the antagonistic protocol (66). This is due to the fact that GnRH agonists not only effectively induce final oocyte maturation (67), but at the same time eliminate the incidence of severe OHSS in an unsupported luteal phase (68). This is the main reason that GnRH antagonists/FSH stimulation combined with GnRH agonist triggering today represents the standard mode of stimulation for oocyte donors (69).

In patients using their own oocytes, however, if embryo transfer is performed in the same cycle under standard luteal-phase support, GnRH agonist triggering is associated with a significantly decreased probability of pregnancy (70,71), due to alterations in the quality of the ensuing luteal phase. To manage this problem, three main approaches have been proposed: stimulation of corpora lutea (72–74); administration of increased doses of sex steroids (75); and freezing of all embryos and embryo transfer in subsequent cycles (76,77).

The strategy of freezing all embryos after GnRH agonist triggering currently appears to be the safest approach regarding the occurrence of severe OHSS, and in addition, this approach maintains a high probability of pregnancy in subsequent frozen–thawed cycles (77).

LUTEAL SUPPORT IN GnRH ANTAGONIST CYCLES

Very low LH levels and endometrium abnormalities are present following oocyte retrieval in both GnRH agonist and antagonist down-regulated cycles. These problems are associated with the supra-physiological sex steroid serum levels after gonadotropin stimulation, and they necessitate luteal phase support for pregnancy achievement (78).

Luteal-phase support is predominantly performed in both GnRH agonist and antagonist cycles by progesterone administration in the form of micronized vaginal progesterone (79) or intramuscular (80) or subcutaneous progesterone (81).

Two RCTs, performed exclusively in GnRH antagonist cycles, did not suggest that addition of estrogens to micronized progesterone increases the probability of ongoing pregnancy (risk ratio (RR): 0.89, 95% CI: 0.61–1.30) (82,83).

NEW CONCEPTS IN OVARIAN STIMULATION USING GnRH ANTAGONISTS

The introduction of GnRH antagonists has facilitated the development of new concepts, such as the modified natural cycle (84), mild IVF (85), and the initiation of antagonist in case of severe established OHSS (86–88), enhancing research and advancing progress in ovarian stimulation.

Moreover, from a patient perspective, the increased safety by eliminating severe OHSS, the improved patient friendliness by simplifying treatment with long-acting FSH and decreasing its duration, and finally the similar efficacy to GnRH agonists regarding the probability of live birth render GnRH antagonists the most attractive way to inhibit a premature LH rise in ovarian stimulation for IVF.

REFERENCES

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* 1978; 2(8085): 366.
2. Trounson AO, Leeton JF, Wood C, Webb J, Wood J. Pregnancies in humans by fertilization *in vitro* and embryo transfer in the controlled ovulatory cycle. *Science* 1981; 212(4495): 681–2.
3. Janssens RM, Lambalk CB, Vermeiden JP et al. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: A prospective, randomized, double-blind, placebo-controlled study. *Hum Reprod* 2000; 15(11): 2333–40.
4. Loumaye E. The control of endogenous secretion of LH by gonadotrophin-releasing hormone agonists during ovarian hyperstimulation for *in-vitro* fertilization and embryo transfer. *Hum Reprod* 1990; 5(4): 357–76.
5. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for *in-vitro* fertilisation using buserelin and gonadotropins. *Lancet* 1984; 2(8414): 1284–5.
6. Schally AV, Arimura A, Baba Y et al. Isolation and properties of the FSH and LH-releasing hormone. *Biochem Biophys Res Commun* 1971; 43(2): 393–9.
7. Hahn DW, McGuire JL, Vale WW, Rivier J. Reproductive/endocrine and anaphylactoid properties of an LHRH-antagonist, ORF 18260 [Ac-DNAL1(2), 4FDPhe2,D-Trp3,D-Arg6]-GnRH. *Life Sci* 1985; 37(6): 505–14.
8. Meldrum DR. Ovulation induction protocols. *Arch Pathol Lab Med* 1992; 116(4): 406–9.
9. Hughes EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA. The routine use of gonadotropin-releasing hormone agonists prior to *in vitro* fertilization and gamete intrafallopian transfer: A meta-analysis of randomized controlled trials. *Fertil Steril* 1992; 58(5): 888–96.
10. Marcus SF, Ledger WL. Efficacy and safety of long-acting GnRH agonists in *in vitro* fertilization and embryo transfer. *Hum Fertil (Camb)* 2001; 4(2): 85–93.
11. Duijkers IJ, Klipping C, Willemsen WN et al. Single and multiple dose pharmacokinetics and pharmacodynamics of the gonadotrophin-releasing hormone antagonist cetrorelix in healthy female volunteers. *Hum Reprod* 1998; 13(9): 2392–8.
12. Mannaerts B, Gordon K. Embryo implantation and GnRH antagonists: GnRH antagonists do not activate the GnRH receptor. *Hum Reprod* 2000; 15(9): 1882–3.
13. Kolibianakis EM, Collins J, Tarlatzis BC, Devroey P, Diedrich K, Griesinger G. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type

of analogue used? A systematic review and meta-analysis. *Hum Reprod Update* 2006; 12(6): 651–71.
14. Al-Inany H, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. *Cochrane Database Syst Rev* 2001; 4: CD001750.
15. Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. *Cochrane Database Syst Rev* 2006; 3: CD001750.
16. Al-Inany HG, Youssef MA, Aboulghar M et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011; 5: CD001750.
17. Deutsches IVF Register. *D.I.R. Jahrbuch*. 2000–2013. http://www.deutsches-ivf-register.de.
18. Rabinovici J, Rothman P, Monroe SE, Nerenberg C, Jaffe RB. Endocrine effects and pharmacokinetic characteristics of a potent new gonadotropin-releasing hormone antagonist (Ganirelix) with minimal histamine-releasing properties: Studies in postmenopausal women. *J Clin Endocrinol Metab* 1992; 75(5): 1220–5.
19. Klingmuller D, Schepke M, Enzweiler C, Bidlingmaier F. Hormonal responses to the new potent GnRH antagonist cetrorelix. *Acta Endocrinol (Copenh)* 1993; 128(1): 15–8.
20. Diedrich K, Diedrich C, Santos E et al. Suppression of the endogenous luteinizing hormone surge by the gonadotrophin-releasing hormone antagonist Cetrorelix during ovarian stimulation. *Hum Reprod* 1994; 9(5): 788–91.
21. Olivennes F, Fanchin R, Bouchard P et al. The single or dual administration of the gonadotropin-releasing hormone antagonist Cetrorelix in an *in vitro* fertilization-embryo transfer program. *Fertil Steril* 1994; 62(3): 468–76.
22. Albano C, Smitz J, Camus M, Riethmuller-Winzen H, Van Steirteghem A, Devroey P. Comparison of different doses of gonadotropin-releasing hormone antagonist cetrorelix during controlled ovarian hyperstimulation. *Fertil Steril* 1997; 67(5): 917–22.
23. A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). The ganirelix dose-finding study group. *Hum Reprod* 1998; 13(11): 3023–31.
24. Olivennes F, Alvarez S, Bouchard P, Fanchin R, Salat-Baroux J, Frydman R. The use of a GnRH antagonist (Cetrorelix) in a single dose protocol in IVF–embryo transfer: A dose finding study of 3 versus 2 mg. *Hum Reprod* 1998; 13(9): 2411–4.
25. Lee TH, Wu MY, Chen HF, Chen MJ, Ho HN, Yang YS. Ovarian response and follicular development for single-dose and multiple-dose protocols for gonadotropin-releasing hormone antagonist administration. *Fertil Steril* 2005; 83(6): 1700–7.
26. Wilcox J, Potter D, Moore M, Ferrande L, Kelly E, CAP IV Investigator Group. Prospective, randomized trial comparing cetrorelix acetate and ganirelix acetate in a programmed, flexible protocol for premature luteinizing hormone surge prevention in assisted reproductive technologies. *Fertil Steril* 2005; 84(1): 108–17.
27. Borm G, Mannaerts B. Treatment with the gonadotrophin-releasing hormone antagonist ganirelix in women undergoing ovarian stimulation with recombinant follicle stimulating hormone is effective, safe and convenient: Results of a controlled, randomized, multicentre trial. The European Orgalutran Study Group. *Hum Reprod* 2000; 15(7): 1490–8.
28. Devroey P, Boostanfar R, Koper NP et al. A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol. *Hum Reprod* 2009; 24(12): 3063–72.
29. Kolibianakis EM, Venetis CA, Kalogeropoulou L, Papanikolaou E, Tarlatzis BC. Fixed versus flexible gonadotropin-releasing hormone antagonist administration in *in vitro* fertilization: A randomized controlled trial. *Fertil Steril* 2011; 95(2): 558–62.
30. Escudero E, Bosch E, Crespo J, Simon C, Remohi J, Pellicer A. Comparison of two different starting multiple dose gonadotropin-releasing hormone antagonist protocols in a selected group of *in vitro* fertilization-embryo transfer patients. *Fertil Steril* 2004; 81(3): 562–6.
31. Ludwig M, Katalinic A, Banz C et al. Tailoring the GnRH antagonist cetrorelix acetate to individual patients' needs in ovarian stimulation for IVF: Results of a prospective, randomized study. *Hum Reprod* 2002; 17(11): 2842–5.
32. Mochtar MH, Dutch Ganirelix Study Group. The effect of an individualized GnRH antagonist protocol on folliculogenesis in IVF/ICSI. *Hum Reprod* 2004; 19(8): 1713–8.
33. European, Middle East Orgalutran Study Group. Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation. *Hum Reprod* 2001; 16(4): 644–51.
34. Fauser BC, Alper MM, Ledger W et al. Pharmacokinetics and follicular dynamics of corifollitropin alfa versus recombinant FSH during ovarian stimulation for IVF. *Reprod Biomed Online* 2010; 21(5): 593–601.
35. Fischl F, Huber JC, Obruca A. Zeitliche Optimierung der kontrollierten Hyperstimulation (KOH) in Kombination mit GnrH-Antagonisten und Ovulationshemmer in einem IVF-Programm. *J Fertil Reprod* 2001; 11: 50–1.
36. Griesinger G, Kolibianakis EM, Venetis C, Diedrich K, Tarlatzis B. Oral contraceptive pre-treatment significantly reduces ongoing pregnancy likelihood in

gonadotropin-releasing hormone antagonist cycles: An updated meta-analysis. *Fertil Steril* 2010; 94(6): 2382–4.
37. Griesinger G, Venetis CA, Tarlatzis B, Kolibianakis EM. To pill or not to pill in GnRH-antagonist cycles: The answer is in the data already! *Reprod Biomed Online* 2015; 31(1): 6–8.
38. Gordon K, Levy MJ, Ledger W, Kolibianakis EM, IJzerman-Boon PC. Reducing the incidence of weekend oocyte retrievals in a rFSH/GnRH antagonist protocol by optimizing the start day of rFSH and delaying human chorionic gonadotropin (hCG) by 1 day. *Fertil Steril* 2011; 95(4): S16–S.
39. Kolibianakis EM, Albano C, Camus M, Tournaye H, Van Steirteghem AC, Devroey P. Prolongation of the follicular phase in *in vitro* fertilization results in a lower ongoing pregnancy rate in cycles stimulated with recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. *Fertil Steril* 2004; 82(1): 102–7.
40. Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. *Hum Reprod Update* 2011; 17(1): 46–54.
41. Olivennes F, Howles CM, Borini A et al. Individualizing FSH dose for assisted reproduction using a novel algorithm: The CONSORT study. *Reprod Biomed Online* 2009; 18(2): 195–204.
42. Popovic-Todorovic B, Loft A, Bredkjaeer HE, Bangsboll S, Nielsen IK, Andersen AN. A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a "standard" dose of 150 IU/day in "standard" patients undergoing IVF/ICSI treatment. *Hum Reprod* 2003; 18(11): 2275–82.
43. Out HJ, David I, Ron-El R et al. A randomized, double-blind clinical trial using fixed daily doses of 100 or 200 IU of recombinant FSH in ICSI cycles. *Hum Reprod* 2001; 16(6): 1104–9.
44. Wikland M, Bergh C, Borg K et al. A prospective, randomized comparison of two starting doses of recombinant FSH in combination with cetrorelix in women undergoing ovarian stimulation for IVF/ICSI. *Hum Reprod* 2001; 16(8): 1676–81.
45. Albano C, Felberbaum RE, Smitz J et al. Ovarian stimulation with HMG: Results of a prospective randomized phase III European study comparing the luteinizing hormone-releasing hormone (LHRH)-antagonist cetrorelix and the LHRH-agonist buserelin. European Cetrorelix Study Group. *Hum Reprod* 2000; 15(3): 526–31.
46. European and Middle East Orgalutran Study Group. Comparable clinical outcome using the GnRH antagonist ganirelix or a long protocol of the GnRH agonist triptorelin for the prevention of premature LH surges in women undergoing ovarian stimulation. *Hum Reprod* 2001; 16(4): 644–51.
47. Levy MJ, Ledger W, Kolibianakis EM, Ijzerman-Boon PC, Gordon K. Is it possible to reduce the incidence of weekend oocyte retrievals in GnRH antagonist protocols? *Reprod Biomed Online* 2013; 26(1): 50–8.
48. Hohmann FP, Macklon NS, Fauser BC. A randomized comparison of two ovarian stimulation protocols with gonadotropin-releasing hormone (GnRH) antagonist cotreatment for *in vitro* fertilization commencing recombinant follicle-stimulating hormone on cycle day 2 or 5 with the standard long GnRH agonist protocol. *J Clin Endocrinol Metab* 2003; 88(1): 166–73.
49. Verberg MF, Eijkemans MJ, Macklon NS et al. The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: A meta-analysis. *Hum Reprod Update* 2009; 15(1): 5–12.
50. Verberg MF, Macklon NS, Nargund G et al. Mild ovarian stimulation for IVF. *Hum Reprod Update* 2009; 15(1): 13–29.
51. Aboulghar MA, Mansour RT, Serour GI, Al-Inany HG, Amin YM, Aboulghar MM. Increasing the dose of human menopausal gonadotrophins on day of GnRH antagonist administration: Randomized controlled trial. *Reprod Biomed Online* 2004; 8(5): 524–7.
52. Propst AM, Bates GW, Robinson RD, Arthur NJ, Martin JE, Neal GS. A randomized controlled trial of increasing recombinant follicle-stimulating hormone after initiating a gonadotropin-releasing hormone antagonist for *in vitro* fertilization-embryo transfer. *Fertil Steril* 2006; 86(1): 58–63.
53. Mochtar MH, Van der V, Ziech M, van Wely M. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2007; 2: CD005070.
54. Lehert P, Kolibianakis EM, Venetis CA et al. Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: Systematic review and meta-analysis. *Reprod Biol Endocrinol* 2014; 12: 17.
55. Kolibianakis EM, Kalogeropoulou L, Griesinger G et al. Among patients treated with FSH and GnRH analogues for *in vitro* fertilization, is the addition of recombinant LH associated with the probability of live birth? A systematic review and meta-analysis. *Hum Reprod Update* 2007; 13(5): 445–52.
56. Baruffi RL, Mauri AL, Petersen CG et al. Recombinant LH supplementation to recombinant FSH during induced ovarian stimulation in the GnRH-antagonist protocol: A meta-analysis. *Reprod Biomed Online* 2007; 14(1): 14–25.
57. Mahmoud Youssef MA, van Wely M, Aboulfoutouh I, El-Khyat W, van der Veen F, Al-Inany H. Is there a place for corifollitropin alfa in IVF/ICSI cycles? A systematic review and meta-analysis. *Fertil Steril* 2012; 97(4): 876–85.

58. Rombauts L, Talmor A. Corifollitropin alfa for female infertility. *Expert Opin Biol Ther* 2012; 12(1): 107–12.
59. Kolibianakis EM, Zikopoulos K, Smitz J et al. Elevated progesterone at initiation of stimulation is associated with a lower ongoing pregnancy rate after IVF using GnRH antagonists. *Hum Reprod* 2004; 19(7): 1525–9.
60. Hamdine O, Macklon NS, Eijkemans MJ et al. Elevated early follicular progesterone levels and *in vitro* fertilization outcomes: A prospective intervention study and meta-analysis. *Fertil Steril* 2014; 102(2): 448–54.e1.
61. Blockeel C, Baumgarten M, De Vos M, Verheyen G, Devroey P. Administration of GnRH antagonists in case of elevated progesterone at initiation of the cycle: A prospective cohort study. *Curr Pharm Biotechnol* 2011; 12(3): 423–8.
62. Kolibianakis EM, Venetis CA, Bontis J, Tarlatzis BC. Significantly lower pregnancy rates in the presence of progesterone elevation in patients treated with GnRH antagonists and gonadotrophins: A systematic review and meta-analysis. *Curr Pharm Biotechnol* 2012; 13(3): 464–70.
63. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: A systematic review and meta-analysis of over 60 000 cycles. *Hum Reprod Update* 2013; 19(5): 433–57.
64. Kolibianakis EM, Collins J, Tarlatzis B, Papanikolaou E, Devroey P. Are endogenous LH levels during ovarian stimulation for IVF using GnRH analogues associated with the probability of ongoing pregnancy? A systematic review. *Hum Reprod Update* 2006; 12(1): 3–12.
65. Griesinger G, Shapiro DB, Kolibianakis EM, Witjes H, Mannaerts BM. No association between endogenous LH and pregnancy in a GnRH antagonist protocol: Part II, recombinant FSH. *Reprod Biomed Online* 2011; 23(4): 457–65.
66. Kolibianakis EM, Griesinger G, Venetis CA. GnRH agonist for triggering final oocyte maturation: Time for a critical evaluation of data. *Hum Reprod Update* 2012; 18(2): 228–9; author reply 229–30.
67. Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: A systematic review and meta-analysis. *Hum Reprod Update* 2006; 12(2): 159–68.
68. Tarlatzis B, Bosdou J, Kolibianakis S. Elimination of OHSS by GnRH agonist and freezing embryos. In Rizk B, and Gerris J, (Eds.), *Complications and Outcomes of Assisted Reproduction* (pp. 141–148). Cambridge: Cambridge University Press. doi:10.1017/9781107295391.015.
69. Bodri D, Sunkara SK, Coomarasamy A. Gonadotropin-releasing hormone agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: A systematic review and meta-analysis. *Fertil Steril* 2011; 95(1): 164–9.
70. Humaidan P, Bredkjaer HE, Bungum L et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: A prospective randomized study. *Hum Reprod* 2005; 20(5): 1213–20.
71. Kolibianakis EM, Schultze-Mosgau A, Schroer A et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. *Hum Reprod* 2005; 20(10): 2887–92.
72. Humaidan P, Polyzos NP, Alsbjerg B et al. GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation: Two prospective randomized controlled multi-centre studies in IVF patients. *Hum Reprod* 2013; 28(9): 2511–21.
73. Papanikolaou EG, Verpoest W, Fatemi H, Tarlatzis B, Devroey P, Tournaye H. A novel method of luteal supplementation with recombinant luteinizing hormone when a gonadotropin-releasing hormone agonist is used instead of human chorionic gonadotropin for ovulation triggering: A randomized prospective proof of concept study. *Fertil Steril* 2011; 95(3): 1174–7.
74. Pirard C, Donnez J, Loumaye E. GnRH agonist as novel luteal support: Results of a randomized, parallel group, feasibility study using intranasal administration of buserelin. *Hum Reprod* 2005; 20(7): 1798–804.
75. Engmann L, Benadiva C. GnRH agonist (buserelin) or HCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: A prospective randomized study. *Hum Reprod* 2005; 20(11): 3258–60; author reply 3260.
76. Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. *Hum Reprod* 2011; 26(10): 2593–7.
77. Griesinger G, Kolibianakis EM, Papanikolaou EG et al. Triggering of final oocyte maturation with gonadotropin-releasing hormone agonist or human chorionic gonadotropin. Live birth after frozen–thawed embryo replacement cycles. *Fertil Steril* 2007; 88(3): 616–21.
78. Beckers NG, Macklon NS, Eijkemans MJ et al. Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in *in vitro* fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. *J Clin Endocrinol Metab* 2003; 88(9): 4186–92.
79. Feinberg EC, Beltsos AN, Nicolaou E, Marut EL, Uhler ML. Endometrin as luteal phase support in assisted reproduction. *Fertil Steril* 2013; 99(1): 174–8.

80. Yanushpolsky E, Hurwitz S, Greenberg L, Racowsky C, Hornstein M. Crinone vaginal gel is equally effective and better tolerated than intramuscular progesterone for luteal phase support in *in vitro* fertilization-embryo transfer cycles: A prospective randomized study. *Fertil Steril* 2010; 94(7): 2596–9.
81. Baker VL, Jones CA, Doody K et al. A randomized, controlled trial comparing the efficacy and safety of aqueous subcutaneous progesterone with vaginal progesterone for luteal phase support of *in vitro* fertilization. *Hum Reprod* 2014; 29(10): 2212–20.
82. Fatemi HM, Kolibianakis EM, Camus M et al. Addition of estradiol to progesterone for luteal supplementation in patients stimulated with GnRH antagonist/rFSH for IVF: A randomized controlled trial. *Hum Reprod* 2006; 21(10): 2628–32.
83. Ceyhan ST, Basaran M, Kemal Duru N, Yilmaz A, Goktolga U, Baser I. Use of luteal estrogen supplementation in normal responder patients treated with fixed multidose GnRH antagonist: A prospective randomized controlled study. *Fertil Steril* 2008; 89(6): 1827–30.
84. Lainas TG, Sfontouris IA, Venetis CA et al. Live birth rates after modified natural cycle compared with high-dose FSH stimulation using GnRH antagonists in poor responders. *Hum Reprod* 2015; 30(10): 2321–30.
85. Fauser BC, Nargund G, Andersen AN et al. Mild ovarian stimulation for IVF: 10 years later. *Hum Reprod* 2010; 25(11): 2678–84.
86. Lainas TG, Sfontouris IA, Zorzovilis IZ et al. Management of severe OHSS using GnRH antagonist and blastocyst cryopreservation in PCOS patients treated with long protocol. *Reprod Biomed Online* 2009; 18(1): 15–20.
87. Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT, Kolibianakis EM. Management of severe early ovarian hyperstimulation syndrome by re-initiation of GnRH antagonist. *Reprod Biomed Online* 2007; 15(4): 408–12.
88. Lainas TG, Sfontouris IA, Zorzovilis IZ et al. Live births after management of severe OHSS by GnRH antagonist administration in the luteal phase. *Reprod Biomed Online* 2009; 19(6): 789–95.

Gonadotropin-releasing hormone agonist triggering

44

LEAH KAYE, CLAUDIO BENADIVA, and LAWRENCE ENGMANN

OVERVIEW

The use of gonadotropin-releasing hormone agonist (GnRHa) has been advocated as a substitute to human chorionic gonadotropin (hCG) for the induction of oocyte maturation and prevention of ovarian hyperstimulation syndrome (OHSS) during *in vitro* fertilization (IVF) cycles since the late 1980s to early 1990s (1–8). However, the subsequent widespread use of GnRHa for pituitary down-regulation during controlled ovarian stimulation limited its use as an option for triggering oocyte maturation.

After GnRH antagonists were introduced for prevention of the luteinizing hormone (LH) surge during controlled ovarian stimulation in the late 1990s, GnRHa could then be used again for the induction of oocyte maturation (9–11). GnRH antagonist blocks the GnRH receptors on the pituitary by competitive inhibition (12). Administration of GnRHa will then displace the antagonist on the receptors and activate them to promote a release of gonadotropins stored in the anterior pituitary (13).

More than 15 years after the first publication regarding the use of GnRHa trigger after GnRH antagonist pretreatment during IVF (11), there are still several questions regarding its effectiveness at inducing oocyte maturation and the ideal luteal-phase supplementation protocol (Table 44.1). Early clinical experiences in the mid-2000s were published using GnRHa for trigger during antagonist stimulation cycles (11). Unfortunately, early studies reported high early pregnancy loss rates and low clinical pregnancy rates (14,15). Additional studies have subsequently been published in an effort to understand the underlying causes of the suboptimal pregnancy rates and to improve the clinical efficacy of the GnRHa trigger. The culmination of the current literature now suggests that the luteolytic properties of GnRHa are effective at preventing OHSS, but are also likely to be the cause of low pregnancy rates when standard luteal support is used. By optimizing the luteal-phase profile for fresh transfer after GnRHa trigger, pregnancy rates can be comparable to those of the hCG trigger while reducing or eliminating the risks of OHSS (16–29).

INDICATIONS

In the current setting of assisted reproduction technology (ART), there are clinical situations in which a GnRHa trigger should be considered first line due to the benefits of safety and comfort for the patient (Table 44.2). In particular, any patient who does not plan to have a fresh embryo transfer may be an ideal candidate for GnRHa trigger, including patients treated for fertility preservation, preimplantation genetic screening/diagnosis, or prematurely elevated progesterone prior to trigger (30–32). Young, healthy women undergoing oocyte donation are also ideal candidates for GnRHa trigger. Moreover, any woman at risk of developing OHSS is an ideal candidate for GnRHa trigger with modified luteal-phase support or subsequent elective cryopreservation of oocytes or embryos.

Some patients are not well suited for use of a GnRHa trigger as it relies on the ability to mount an endogenous surge of gonadotropins. As a result, patients with hypothalamic dysfunction are not ideal candidates for GnRHa trigger for oocyte maturation. Moreover, women who have had long-term suppression of the hypothalamus and pituitary may have a failed or suboptimal response because they may not be able to mount an optimal LH surge after GnRHa trigger (33).

PHYSIOLOGY

Natural versus GnRHa-induced mid-cycle surge

A single bolus of GnRHa will interact with the GnRH receptors and cause the endogenous release or "flare" of gonadotropins from the anterior pituitary. The resultant surge of LH and follicle-stimulating hormone (FSH) resembles the natural mid-cycle surge of gonadotropins seen shortly before ovulation, and thus a bolus of GnRHa can "trigger" ovulation (34). While the role of the FSH surge is not completely elucidated in humans, there are animal and human cell studies suggesting that FSH plays a role in oocyte maturation and resumption of meiosis (35,36), function of the oocyte–cumulus complex and facilitation of its detachment from the follicle wall (37), and generation of LH receptors on granulosa cells (38). Thus, there may be advantages to an ovulation trigger that result in a surge of both LH and FSH.

A natural ovulatory surge consists traditionally of three phases: abrupt onset (14 hours), LH peak/plateau (14 hours), and gradual descent to baseline (20 hours), lasting a mean duration of 48 hours (Figure 44.1) (39). In contrast, the surge after GnRHa occurs in two phases: rapid ascent and moderate descent, lasting 24–36 hours (3). An early study by Itskovitz et al. (3) showed that GnRHa causes LH to rise over four hours and FSH to rise over 12 hours, with significant elevation lasting 24 hours before a return of LH to baseline levels (3,20). The relatively short duration of the LH surge is capable of inducing oocyte maturation and ovulation but may result in defective formation of the corpus luteum (40).

Table 44.1 Controversies surrounding use of gonadotropin-releasing hormone agonist (GnRHa) trigger

1. What is the ideal dose of GnRHa trigger?
2. Is it effective at inducing oocyte maturation?
3. Are there any post-trigger serum luteinizing hormone or progesterone levels that will predict trigger failure?
4. Does it eliminate the risk of ovarian hyperstimulation syndrome?
5. Should fresh embryo transfer be performed or should all oocytes/embryos be frozen after GnRHa trigger?
6. What is the ideal luteal-phase supplementation regimen?

Table 44.2 Indications for gonadotropin-releasing hormone agonist trigger

High risk for ovarian hyperstimulation syndrome development

Oocyte donors

Elective cryopreservation of oocytes or embryos

- Fertility preservation for medical reasons (e.g., cancer)
- Fertility preservation for social reasons
- Trophectoderm biopsy for preimplantation genetic screening/preimplantation genetic diagnosis
- Premature serum progesterone rise prior to induction of oocyte maturation

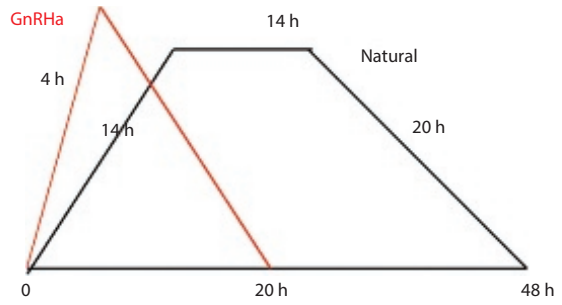

Figure 44.1 Luteinizing hormone surge in a natural cycle and after GnRHa trigger. *Abbreviation*: GnRHa, gonadotropin-releasing hormone agonist. (From Humaidan P et al., *Hum Reprod Update*, GnRH agonist for triggering of final oocyte maturation: time for a change of practice?, 17(4): 510–24, 2011, by permission of Oxford University Press.)

Follicular fluid and granulosa/luteal cells after GnRHa trigger

Differences in follicular fluid dynamics between GnRHa and hCG triggers may explain potential differences in the induction of oocyte maturation, prevention of OHSS, and pregnancy rates. Follicular fluid after GnRHa trigger is noted to have significantly higher levels of LH and FSH than those after hCG trigger due to the combined surge of both gonadotropins (41). Progesterone levels are reduced by 25%, attributed to a lack of LH stimulus on luteal cells in the GnRHa trigger group (41,42). Levels of estrogen, inhibin-A and inhibin-B have been shown to be similar after both triggers (42). These differences in follicular fluid dynamics may represent a larger difference between the signal required for oocyte maturation versus the signal needed for ovulation; although they are typically two closely related events, they may require slightly different signals (42). The follicular fluid studies reflect the similarity between the GnRHa surge and the natural mid-cycle surge, with an endogenous surge of LH and FSH and resultant oocyte maturity, but also how pregnancy rates may be affected by decreased luteal-phase progesterone seen in both the follicular fluid and in the circulation.

Amphiregulin and other members of the epidermal growth factor-like family rapidly increase in follicular fluid in response to LH/hCG and are felt to play a role in oocyte maturation by mediating the LH effects within the follicle (43). Levels of amphiregulin in follicular fluid after GnRHa trigger are much lower than in follicles triggered with hCG and approach the level of a natural cycle (44). Vascular endothelial growth factor (VEGF) is also noted to be significantly decreased in follicular fluid after GnRHa trigger, and expression of VEGF mRNA in the granulosa cells is decreased when compared to hCG trigger (45). VEGF is one of the key vasoactive substances and works in part by modulating endothelial cell permeability and hyperpermeability via the cell adhesion molecule VE-cadherin within the ovarian cells (46). The significant decrease in VEGF and vascular permeability after GnRHa trigger play major roles in the prevention of OHSS (45). Closely related is angiopoietin-2 (Ang-2), which causes vascular destabilization and may work synergistically with VEGF to promote the leakage of fluid into the third space that occurs in OHSS. Cerrillo and colleagues found a non-significant decrease in Ang-2 levels in follicular fluid when using a GnRHa trigger, further explaining the effect of GnRHa trigger on OHSS prevention (45,47). More recently, it has been shown that GnRHa induces a direct effect on granulosa cell expression of an antiangiogenic factor, pigment epithelium-derived factor (PEDF), thereby increasing the PEDF to VEGF ratio and creating a more antiangiogenic environment, which may result in impairment of corpora lutea function and hence the onset of OHSS (48).

Although rapid luteolysis occurs after GnRHa trigger, granulosa/luteal cells maintain similar functionality and viability within the first two days after trigger when compared with hCG trigger (49). Engmann et al. analyzed luteal cells collected at oocyte retrieval and noted no significant difference in the proportion of apoptotic cells (49). Finally, the authors showed that luteal cells after both triggers remained responsive and, when exposed to hCG *in vitro*, were able to increase progesterone production (49).

ADMINISTRATION

A number of different GnRH agonists are available for subcutaneous injection, including triptorelin, buserelin, leuprorelin, and nafarelin. Buserelin and nafarelin are

available as intranasal sprays. All must be used in IVF stimulation protocols that utilize a GnRH antagonist for suppression of the LH surge.

Very few studies have been performed to determine the optimal trigger dose that will effectively induce oocyte maturation and prevent OHSS by minimizing luteolysis. Different doses of subcutaneous leuprorelin have been used in the literature and range from 0.5 to 4 mg (19,20,29,31,50–52). Although some studies have used a higher dose of leuprorelin 4 mg (29) and others have used two doses 12 hours apart (53), a single dose of 1 mg is effective at inducing optimal mature oocyte yield (54). The dose of triptorelin has consistently been 0.2 mg in the literature (11,15,16,20,55,56). However, a randomized dose-finding study of 0.2, 0.3, and 0.4 mg of triptorelin in oocyte donors showed similar rates of mature oocytes and top-quality embryos regardless of dose (57). Although different doses have been used for intranasal buserelin, Buckett et al. showed that a dose of 50 μg is the most effective minimal dose to induce an endogenous surge consistently (58). Given the overall equivalent findings, availability and cost should be considered in choosing the type and dose of GnRHa to use for trigger of oocyte maturation. As there may be differences in the endocrine profiles of the luteal phase due to differences in trigger dose, further fine-tuning of the trigger dose could enhance the function of the corpora lutea and overall outcomes.

OOCYTE YIELD AFTER GnRHa TRIGGER

GnRHa trigger has been shown to be as effective as hCG trigger with respect to oocyte yield and maturity in both autologous and donor cycles (Table 44.3). Some studies suggest that a GnRHa trigger may result in more mature oocytes (14,24,32,59–61), though other studies do not (17,19,27,28,62,63). Humaidan et al. found in a randomized trial of 122 patients that GnRHa trigger resulted in 16% more metaphase II (MII) oocytes than hCG ($p < 0.02$) (14). A later study by the same group resulted in 14% more MII oocytes and 11% more embryos suitable for transfer after GnRHa trigger compared to hCG (44).

There are published reports of failed oocyte maturation after GnRHa trigger, often detected with low serum LH on the day after trigger (64). Rates of empty follicle syndrome (EFS) after GnRHa were between 1.4% and 3.5% in two studies, which did not differ significantly from rates of EFS after hCG trigger (0.1%–2%) (54,65–70). A survey of practitioners from clinics worldwide reported that 11% of physicians who use a GnRHa trigger have encountered a case of EFS (71). Predicting the probability of not obtaining oocytes after GnRHa is therefore very important when deciding whether to proceed with retrieval or administer a rescue hCG dose.

In a study including 508 cycles triggered with only GnRHa, Kummer et al. found that there were no clear serum predictors for oocyte yield, but post-trigger LH and progesterone strongly correlated with total oocytes and mature oocytes retrieved (54). The authors showed that all cases of EFS had an LH <15 IU/L and progesterone ≤3.5 ng/mL measured 8–12 hours after trigger. The probability of EFS occurring with a post-trigger LH less than 15 IU/L was 18.8%. A similar study evaluating post-trigger LH noted that an LH ≤15 IU/L resulted in a lower oocyte yield than cycles with a serum LH above 15 IU/L, but no differences in oocyte maturity (72). Candidates for a GnRHa trigger must be evaluated for the presence or possibility of hypothalamic dysfunction or amenorrhea. Meyer et al. examined risk factors for a low post-trigger LH ≤15 IU/L and found that patients with a suboptimal response were more likely to have low serum FSH and LH levels at the start of the cycle, low LH on the day of trigger, irregular menses at baseline, and were more likely to be on long-term oral contraception (33).

Post-trigger serum levels of LH and progesterone drawn approximately 12 hours after trigger can provide warning for a failed endogenous response to the trigger injection

Table 44.3 Trials demonstrating effect of gonadotropin-releasing hormone agonist (GnRHa) trigger on oocyte yield and maturation

	Oocyte yield		Oocyte maturation (%)	
Study	**GnRHa**	**hCG**	**GnRHa**	**hCG**
Fauser et al. (2002)	8.7 ± 4.5	8.3 ± 3.3	87 ± 17	86 ± 17
Humaidan et al. (2005)	8.4	9.7	84 ± 18	68.0 ± 22.0[a]
Kolibianakis et al. (2005)	10.2 ± 7.0	10.6 ± 6.3	73.5 ± 4.5	78.7 ± 3.3
Babayof et al. (2006)	19.8 ± 2.5	19.5 ± 1.9	89.4	84.1
Acevedo et al. (2006)	9.1 ± 4.0	10.3 ± 6.3	70	76
Engmann et al. (2008)	20.2 ± 9.9	18.8 ± 10.4	81.0 ± 16.3	83.8 ± 13.2
Galindo et al. (2009)	11.4 ± 6.4	12.0 ± 6.3	67.1 ± 20.9	67.2 ± 20.4
Melo et al. (2009)	17.1 ± 2.7	18.7 ± 3.1	75.4	78.6
Sismanoglu et al. (2009)	38.2 ± 14.5	36.6 ± 11.1	81.1	79.4
Papanikolaou et al. (2011)	11.7 ± 1.9	13.8 ± 1.8	67.5	60.1

[a] Findings statistically significantly different.
Abbreviation: hCG, human chorionic gonadotropin.

and intervention may be possible. If there is no LH surge and/or progesterone rise after GnRHa trigger, repeat trigger with hCG and oocyte retrieval 35 hours later have been shown to result in successful retrieval of oocytes (54). If there is a suboptimal LH rise with values less than 15 IU/L, repeat trigger with hCG can be given as soon as possible to proceed with retrieval as planned or the cycle may be cancelled. Alternatively, the patient can proceed with unilateral follicle aspiration and, if there are no oocytes, re-trigger with hCG and repeat oocyte retrieval of the contralateral ovary 34 hours later (64).

Addition of a standard or low-dose hCG to GnRHa trigger in a "dual trigger" protocol demonstrated an improvement in the number and proportion of mature oocytes (73), and has been adopted for wide use in some clinics to reduce the chances of EFS with GnRHa trigger (33). However, adjuvant hCG in addition to GnRHa trigger should be used with caution in patients at high risk of OHSS development.

LUTEAL-PHASE STEROID PROFILE AFTER NATURAL CYCLE AND GnRHa TRIGGER

In the luteal phase of a natural menstrual cycle, LH acts as a luteotropic hormone that supports the growth and function of the corpus luteum and steroidogenesis after ovulation (74). Luteal-phase LH increases growth factors and cytokines necessary for implantation, such as (VEGF-A) and fibroblast growth factor 2 (75,76). The circulating LH also promotes action via LH receptors located outside the ovary: in the endometrium, fallopian tubes, early fetal cells, placenta, and numerous other tissues. As a result, LH has many regulatory roles before and during pregnancy, including the synthesis of prostaglandins and tubal glycoproteins, stimulation of embryonic growth in the tube, and initiation and maintenance of pregnancy in the uterus (77).

In a natural cycle, if pregnancy does not occur and hCG is not available to continue to support the function of the corpus luteum, withdrawal of LH will result in luteolysis and then menses. In the setting of IVF, use of any trigger for oocyte maturation without luteal-phase support in an IVF cycle using a GnRH antagonist will significantly reduce the length of the luteal phase (16). The median duration of the luteal phase after GnRHa trigger may be as short as nine days compared to 13 days after hCG trigger (16). The duration of the LH surge after GnRHa trigger is short, with a median serum LH <2 IU/L on day 4 after trigger, and a shortened surge correlates with decreased production of progesterone throughout the luteal phase (3,16). Serum levels of progesterone and estrogen throughout the luteal phase are significantly lower with GnRHa trigger than after an hCG trigger (3,14,16).

The shortened duration of the LH surge after GnRHa trigger is enough to induce maturation of oocytes, but not sufficient to induce and maintain adequate corpora lutea to resemble a natural luteal phase (40,78,79). After the trigger, GnRHa may partially down-regulate the pituitary, continuing to inhibit the release of endogenous LH (80). By an additional mechanism common to most IVF protocols, supraphysiologic levels of progesterone and estrogen from ovarian stimulation also suppress LH release from the pituitary (16,81). All these factors together result in early luteolysis. Unfortunately, even if pregnancy does occur after GnRHa trigger, the luteolytic process is profound and significant enough that the corpora lutea cannot reliably be rescued by the time endogenous hCG from an implanting embryo is detected in the circulation (82). Nevo et al. measured levels of inhibin A and pro-αC, which are markers of corpus luteum function, and found that in the late luteal phase, the onset of pregnancy and the presence of hCG did not correlate with an increase in these corpora lutea markers (82). In fact, endometrial gene expression studies have shown significant alteration in gene expression after GnRHa trigger (83,84).

The above holds true in the normogonadotropic woman, but it should also be noted that the luteal phase of select patients may differ somewhat in a way that alters the hormonal milieu after GnRHa trigger. It is possible that polycystic ovary syndrome (PCOS) patients may have an elevated serum LH through both the follicular and luteal phases compared with normogonadotropic women; additionally, they may have decreased sensitivity at the hypothalamus to inhibition by ovarian steroids such as progesterone (85). These factors may contribute to a favorable response after GnRHa trigger and should be considered when discussing the luteal-phase steroid profile of the infertile and subfertile population.

STRATEGIES FOR MODIFYING THE LUTEAL PHASE AND PREGNANCY RATES

After early studies suggested that the luteal phase was suboptimal to achieve excellent clinical and ongoing pregnancy rates after GnRHa trigger (86), numerous strategies have been proposed to modify the standard luteal support in order to increase pregnancy rates after fresh embryo transfer without significantly increasing the risk for OHSS. These modifications include intensive exogenous luteal-phase steroid support and close monitoring of serum estrogen and progesterone levels (19,56,87,88), an adjuvant low dose of hCG given at the time of GnRHa trigger or at the time of retrieval (21,22,24,88–91), or luteal-phase recombinant LH administration (28).

Standard luteal support

As mentioned above, supraphysiologic levels of steroid hormones during a stimulated cycle provide negative feedback on the pituitary, resulting in a decrease in endogenous LH and the potential for early luteolysis (81). As a result, standard luteal support is generally given during IVF cycles. Standard luteal-phase support used after GnRHa may vary between centers, but may include a regimen of progesterone alone, or in combination with estrogen supplementation.

In the mid-2000s, a meta-analysis reviewed the outcomes after GnRHa trigger with the use of conventional luteal support. The review included three publications; two were stopped early due to significant differences in clinical

outcomes in favor of the hCG groups (14,15,20). Their luteal support protocols differed, but primarily involved vaginal micronized progesterone with or without oral estrogen starting after transfer and discontinued between the first positive pregnancy test and seven weeks of gestation. The meta-analysis revealed a clinical pregnancy rate of 7.9% in the GnRHa group compared to 30.14% in the hCG group (86). Early pregnancy loss rates were also noted to be higher than those of the hCG group (86).

Intensive luteal support

Knowing that the serum levels of estrogen and progesterone after GnRHa trigger decrease significantly, a strategy to improve the dysfunctional luteal phase includes a more intensive luteal-phase support protocol. This has been described as supplementation with both estrogen and progesterone in addition to close monitoring of serum steroid levels to adjust doses as necessary. The supplementation protocol that has been described by Engmann et al (19) in a randomized controlled study of 66 PCOS or high-responding patients begins with initiation on the day after retrieval of 50 mg intramuscular (i.m.) progesterone daily and three 0.1 mg estradiol (E2) transdermal patches replaced every other day (Figure 44.2). Serum levels of E2 and progesterone were evaluated on days 3 and 7 after oocyte retrieval and weekly thereafter, with continuation of i.m. progesterone and transdermal estrogen supplementation until approximately 10 weeks of gestational age. Based on serum levels, doses of i.m. progesterone were increased to a maximum of 75 mg daily, with the addition of micronized vaginal progesterone daily as needed to maintain serum progesterone above 20 ng/mL. Similarly, estrogen patches could be increased to four 0.1 mg patches every other day, with addition of oral micronized E2 (2–8 mg) daily to maintain serum E2 above 200 pg/mL (19). This study, which compared an intensive luteal-phase support after GnRHa trigger with standard luteal-phase support after hCG trigger, resulted in a 53% ongoing pregnancy rate, comparable to 48.3% in the hCG group.

These results have been corroborated by other investigators (56,90,92). Imbar et al. (56) described an intensive luteal support of 50 mg i.m. progesterone in oil as well as 6 mg oral E2 started on the day of retrieval and continued until 10 weeks of gestation. With 70 patients in the study arm, a clinical pregnancy rate of 37% and a live birth rate of 27.1% were found, comparable to patients who underwent cryopreservation with subsequent frozen–thaw embryo transfer. In a retrospective cohort study, Iliodromiti et al. noted equivalent live birth rates of 29% in both GnRHa and hCG groups (92). Shapiro et al. reported a 50% ongoing pregnancy rate in GnRHa trigger patients receiving enhanced luteal support, a significant improvement over women with agonist trigger alone and standard luteal support (25.3% ongoing pregnancy rate) and comparable to a 57.7% ongoing pregnancy rate in dual trigger patients, as described below (90).

The availability of i.m. progesterone is not universal and must be considered when planning to provide intensive luteal supplementation. In protocols utilizing an hCG trigger, studies suggest that there is no superiority of i.m. progesterone over vaginal progesterone (93,94); however, this may be essential after GnRHa trigger.

Adjuvant low-dose hCG

As it is the activity of LH in the luteal phase that supports steroidogenesis from the corpus luteum, a number of strategies have been described to restore or replace the function of LH in the luteal phase after use of a GnRHa trigger, often in addition to providing the luteal phase steroids exogenously. Any dose of hCG in addition to GnRHa trigger should be used cautiously since it may potentially increase the risk of OHSS development.

Dual trigger with hCG

The concept of dual trigger with low-dose hCG and GnRHa is to provide a small amount of hCG to help rescue the corpora lutea by providing the additional signal necessary for adequate luteinization. Shapiro et al. described a dual trigger protocol with an hCG dose $\leq$33 IU/kg body weight (ranging between 1000 and 2500 IU) with an ongoing pregnancy rate of 53.3% (89). The same group later published another similar study reporting a 57.7% ongoing

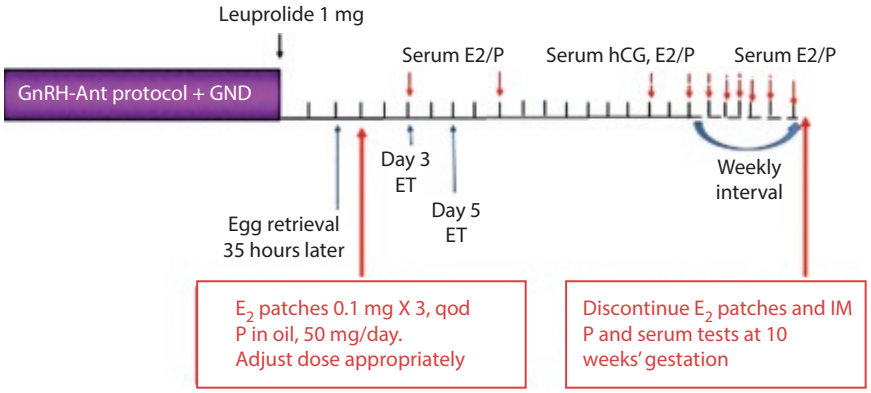

Figure 44.2 Components of intensive luteal-phase support. *Abbreviations*: GND, gonadotropins; E2, estradiol; P, progesterone; hCG, human chorionic gonadotropin; ET, embryo transfer; IM, intramuscular; GnRH-ant, gonadotropin-releasing hormone antagonist.

pregnancy rate with one case of clinically significant OHSS (90). In order to simplify the regimen and reduce the risk of OHSS, Griffin et al. recommended a standard low hCG dose of 1000 IU given with GnRHa trigger and intensive luteal steroid support. The live birth rate of 52.9% was significantly higher than the 30.9% rate noted after GnRHa trigger alone in patients with serum E2 <4000 pg/mL (21). The authors noted one case of mild OHSS in the dual trigger group versus no OHSS in the GnRHa-alone group (21). The added benefit of the dual trigger is to serve as a "back-up" in the case of GnRHa trigger failure (33).

Adjuvant hCG at time of oocyte retrieval

Humaidan and colleagues have described in multiple studies the use of a single bolus of 1500 IU hCG given on the day of oocyte retrieval, typically within one hour of retrieval, in addition to standard luteal-phase support (22–24). It has been previously shown that the granulosa/luteal cells are viable and able to respond to hCG on the day of retrieval (49). A randomized trial of 302 IVF cycles comparing one bolus of hCG 1500 IU after GnRHa trigger with hCG trigger showed no significant difference in delivery rates of 24% versus 31%, respectively (24). Large retrospective studies report clinical pregnancy rates of 41.8%–52.1% while maintaining low rates of severe OHSS (52,88). Radesic and Tremellen reported one case of severe OHSS among 71 women at high risk of OHSS receiving 1500 IU hCG within one hour after vaginal oocyte retrieval (52). Iliodromiti et al. reported two cases of severe OHSS out of 275 cycles using the same trigger protocol (92). However, Seyhan et al. evaluated 23 women at high risk of OHSS with mean E2 4891 pg/mL on the day of trigger who received GnRHa trigger and hCG 1500 IU administered within one hour of oocyte retrieval and reported a high severe OHSS rate of 26% (95).

Very low hCG dose

More recently, a very low dose of daily hCG has also been described, which resulted in good clinical pregnancy rates by rescuing corpora lutea function without the need for additional supplementation of progesterone or E2. Recombinant hCG 125 IU was given daily starting on either day 2 or day 6 of stimulation and continued daily throughout the luteal phase (96,97). This protocol, in a proof-of-concept study of normal responders, showed significantly higher luteal progesterone levels without exogenous supplementation compared with a standard luteal-phase protocol, and pregnancy outcomes were the same in the study arm versus the control arm using standard luteal support (97). Additional confirmatory studies are necessary before incorporating this approach into common practice. Very low doses of hCG are not currently commercially available in most countries.

Recombinant LH

When recombinant LH is available, this can also be considered for luteal-phase supplementation, perhaps with the benefit of a shorter half-life than hCG to further minimize OHSS risk. However, only one study has been published describing the dose and timing of its use in normal-responder patients. While comparable delivery rates were noted and there were no cases of OHSS compared to an hCG trigger control group, these findings have not been corroborated (28).

Luteal coasting

Using a similar strategy to coasting at the end of stimulation in high-responder patients, Kol et al. obtained pregnancies after fresh transfer through luteal coasting after trigger (98). In their case series of 21 high-responder patients, no luteal-phase steroid supplementation was provided unless monitored serum progesterone levels dropped significantly, at which time a bolus of 1500 IU hCG was administered (98). This approach individualizes the luteal supplementation, providing exogenous support when indicated and avoiding excessive stimulus when the risk for OHSS is elevated, but requires additional studies to confirm its efficacy.

Cycle segmentation: Cryopreservation of all oocytes or embryos

In an attempt to overcome the suboptimal luteal phase after GnRHa trigger, a freeze-all policy with transfer after thaw during a subsequent cycle has been proposed (99–103). Not only can segmentation of the IVF process avoid early- or late-onset OHSS in high responders, but implantation and pregnancy rates can also be optimized. Manzanares et al. reported a 33% pregnancy rate in PCOS patients with previous cycle cancelations after freezing all embryos with a subsequent thaw and transfer cycle (103). However, the study did not include a control group. Garcia-Velasco reported a 50% clinical pregnancy rate for patients at high risk for OHSS who opted to freeze all oocytes and undergo thaw and transfer of embryos in a subsequent natural cycle, compared to 29.5% in high-risk patients after coasting and fresh embryo transfer (100). The segmentation approach has become a feasible option in view of studies that have shown excellent pregnancy rates after freeze-all cycles. This approach must consider factors associated with the cost of additional frozen embryo transfer cycles and may be best suited for specific clinical situations.

Individualization of protocols to improve conception rates

In view of the different approaches that have been recommended by various researchers, it is important to develop an individualized approach to managing the luteal phase and optimizing conception rates without increasing the risk of OHSS development (Figure 44.3). Previous studies have attempted to determine the predictors of clinical outcomes in an attempt to formulate management guidelines that are tailored to a patient's response. One study found that the most important predictors of pregnancy success after GnRHa trigger and intensive luteal support were a peak E2 ≥4000 pg/mL and an elevated LH on the day of trigger (104), suggesting that the elevated LH at trigger

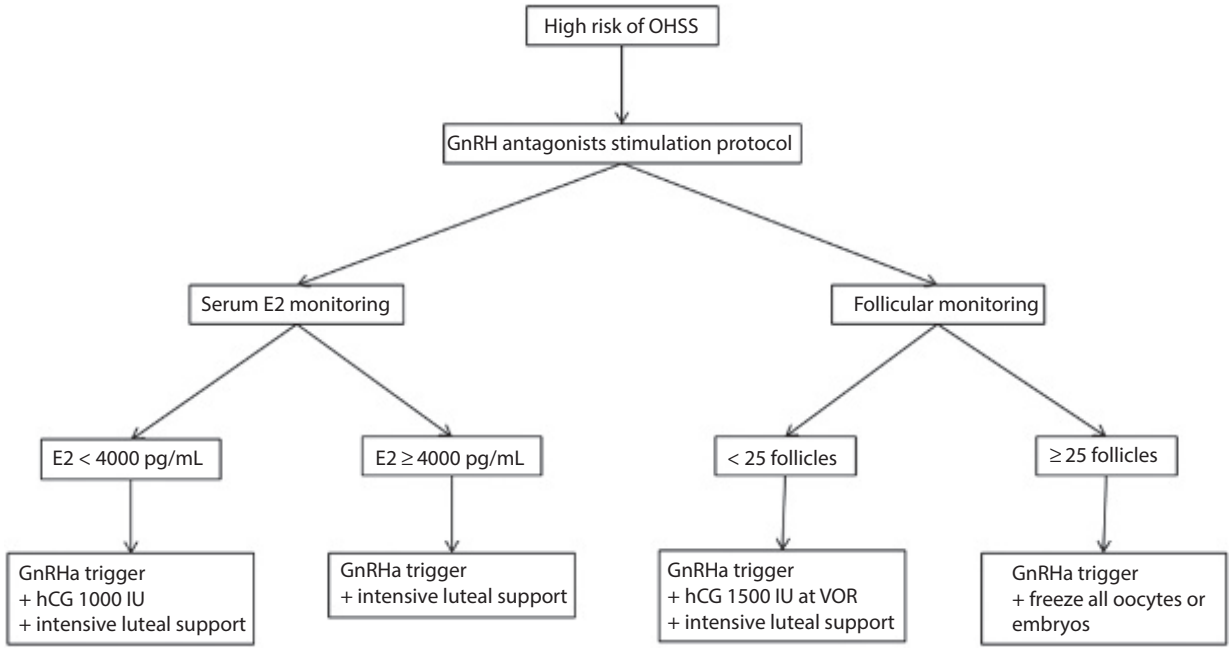

Figure 44.3 Suggested luteal-phase support protocols by high-responder characteristics. *Abbreviations*: OHSS, ovarian hyperstimulation syndrome; GnRH, gonadotropin-releasing hormone; E2, estradiol; GnRHa, gonadotropin-releasing hormone agonist; hCG, human chorionic gonadotropin; VOR, vaginal oocyte retrieval.

functions to rescue some corpora lutea and results in increased rates of conception. In that study, women with peak serum E2 of ≥4000 pg/mL had a significantly higher clinical pregnancy rate of 53.6% compared with 38.1% in women with peak E2 of <4000 pg/mL. A study by Griffin et al. showed that the use of a dual trigger GnRHa with low-dose hCG of 1000 IU results in a significantly higher live birth rate compared with GnRHa trigger alone in women with peak E2 of <4000 pg/mL (21).

For patients with a peak serum E2 of ≥4000 pg/mL, intensive luteal-phase supplementation with progesterone and E2 may be all that is necessary to optimize conception rates, or consider a 'freeze all' strategy. However, for women with a peak E2 less than this threshold (21), an adjuvant low dose of hCG may have an additional benefit on pregnancy rates.

The alternative criterion is the number of follicles on the day of trigger to determine whether to use an hCG bolus of 1500 IU on the day of retrieval or to freeze all oocytes/embryos (105). Seyhan et al. proposed that women with more than 18 follicles measuring between 10 and 14 mm should avoid hCG bolus and undergo oocyte/embryo cryopreservation based on a risk of severe OHSS of 26% after the use of 1500-IU bolus at the time of retrieval (95). Other studies have also suggested that women with more than 25 follicles greater than 11 mm in diameter should be considered for a "freeze-all" strategy in order to eliminate the risk of OHSS (91,106).

OVARIAN HYPERSTIMULATION SYNDROME

The short duration of the LH surge results in inadequate corpus luteum formation and early corpus luteum demise after GnRHa trigger, which has been shown to be effective in the prevention of OHSS.

Table 44.4 (107) lists various publications regarding OHSS rates after GnRHa trigger compared to hCG trigger. Overall, the elimination of OHSS is noted after GnRHa trigger (107), corroborated by a recent Cochrane review from Youssef et al. (108).

Despite the use of a GnRHa for trigger, there are still a few cases of moderate to severe OHSS that persist. Some of these cases result from the use of low-dose hCG supplementation in the luteal phase. However, some cases of OHSS after the use of GnRHa alone have been reported and require additional exploration, including mutations in the GnRH, FSH, or LH receptors, or variations in the genes for VEGF, its receptor, or other important vasoactive substances. Ling et al. described a case of early-onset severe OHSS occurring shortly after oocyte retrieval in a woman with an anti-Mullerian hormone level of 64.5 ng/mL who received a leuprorelin trigger as well as a freeze-all segmentation strategy (50). Fatemi et al. also described two cases of severe OHSS after GnRHa trigger alone without any adjuvant hCG and who did not have fresh embryo transfers (109). Activating mutations of the FSH receptor or the GnRH receptor could predispose patients to OHSS despite the use of GnRHa trigger (109).

USE OF GnRHa TRIGGER IN SPECIFIC CLINICAL SITUATIONS

Oocyte donation cycles

Several retrospective cohort and prospective randomized trials in donor oocyte cycles have shown no differences in the number of oocytes retrieved, proportion of mature

Table 44.4 Ovarian hyperstimulation syndrome (OHSS) incidence after gonadotropin-releasing hormone agonist triggering of final oocyte maturation versus human chorionic gonadotropin (hCG) triggering in published trials

Study	Study design	OHSS risk	Agonist trigger arm	hCG trigger arm
Fresh IVF cycles with ET				
Fauser 2002 (20)	RCT	Normal	0% (0/32)	0% (0/15)
Humaidan 2005 (14)	RCT	Normal	0% (0/55)	0% (0/67)
Kolibianakis 2005 (15)	RCT	Normal	0% (0/52)	0% (0/54)
Pirard 2006 (117)	RCT	Normal	0% (0/06)	0% (0/06)
Humaidan 2006 (23)	RCT	Normal	0% (0/13)	0% (0/15)
Babayof 2006 (55)	RCT	High	0% (0/15)	31.0% (4/13)
Engmann 2008 (19)	RCT	High	0% (0/33)	31.0% (10/32)
Humaidan 2010 (24)	RCT	Normal/high	0% (0/152)	2.0% (3/150)
Papanikolaou 2011 (28)	RCT	Normal	0% (0/17)	0% (0/18)
Donor IVF cycles (no ET)				
Acevedo 2006 (62)	RCT	Normal	0% (0/30)	17.0% (5/30)
Galindo 2011 (111)	RCT	Normal	0% (0/106)	8.5% (9/106)
Melo 2009 (27)	RCT	Very high	0% (0/50)	4.0% (2/50)
Sismanoglu 2009 (63)	RCT	Very high	0% (0/44)	6.8% (3/44)
Total embryo freezing (no ET)				
Griesinger 2007 (118)	Observational	Very high	0% (0/20)	—
Manzanares 2010 (103)	Observational	Very high	0% (0/42)	—

Source: From Humaidan P et al. *Fertil Steril* 2015; 103(4): 879–85, with permission.
Abbreviations: IVF, *in vitro* fertilization; ET, embryo transfer; RCT, randomized controlled trial.

oocytes, fertilization rates, implantation, pregnancy rates, and live birth rates between cycles resulting from GnRHa compared to hCG triggers (25,58,60,61,104,108,110). In recipient patients, pregnancy rates ranged from 38%–55% (compared to 38%–59% after hCG trigger), with a miscarriage rate of 15.4%–22.2% (27,62,111).

Use of the GnRHa trigger in oocyte donors with normal or high responses to ovarian stimulation has a clear advantage in the prevention of OHSS (17,27,112). Randomized clinical trials (27,62,108,111) as well as retrospective cohort studies (60,61,110) comparing GnRHa and hCG trigger have shown a clear advantage in reducing the risk of OHSS. Rates of OHSS in the hCG trigger arms ranged from 4.0% to 17.0% in a population of women undergoing elective controlled ovarian stimulation for the purpose of oocyte donation. The GnRHa trigger arms had no cases of moderate or severe OHSS out of 186 women reviewed by Youssef et al. (108).

Breast cancer patients

Patients diagnosed with estrogen receptor-positive breast cancer may elect to undergo cryopreservation of embryos or oocytes and may be good candidates for GnRHa trigger. A study by Oktay et al. found that, after stimulation with gonadotropins and an aromatase inhibitor to minimize systemic estrogen exposure, GnRHa trigger not only minimized the risk for OHSS such that patients could recover quickly after stimulation to proceed with cancer therapy, but GnRHa trigger resulted in significantly lower serum E2 levels in the luteal phase (31).

SAFETY OF GnRHa USE

When compared with an hCG trigger, maternal and neonatal outcomes are likely equivalent, but there is little published evidence. In a retrospective study, Budinetz et al. found no significant differences in the rate of congenital anomalies between GnRHa and hCG triggers (6.6% vs. 9.2%) (113). There were also no differences in maternal complications (27.6% vs. 20.8%) or minor or major neonatal complications (19.7% vs. 20.0%) between the GnRHa and hCG trigger groups (113).

OTHER ADVANTAGES

Multiple studies have reported improvements in patient comfort after GnRHa trigger compared to hCG (19,61,114). GnRHa trigger alters the undesirable characteristics common in the luteal phase, resulting in smaller ovarian volumes and decreased fluid in the pelvis, thus reducing abdominal bloating and pain. The duration of the uncomfortable luteal phase is also shortened with earlier menses, which can improve patient satisfaction, especially for oocyte donors and women who are not planning a fresh transfer (19,61,114).

GnRHa trigger in addition to a standard dose of hCG has the advantage of providing an additional option for patients with a history of immature oocyte or EFS after

hCG trigger. The dual surge of LH and FSH may have benefits in its resemblance to a natural cycle surge that could assist in strategies to prevent recurrent failed cycles (73,115,116).

CONCLUSION

The increasingly successful use of the GnRHa trigger has changed the practice and goals of ART. The Copenhagen GnRH Agonist Triggering Workshop Group meeting in 2009 has noted that with the remarkable prevention of OHSS after use of GnRHa trigger when appropriate, a new definition of success in ART should be the achievement of pregnancy, without OHSS, that results in a healthy singleton live birth at term (107). Additionally, reporting systems can be modified to incorporate OHSS in success rates, which could encourage practices to take additional steps to avoid OHSS, particularly among high responders such as women with PCOS, women undergoing elective cryopreservation, and oocyte donors, for whom safety is the primary concern.

REFERENCES

1. Emperaire JC. [Therapeutic induction of ovulation: Towards the replacement of hCG with LH]. *Contracept Fertil Sex* 1994; 22(7–8): 459–67.
2. Gonen Y, Balakier H, Powell W et al. Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for *in vitro* fertilization. *J Clin Endocrinol Metab* 1990; 71(4): 918–22.
3. Itskovitz J, Boldes R, Levron J et al. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. *Fertil Steril* 1991; 56(2): 213–20.
4. Itskovitz-Eldor J, Levron J, Kol S. Use of gonadotropin-releasing hormone agonist to cause ovulation and prevent the ovarian hyperstimulation syndrome. *Clin Obstet Gynecol* 1993; 36(3): 701–10.
5. Lanzone A, Fulghesu AM, Apa R et al. LH surge induction by GnRH agonist at the time of ovulation. *Gynecol Endocrinol* 1989; 3(3): 213–20.
6. Lewit N, Kol S, Manor D et al. Comparison of gonadotrophin-releasing hormone analogues and human chorionic gonadotrophin for the induction of ovulation and prevention of ovarian hyperstimulation syndrome: A case–control study. *Hum Reprod* 1996; 11(7): 1399–402.
7. Segal S, Casper RF. Gonadotropin-releasing hormone agonist versus human chorionic gonadotropin for triggering follicular maturation in *in vitro* fertilization. *Fertil Steril* 1992; 57(6): 1254–8.
8. van der Meer S, Gerris J, Joostens M et al. Triggering of ovulation using a gonadotrophin-releasing hormone agonist does not prevent ovarian hyperstimulation syndrome. *Hum Reprod* 1993; 8(10): 1628–31.
9. Albano C, Smitz J, Camus M et al. Comparison of different doses of gonadotropin-releasing hormone antagonist cetrorelix during controlled ovarian hyperstimulation. *Fertil Steril* 1997; 67(5): 917–22.
10. A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). The ganirelix dose-finding study group. *Hum Reprod* 1998; 13(11): 3023–31.
11. Itskovitz-Eldor J, Kol S, Mannaerts B. Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: Preliminary report: Short communication. *Hum Reprod* 2000; 15(9): 1965–8.
12. Reissmann T, Felberbaum R, Diedrich K et al. Development and applications of luteinizing hormone-releasing hormone antagonists in the treatment of infertility: An overview. *Hum Reprod* 1995; 10(8): 1974–81.
13. Porter RN, Smith W, Craft IL et al. Induction of ovulation for *in-vitro* fertilisation using buserelin and gonadotropins. *Lancet* 1984; 2(8414): 1284–5.
14. Humaidan P, Bredkjaer HE, Bungum L et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: A prospective randomized study. *Hum Reprod* 2005; 20(5): 1213–20.
15. Kolibianakis EM, Schultze-Mosgau A, Schroer A et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. *Hum Reprod* 2005; 20(10): 2887–92.
16. Beckers NG, Macklon NS, Eijkemans MJ et al. Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in *in vitro* fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. *J Clin Endocrinol Metab* 2003; 88(9): 4186–92.
17. Bodri D, Sunkara SK, Coomarasamy A. Gonadotropin-releasing hormone agonists versus antagonists for controlled ovarian hyperstimulation in oocyte donors: A systematic review and meta-analysis. *Fertil Steril* 2011; 95(1): 164–9.
18. DiLuigi AJ, Engmann L, Schmidt DW et al. Gonadotropin-releasing hormone agonist to induce final oocyte maturation prevents the development of ovarian hyperstimulation syndrome in high-risk patients and leads to improved clinical outcomes compared with coasting. *Fertil Steril* 2010; 94(3): 1111–4.
19. Engmann L, DiLuigi A, Schmidt D et al. The use of gonadotropin-releasing hormone (GnRH) agonist

to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing *in vitro* fertilization prevents the risk of ovarian hyperstimulation syndrome: A prospective randomized controlled study. *Fertil Steril* 2008; 89(1): 84–91.
20. Fauser BC, de Jong D, Olivennes F et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for *in vitro* fertilization. *J Clin Endocrinol Metab* 2002; 87(2): 709–15.
21. Griffin D, Benadiva C, Kummer N et al. Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders. *Fertil Steril* 2012; 97(6): 1316–20.
22. Humaidan P. Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose HCG: A pilot study. *Reprod Biomed Online* 2009; 18(5): 630–4.
23. Humaidan P, Bungum L, Bungum M et al. Rescue of corpus luteum function with peri-ovulatory HCG supplementation in IVF/ICSI GnRH antagonist cycles in which ovulation was triggered with a GnRH agonist: A pilot study. *Reprod Biomed Online* 2006; 13(2): 173–8.
24. Humaidan P, Ejdrup Bredkjaer H, Westergaard LG et al. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: A prospective, randomized, controlled study. *Fertil Steril* 2010; 93(3): 847–54.
25. Humaidan P, Polyzos NP, Alsbjerg B et al. GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation: Two prospective randomized controlled multi-centre studies in IVF patients. *Hum Reprod* 2013; 28(9): 2511–21.
26. Kol S, Humaidan P, Itskovitz-Eldor J. GnRH agonist ovulation trigger and hCG-based, progesterone-free luteal support: A proof of concept study. *Hum Reprod* 2011; 26(10): 2874–7.
27. Melo M, Busso CE, Bellver J et al. GnRH agonist versus recombinant HCG in an oocyte donation programme: A randomized, prospective, controlled, assessor-blind study. *Reprod Biomed Online* 2009; 19(4): 486–92.
28. Papanikolaou EG, Verpoest W, Fatemi H et al. A novel method of luteal supplementation with recombinant luteinizing hormone when a gonadotropin-releasing hormone agonist is used instead of human chorionic gonadotropin for ovulation triggering: A randomized prospective proof of concept study. *Fertil Steril* 2011; 95(3): 1174–7.
29. Shapiro BS, Daneshmand ST, Restrepo H et al. Efficacy of induced luteinizing hormone surge after "trigger" with gonadotropin-releasing hormone agonist. *Fertil Steril* 2011; 95(2): 826–8.
30. Shapiro BS, Daneshmand ST, Garner FC et al. Embryo cryopreservation rescues cycles with premature luteinization. *Fertil Steril* 2010; 93(2): 636–41.
31. Oktay K, Turkcuoglu I, Rodriguez-Wallberg KA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. *Reprod Biomed Online* 2010; 20(6): 783–8.
32. Reddy J, Turan V, Bedoschi G et al. Triggering final oocyte maturation with gonadotropin-releasing hormone agonist (GnRHa) versus human chorionic gonadotropin (hCG) in breast cancer patients undergoing fertility preservation: An extended experience. *J Assist Reprod Genet* 2014; 31(7): 927–32.
33. Meyer L, Murphy LA, Gumer A et al. Risk factors for a suboptimal response to gonadotropin-releasing hormone agonist trigger during *in vitro* fertilization cycles. *Fertil Steril* 2015; 104(3): 637–42.
34. Nakano R, Mizuno T, Kotsuji F et al. "Triggering" of ovulation after infusion of synthetic luteinizing hormone releasing factor (LRF). *Acta Obstet Gynecol Scand* 1973; 52(3): 269–72.
35. Yding Andersen C. Effect of FSH and its different isoforms on maturation of oocytes from pre-ovulatory follicles. *Reprod Biomed Online* 2002; 5(3): 232–9.
36. Zelinski-Wooten MB, Hutchison JS, Hess DL et al. Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. *Hum Reprod* 1995; 10(7): 1658–66.
37. Eppig JJ. FSH stimulates hyaluronic acid synthesis by oocyte-cumulus cell complexes from mouse preovulatory follicles. *Nature* 1979; 281(5731): 483–4.
38. Richards JS, Jahnsen T, Hedin L et al. Ovarian follicular development: From physiology to molecular biology. *Recent Prog Horm Res* 1987; 43: 231–76.
39. Hoff JD, Quigley ME, Yen SS. Hormonal dynamics at midcycle: A reevaluation. *J Clin Endocrinol Metab* 1983; 57(4): 792–6.
40. Chandrasekher YA, Brenner RM, Molskness TA et al. Titrating luteinizing hormone surge requirements for ovulatory changes in primate follicles. II. Progesterone receptor expression in luteinizing granulosa cells. *J Clin Endocrinol Metab* 1991; 73(3): 584–9.
41. Yding Andersen C, Westergaard LG, Figenschau Y et al. Endocrine composition of follicular fluid comparing human chorionic gonadotrophin to a gonadotrophin-releasing hormone agonist for ovulation induction. *Hum Reprod* 1993; 8(6): 840–3.
42. Andersen CY, Humaidan P, Ejdrup HB et al. Hormonal characteristics of follicular fluid from women receiving either GnRH agonist or hCG for ovulation induction. *Hum Reprod* 2006; 21(8): 2126–30.
43. Park JY, Su YQ, Ariga M et al. EGF-like growth factors as mediators of LH action in the ovulatory follicle. *Science* 2004; 303(5658): 682–4.

44. Humaidan P, Westergaard, LG, Mikkelsen AL, Fukuda M, Yding Andersen C. Levels of the EGF-like peptide amphiregulin are significantly reduced in follicular fluid after GnRHa triggering of final oocyte maturation. *Hum Reprod* 2009; 24: O-284.
45. Cerrillo M, Rodriguez S, Mayoral M et al. Differential regulation of VEGF after final oocyte maturation with GnRH agonist versus hCG: A rationale for OHSS reduction. *Fertil Steril* 2009; 91(4 Suppl): 1526–8.
46. Villasante A, Pacheco A, Ruiz A et al. Vascular endothelial cadherin regulates vascular permeability: Implications for ovarian hyperstimulation syndrome. *J Clin Endocrinol Metab* 2007; 92(1): 314–21.
47. Cerrillo M, Pacheco A, Rodriguez S et al. Effect of GnRH agonist and hCG treatment on VEGF, angiopoietin-2, and VE-cadherin: Trying to explain the link to ovarian hyperstimulation syndrome. *Fertil Steril* 2011; 95(8): 2517–9.
48. Miller I, Chuderland D, Ron-El R et al. GnRH agonist triggering modulates PEDF to VEGF ratio inversely to hCG in granulosa cells. *J Clin Endocrinol Metab* 2015; 100(11): E1428–36.
49. Engmann L, Romak J, Nulsen J et al. *In vitro* viability and secretory capacity of human luteinized granulosa cells after gonadotropin-releasing hormone agonist trigger of oocyte maturation. *Fertil Steril* 2011; 96(1): 198–202.
50. Ling LP, Phoon JW, Lau MS et al. GnRH agonist trigger and ovarian hyperstimulation syndrome: Relook at "freeze-all strategy". *Reprod Biomed Online* 2014; 29(3): 392–4.
51. Castillo JC, Dolz M, Bienvenido E et al. Cycles triggered with GnRH agonist: Exploring low-dose HCG for luteal support. *Reprod Biomed Online* 2010; 20(2): 175–81.
52. Radesic B, Tremellen K. Oocyte maturation employing a GnRH agonist in combination with low-dose hCG luteal rescue minimizes the severity of ovarian hyperstimulation syndrome while maintaining excellent pregnancy rates. *Hum Reprod* 2011; 26(12): 3437–42.
53. Parneix I, Emperaire JC, Ruffie A et al. [Comparison of different protocols of ovulation induction, by GnRH agonists and chorionic gonadotropin]. *Gynecol Obstet Fertil* 2001; 29(2): 100–5.
54. Kummer NE, Feinn RS, Griffin DW et al. Predicting successful induction of oocyte maturation after gonadotropin-releasing hormone agonist (GnRHa) trigger. *Hum Reprod* 2013; 28(1): 152–9.
55. Babayof R, Margalioth EJ, Huleihel M et al. Serum inhibin A, VEGF and TNFalpha levels after triggering oocyte maturation with GnRH agonist compared with HCG in women with polycystic ovaries undergoing IVF treatment: A prospective randomized trial. *Hum Reprod* 2006; 21(5): 1260–5.
56. Imbar T, Kol S, Lossos F et al. Reproductive outcome of fresh or frozen–thawed embryo transfer is similar in high-risk patients for ovarian hyperstimulation syndrome using GnRH agonist for final oocyte maturation and intensive luteal support. *Hum Reprod* 2012; 27(3): 753–9.
57. Vuong TN, Ho MT, Ha TD et al. Gonadotropin-releasing hormone agonist trigger in oocyte donors co-treated with a gonadotropin-releasing hormone antagonist: A dose-finding study. *Fertil Steril* 2016; 105(2): 356–63.
58. Buckett WM, Bentick B, Shaw RW. Induction of the endogenous gonadotrophin surge for oocyte maturation with intra-nasal gonadotrophin-releasing hormone analogue (buserelin): Effective minimal dose. *Hum Reprod* 1998; 13(4): 811–4.
59. Oktay K, Buyuk E, Rodriguez-Wallberg KA et al. *In vitro* maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. *Reprod Biomed Online* 2010; 20(5): 634–8.
60. Hernandez ER, Gomez-Palomares JL, Ricciarelli E. No room for cancellation, coasting, or ovarian hyperstimulation syndrome in oocyte donation cycles. *Fertil Steril* 2009; 91(4 Suppl): 1358–61.
61. Bodri D, Guillen JJ, Galindo A et al. Triggering with human chorionic gonadotropin or a gonadotropin-releasing hormone agonist in gonadotropin-releasing hormone antagonist-treated oocyte donor cycles: Findings of a large retrospective cohort study. *Fertil Steril* 2009; 91(2): 365–71.
62. Acevedo B, Gomez-Palomares JL, Ricciarelli E et al. Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates. *Fertil Steril* 2006; 86(6): 1682–7.
63. Sismanoglu A, Tekin HI, Erden HF et al. Ovulation triggering with GnRH agonist vs. hCG in the same egg donor population undergoing donor oocyte cycles with GnRH antagonist: A prospective randomized cross-over trial. *J Assist Reprod Genet* 2009; 26(5): 251–6.
64. Asada Y, Itoi F, Honnma H et al. Failure of GnRH agonist-triggered oocyte maturation: Its cause and management. *J Assist Reprod Genet* 2013; 30(4): 581–5.
65. Castillo JC, Garcia-Velasco J, Humaidan P. Empty follicle syndrome after GnRHa triggering versus hCG triggering in COS. *J Assist Reprod Genet* 2012; 29(3): 249–53.
66. Baum M, Machtinger R, Yerushalmi GM et al. Recurrence of empty follicle syndrome with stimulated IVF cycles. *Gynecol Endocrinol* 2012; 28(4): 293–5.
67. Ben-Shlomo I, Schiff E, Levran D et al. Failure of oocyte retrieval during *in vitro* fertilization: A sporadic event rather than a syndrome. *Fertil Steril* 1991; 55(2): 324–7.
68. Mesen TB, Yu B, Richter KS et al. The prevalence of genuine empty follicle syndrome. *Fertil Steril* 2011; 96(6): 1375–7.

69. Quintans CJ, Donaldson MJ, Blanco LA et al. Empty follicle syndrome due to human errors: Its occurrence in an in-vitro fertilization programme. *Hum Reprod* 1998; 13(1O): 2703–5.
70. Zegers-Hochschild F, Fernandez E, Mackenna A et al. The empty follicle syndrome: A pharmaceutical industry syndrome. *Hum Reprod* 1995; 10(9): 2262–5.
71. IVF Worldwide. Survey on vitrification, GnRH trigger and differed embryo transfer. 2013. http://www.ivf-worldwide.com/survey/vitrification,-gnrh-trigger-and-differed-et/results-vitrification,-gnrh-trigger-and-differed-et.html
72. Chen SL, Ye DS, Chen X et al. Circulating luteinizing hormone level after triggering oocyte maturation with GnRH agonist may predict oocyte yield in flexible GnRH antagonist protocol. *Hum Reprod* 2012; 27(5): 1351–6.
73. Griffin D, Feinn R, Engmann L et al. Dual trigger with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin to improve oocyte maturity rates. *Fertil Steril* 2014; 102(2): 405–9.
74. McCracken JA, Custer EE, Lamsa JC. Luteolysis: A neuroendocrine-mediated event. *Physiol Rev* 1999; 79(2): 263–323.
75. Wang TH, Horng SG, Chang CL et al. Human chorionic gonadotropin-induced ovarian hyperstimulation syndrome is associated with up-regulation of vascular endothelial growth factor. *J Clin Endocrinol Metab* 2002; 87(7): 3300–8.
76. Sugino N, Kashida S, Takiguchi S et al. Expression of vascular endothelial growth factor and its receptors in the human corpus luteum during the menstrual cycle and in early pregnancy. *J Clin Endocrinol Metab* 2000; 85(10): 3919–24.
77. Rao CV. Multiple novel roles of luteinizing hormone. *Fertil Steril* 2001; 76(6): 1097–100.
78. Zelinski-Wooten MB, Lanzendorf SE, Wolf DP et al. Titrating luteinizing hormone surge requirements for ovulatory changes in primate follicles. I. Oocyte maturation and corpus luteum function. *J Clin Endocrinol Metab* 1991; 73(3): 577–83.
79. Zelinski-Wooten MB, Hutchison JS, Chandrasekher YA et al. Administration of human luteinizing hormone (hLH) to macaques after follicular development: Further titration of LH surge requirements for ovulatory changes in primate follicles. *J Clin Endocrinol Metab* 1992; 75(2): 502–7.
80. Kol S. Luteolysis induced by a gonadotropin-releasing hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. *Fertil Steril* 2004; 81(1): 1–5.
81. Fauser BC, Devroey P. Reproductive biology and IVF: Ovarian stimulation and luteal phase consequences. *Trends Endocrinol Metab* 2003; 14(5): 236–42.
82. Nevo O, Eldar-Geva T, Kol S et al. Lower levels of inhibin A and pro-alphaC during the luteal phase after triggering oocyte maturation with a gonadotropin-releasing hormone agonist versus human chorionic gonadotropin. *Fertil Steril* 2003; 79(5): 1123–8.
83. Bermejo A, Cerrillo M, Ruiz-Alonso M et al. Impact of final oocyte maturation using gonadotropin-releasing hormone agonist triggering and different luteal support protocols on endometrial gene expression. *Fertil Steril* 2014; 101(1): 138–46.e3.
84. Humaidan P, Van Vaerenbergh I, Bourgain C et al. Endometrial gene expression in the early luteal phase is impacted by mode of triggering final oocyte maturation in recFSH stimulated and GnRH antagonist co-treated IVF cycles. *Hum Reprod* 2012; 27(11): 3259–72.
85. McCartney CR, Eagleson CA, Marshall JC. Regulation of gonadotropin secretion: Implications for polycystic ovary syndrome. *Semin Reprod Med* 2002; 20(4): 317–26.
86. Griesinger G, Diedrich K, Devroey P et al. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: A systematic review and meta-analysis. *Hum Reprod Update* 2006; 12(2): 159–68.
87. Engmann L, Siano L, Schmidt D et al. GnRH agonist to induce oocyte maturation during IVF in patients at high risk of OHSS. *Reprod Biomed Online* 2006; 13(5): 639–44.
88. Iliodromiti S, Blockeel C, Tremellen KP et al. Consistent high clinical pregnancy rates and low ovarian hyperstimulation syndrome rates in high-risk patients after GnRH agonist triggering and modified luteal support: A retrospective multicentre study. *Hum Reprod* 2013; 28(9): 2529–36.
89. Shapiro BS, Daneshmand ST, Garner FC et al. Gonadotropin-releasing hormone agonist combined with a reduced dose of human chorionic gonadotropin for final oocyte maturation in fresh autologous cycles of *in vitro* fertilization. *Fertil Steril* 2008; 90(1): 231–3.
90. Shapiro BS, Daneshmand ST, Garner FC et al. Comparison of "triggers" using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. *Fertil Steril* 2011; 95(8): 2715–7.
91. Humaidan P, Thomsen LH, Alsbjerg B. GnRHa trigger and modified luteal support with one bolus of hCG should be used with caution in extreme responder patients. *Hum Reprod* 2013; 28(9): 2593–4.
92. Iliodromiti S, Lan VT, Tuong HM et al. Impact of GnRH agonist triggering and intensive luteal steroid support on live-birth rates and ovarian hyperstimulation syndrome: A retrospective cohort study. *J Ovarian Res* 2013; 6(1): 93.
93. Fatemi HM. The luteal phase after 3 decades of IVF: What do we know? *Reprod Biomed Online* 2009; 19(Suppl 4): 4331.
94. Mitwally MF, Diamond MP, Abuzeid M. Vaginal micronized progesterone versus intramuscular progesterone for luteal support in women undergoing *in vitro* fertilization-embryo transfer. *Fertil Steril* 2010; 93(2): 554–69.

95. Seyhan A, Ata B, Polat M et al. Severe early ovarian hyperstimulation syndrome following GnRH agonist trigger with the addition of 1500 IU hCG. *Hum Reprod* 2013; 28(9): 2522–8.
96. Elbaek HO AB, Laursen R, Povlsen BB, Mikkelsen AT, Andersen CY, Humaidan P. The exogenous progesterone free luteal phase in IVF—Exploring a new concept. *Hum Reprod* 2014; 29(i326): P–502.
97. Andersen CY, Elbaek HO, Alsbjerg B et al. Daily low-dose hCG stimulation during the luteal phase combined with GnRHa triggered IVF cycles without exogenous progesterone: A proof of concept trial. *Hum Reprod* 2015; 30(10): 2387–95.
98. Kol S, Breyzman T, Segal L et al. "Luteal coasting" after GnRH agonist trigger—Individualized, HCG-based, progesterone-free luteal support in "high responders": A case series. *Reprod Biomed Online* 2015; 31(6): 747–51.
99. Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. *Hum Reprod* 2011; 26(10): 2593–7.
100. Garcia-Velasco JA. Agonist trigger: What is the best approach? Agonist trigger with vitrification of oocytes or embryos. *Fertil Steril* 2012; 97(3): 527–8.
101. Griesinger G, Berndt H, Schultz L et al. Cumulative live birth rates after GnRH-agonist triggering of final oocyte maturation in patients at risk of OHSS: A prospective, clinical cohort study. *Eur J Obstet Gynecol Reprod Biol* 2010; 149(2): 190–4.
102. Griesinger G, Schultz L, Bauer T et al. Ovarian hyperstimulation syndrome prevention by gonadotropin-releasing hormone agonist triggering of final oocyte maturation in a gonadotropin-releasing hormone antagonist protocol in combination with a "freeze-all" strategy: A prospective multicentric study. *Fertil Steril* 2011; 95(6): 2029–33, 33 e1.
103. Manzanares MA, Gomez-Palomares JL, Ricciarelli E et al. Triggering ovulation with gonadotropin-releasing hormone agonist in *in vitro* fertilization patients with polycystic ovaries does not cause ovarian hyperstimulation syndrome despite very high estradiol levels. *Fertil Steril* 2010; 93(4): 1215–9.
104. Kummer N, Benadiva C, Feinn R et al. Factors that predict the probability of a successful clinical outcome after induction of oocyte maturation with a gonadotropin-releasing hormone agonist. *Fertil Steril* 2011; 96(1): 63–8.
105. Bodri D. Low-dose hCG supplementation after GnRH agonist triggering: Don't be too quick on the trigger. *Hum Reprod* 2013; 28(9): 2315–7.
106. Humaidan P, Engmann L, Benadiva C. Luteal phase supplementation after gonadotropin-releasing hormone agonist trigger in fresh embryo transfer: The American versus European approaches. *Fertil Steril* 2015; 103(4): 879–85.
107. Humaidan P, Kol S, Papanikolaou EG et al. GnRH agonist for triggering of final oocyte maturation: Time for a change of practice? *Hum Reprod Update* 2011; 17(4): 510–24.
108. Youssef MA, Van der Veen F, Al-Inany HG et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. *Cochrane Database Syst Rev* 2014; 10: CD008046.
109. Fatemi HM, Popovic-Todorovic B, Humaidan P et al. Severe ovarian hyperstimulation syndrome after gonadotropin-releasing hormone (GnRH) agonist trigger and "freeze-all" approach in GnRH antagonist protocol. *Fertil Steril* 2014; 101(4): 1008–11.
110. Shapiro BS, Daneshmand ST, Garner FC et al. Comparison of human chorionic gonadotropin and gonadotropin-releasing hormone agonist for final oocyte maturation in oocyte donor cycles. *Fertil Steril* 2007; 88(1): 237–9.
111. Galindo A, Bodri D, Guillen JJ et al. Triggering with hCG or GnRH agonist in GnRH antagonist treated oocyte donation cycles: A randomised clinical trial. *Gynecol Endocrinol* 2009; 25(1): 60–6.
112. Bodri D, Guillen JJ, Trullenque M et al. Early ovarian hyperstimulation syndrome is completely prevented by gonadotropin releasing-hormone agonist triggering in high-risk oocyte donor cycles: A prospective, luteal-phase follow-up study. *Fertil Steril* 2010; 93(7): 2418–20.
113. Budinetz TH, Mann JS, Griffin DW et al. Maternal and neonatal outcomes after gonadotropin-releasing hormone agonist trigger for final oocyte maturation in patients undergoing *in vitro* fertilization. *Fertil Steril* 2014; 102(3): 753–8.
114. Garcia-Velasco JA, Motta L, Lopez A et al. Low-dose human chorionic gonadotropin versus estradiol/progesterone luteal phase support in gonadotropin-releasing hormone agonist-triggered assisted reproductive technique cycles: Understanding a new approach. *Fertil Steril* 2010; 94(7): 2820–3.
115. Castillo JC MJ, Dolz M, Bonilla-Musoles F. Successful pregnancy following dual triggering concept (rhCG & GnRH agonist) in a patient showing repetitive immature oocytes and empty follicle syndrome: Case report. *J Med Cases* 2013; 5: 221–6.
116. Lok F, Pritchard J, Lashen H. Successful treatment of empty follicle syndrome by triggering endogenous LH surge using GnRH agonist in an antagonist down-regulated IVF cycle. *Hum Reprod* 2003; 18(10): 2079–81.
117. Pirard X, Donnez J, Loumaye E. GnRH agonist as luteal phase support in assisted reproduction technique cycles: Results of a pilot study. *Hum Reprod* 2006; 21(7): 1894–900.
118. Griesinger G, Kolibianakis EM, Papanikolaou EG, Diedrich K, Van Steirteghem A, Devroey P, Ejdrup Bredkjaer H, Humaidan P. Triggering of final oocyte maturation with gonadotropin-releasing hormone agonist or human chorionic gonadotropin. Live birth after frozen–thawed embryo replacement cycles. *Fertil Steril* 2007; 88(3): 616–21.

Segmentation of *in vitro* fertilization treatment

45

BRUCE SHAPIRO, CARRIE E. BEDIENT, and FOREST GARNER

INTRODUCTION

Traditional *in vitro* fertilization (IVF), in which fresh embryos are transferred to the patient's uterus following controlled ovarian stimulation (COS), is limited by a temporally inflexible relationship between embryonic and uterine development. COS results in supraphysiologic hormone levels that may accelerate uterine histological development and impair uterine receptivity by inducing embryo–endometrium asynchrony, particularly when embryos develop slowly. Additionally, genetic analyses of embryos sometimes require a delay that might preclude fresh transfer, necessitating cryopreservation. Furthermore, fresh autologous embryo transfer is associated with increased risk of ovarian hyperstimulation syndrome (OHSS) and also with certain increased perinatal risks. For these reasons, it is becoming increasingly common to "segment" the IVF cycle by temporally separating the transfer from the retrieval through embryo or oocyte cryopreservation.

TRADITIONAL IVF

By far the most common type of IVF cycle performed to date has been the fresh autologous cycle. For reporting year 2013, the U.S. Centers for Disease Control and Prevention reported 73,571 fresh autologous embryo transfers, 46,779 autologous transfers of thawed embryos, 8597 fresh transfers of fresh embryos derived from oocyte donation, and 9499 transfers of thawed embryos derived from donor oocytes (1).

In the fresh autologous cycle, the patient typically undergoes COS, has oocytes collected and inseminated, and one or more resulting embryos are transferred to her uterus shortly after oocyte retrieval to achieve implantation and subsequent live birth. However, there are potential obstacles. Successful embryo implantation in IVF cycles requires a viable embryo transferred into a synchronous, receptive uterine environment.

About half of human fertilized oocytes do not develop to the blastocyst stage, and those that do form blastocysts are often genetically abnormal. A recent retrospective study found that 6168 (40.7%) of 15,169 biopsied expanded blastocysts had aneuploid test results (2). Therefore, the probability of live birth with a single collected oocyte is low. For this reason, COS with exogenous follicle-stimulating hormone (FSH) is routinely used so that many follicles may develop and many eggs can be collected. COS increases the probability of obtaining at least one viable embryo, therefore increasing IVF success rates when compared to non-stimulated IVF cycles. However, there are potential risks of COS.

Even with COS, many of the embryos generated are non-viable. One method to increase the probability that a transferred embryo will be viable is preimplantation genetic screening (PGS). With PGS, one or more cells are removed from each embryo so that embryo ploidy may be assessed. The ploidy of each embryo is used to decide which embryos are eligible for transfer. Techniques for embryo biopsy and genetic testing have recently advanced, and it appears that the recent techniques increase the probability of selecting a viable embryo that will implant compared to selection based on embryo morphology alone. One recent randomized trial compared 72 blastocyst transfers after PGS to 83 blastocyst transfers in controls without PGS and found a sustained implantation rate (live birth rate per transferred embryo) of 66.4% with PGS and 47.9% in controls (3).

Genetic analysis of the biopsied tissue can require several hours and the biopsies are typically shipped overnight to a laboratory for analysis. Therefore, the transfer of biopsied embryos is typically delayed by one day or longer, depending on techniques and circumstances. The resulting delay may be suboptimal for various reasons, and many centers have elected to cryopreserve the embryos while genetic test results are obtained and used to form decisions.

DRAWBACKS OF TRADITIONAL IVF

Impaired endometrial receptivity

During COS, supraphysiologic numbers of developing follicles significantly alter the hormonal milieu. Enhanced follicular development results in supraphysiologic levels of estradiol and other hormones. Supraphysiologic estradiol and progesterone levels also follow the ovulatory trigger. Estradiol and progesterone are known to control endometrial development. Endometrial histology is advanced by approximately one to two days after COS, when compared to natural cycles, and this advancement is correlated with premature progesterone elevation (4).

Endometrial advancement following COS is a potential source of implantation failure following fresh autologous embryo transfer. Under this hypothesis, viable but slowly developing transferred embryos fail to implant because the endometrial receptive phase might expire before the embryo is able to initiate viable trophoblast invasion. This effect is demonstrated by day-5 blastocysts implanting more readily than day-6 blastocysts in fresh autologous

cycles, but not in donor or frozen–thawed embryo cycles (5). In addition, frozen–thawed day-6 blastocysts implant more readily than do fresh day-6 blastocysts (5–8). A randomized trial found greater implantation and clinical pregnancy rates per transfer in normal-responder patients who had entire cohorts of bi-pronuclear oocytes cryopreserved for subsequent thaw and transfer of two resulting blastocysts compared to patients who had two fresh blastocysts transferred (9). That study focused only on endometrial receptivity and therefore only on cycles receiving an embryo transfer.

A subsequent meta-analysis of three randomized trials compared ongoing pregnancy rate per randomized subject through the first transfer attempt, and reported a risk ratio of 1.32 (95% confidence interval: 1.10–1.59) in favor of embryo cryopreservation and subsequent transfer in a later cycle (10). However, since that report was published, one of those three randomized trials was withdrawn.

Two subsequent comparisons of fresh and frozen–thawed embryo transfers, matched on embryo quality and patient parameters, reported that embryos transferred in thaw cycles were more likely to implant than embryos of matched quality in fresh transfers (11,12). One of those studies (11) featured a sub-analysis that found that the difference could be explained by day-6 blastocysts having much greater implantation rates in thaw transfers than in fresh transfers, while the day-5 blastocysts were roughly comparable between thaw and fresh transfers.

A meta-analysis confirmed that premature progesterone elevation is associated with reduced IVF success rates (13). However, premature progesterone elevation is not associated with a detrimental effect in oocyte donation cycles (14), suggesting that the detrimental effect is through impaired endometrial receptivity, and not a detrimental effect on the embryo cohort. This hypothesis has been confirmed by genomic analysis (15). Embryo cryopreservation circumvents the effect of premature progesterone elevation in autologous patients (16), and premature progesterone elevation in the stimulated cycle is even predictive of success in subsequent thaw cycles (17). The impaired endometrial receptivity in fresh autologous transfers following premature progesterone elevation is largely mitigated in cycles with excellent embryo cohorts (18), apparently due to rapidly developing embryos compensating for advanced endometrial development (8).

Ovarian hyperstimulation syndrome

Another risk associated with COS is OHSS. OHSS can occur when a large number of developing follicles are exposed to prolonged luteinizing hormone (LH) activity, such as when human chorionic gonadotropin (hCG) is used for final oocyte maturation. Risk factors for OHSS include a large number of developing follicles, extremely elevated estradiol level during stimulation, young age, a history of OHSS, and exposure to hCG for the ovulatory "trigger" (19).

A common and effective method to reduce OHSS risk is to use a gonadotropin-releasing hormone (GnRH) agonist instead of hCG for final oocyte maturation (20). This technique does not work in cycles continuing daily GnRH agonist for down-regulation, and therefore it is best to use GnRH antagonists for pituitary suppression in patients at risk of OHSS so that the agonist trigger option is maintained. The agonist "trigger" almost eliminates OHSS risk through the much more rapid clearance of LH than hCG, and subsequent greatly abbreviated exposure to LH activity. In the absence of LH activity, rapid, complete, and irreversible luteolysis typically terminates follicular production of numerous hormones, including those presumed to be on the causal pathway of OHSS (21).

The GnRH agonist trigger is associated with success rates comparable to hCG trigger in oocyte donation cycles (22). Unfortunately, reduced live birth rates have been reported with fresh autologous embryo transfer following agonist trigger (23). This might be largely addressed in very high responders by applying intensive luteal support (24,25).

An alternative to agonist-only trigger is to administer GnRH agonist as a "dual trigger" in combination with low-dose hCG, or else to follow the agonist trigger 36 hours later with a low dose of hCG for luteal rescue. The dual trigger of agonist and low-dose hCG has been reported to have a 53%–59% ongoing pregnancy rate per transfer (25–27), while the luteal rescue approach has been reported to have live birth rates of 26%–50% (28,29), each being a reportedly acceptable success rate for the respective patient populations. Both of these approaches are also associated with some OHSS risk, although that risk may be lower than it would have been with a large bolus of hCG as routinely used in typical IVF cycles (27).

Another approach is to freeze all the resulting embryos after GnRH agonist trigger and transfer those embryos in a subsequent cycle. Good success rates have been reported in such cycles (30). Elimination of the fresh autologous transfer precludes late-onset OHSS, regardless of the trigger medication used.

Maternal and perinatal risks

Ectopic pregnancy risk is increased in fresh autologous IVF pregnancies when compared to spontaneous pregnancies. A 2012 meta-analysis of pregnancy outcomes in elective single embryo transfer (eSET) IVF cycles compared to spontaneous conception showed a relative risk of 6.4 for ectopic pregnancy; however, this was based on a single observational study (31). This increased risk might result from COS exposure and the resulting effects of supraphysiologic estradiol levels on uterine contractions, with a predominance of cervico-fundal uterine contractions noted during portions of the cycle with elevated estrogen levels (32). Alternatively, supraphysiologic progesterone levels depress ciliary beat frequency by approximately half compared to controls (33), potentially elevating the risk of implantation in an inappropriate location. It is possible that reduced uterine receptivity following COS (9) allows embryos to implant elsewhere. One recent study found ectopic pregnancy risk was associated with thin

endometrium following COS (34), one marker of poor endometrial receptivity. Another recent study found ectopic pregnancy risk was correlated with increasing oocyte yield in autologous cycles but not in oocyte donation cycles (35), suggesting a relationship with hormone levels following COS exposure.

There have been several reports that frozen embryo transfer (FET) has a reduced risk of ectopic pregnancy when compared to fresh transfer (36–44), although others have reported no significant difference (45–47). The difference, if any, might be specific to methodology.

Other risks associated with fresh autologous cycles include pre-eclampsia, low birthweight (LBW), small for gestational age (SGA), prematurity, pre-term LBW, antepartum hemorrhage, placental abruption, and perinatal death (48–66). When compared to fresh-transfer pregnancies, FET pregnancies are associated with reduced risks of preterm birth (relative risk [RR] 0.84), SGA (RR 0.45), LBW (RR 0.69), perinatal mortality (RR 0.68), placental abruption (RR 0.44), and placenta previa (RR 0.71) (48). In recipients of donor oocytes who were not exposed to COS, no differences in birthweight between fresh transfer and FET pregnancies were noted (57), further implicating supraphysiologic hormone levels in abnormal implantation. Some of these risks may be elevated through a uterine mechanism of altered placentation in cycles with COS exposure (57). Research in the mouse model has associated COS exposure with reduced placental and fetal weights (67).

CYCLE SEGMENTATION

Temporal separation of the traditional cycle into distinct oocyte retrieval and embryo transfer cycles may confer the advantages of reduced OHSS risk, improved endometrial receptivity, and ample time in which to complete genetic screening. Furthermore, there have been reported reductions in certain perinatal risks with transfer of frozen embryos when compared to fresh autologous transfer, as discussed previously (48). However, the relative risk of large for gestational age has been reported to be 1.48 with frozen–thawed embryo transfer when compared to fresh autologous transfer (49).

The improved implantation rates conferred with cryopreservation (9) and genetic screening (68) support routine elective single-embryo transfer. The historic routine use of multiple embryo transfer by IVF centers has resulted in an "epidemic" of multiple births (69,70). Multiple birth is associated with numerous increased obstetric and pediatric risks, including greater incidence of preterm delivery, pre-eclampsia, gestational diabetes, and pediatric complications of prematurity (69,71). Iatrogenic multiple births increase costs per delivery and for society as a whole, with annual costs estimated to exceed $6 billion in the U.S.A. (72,73).

Another advantage of cycle segmentation, particularly with the agonist trigger option, is that follicular development need not be compromised due to concerns of OHSS risk or impaired endometrial receptivity. OHSS risk is very low with the agonist trigger followed by cryopreservation, and few cases have been reported (74). Previously, some centers halted exogenous FSH administration late in the stimulation cycle of high responders, "coasting" to starve developing follicles of FSH in order to reduce markers of OHSS risk (75). This is unnecessary with the option of agonist trigger and cohort cryopreservation.

Compromised endometrial receptivity, indicated by early rising progesterone levels (76,77), for example, should not motivate premature trigger if the embryos will all be frozen for later use. Moderately extended stimulation of autologous patients might result in supernumerary embryos for additional transfers without the need for additional retrievals, while in oocyte donors might result in sufficient oocytes to support additional recipients.

Cryopreservation of oocytes or embryos allows autologous pregnancy potential to be preserved indefinitely, even while ovarian reserve declines naturally or through medical interventions. This includes cancer treatment regimens with chemotherapy, surgery, or radiation therapy that can impact the ovary and for conditions such as endometriosis or other benign pathology. Similar oocyte and embryo survival and subsequent pregnancy rates were seen in studies examining the impact of cryopreservation duration on success rates (78–80).

Preimplantation genetic screening

PGS is an increasingly popular technique for identifying euploid embryos that are likely to implant and result in live birth. The recent increased usage is related to the confluence of three methodological improvements. The first of these improvements was trophectoderm biopsy of the outer cells of a blastocyst, a technique reported to cause less embryonic damage than cleavage-stage biopsy (81). The second improvement was the ability to routinely test for the presence of all 46 chromosomes (3,82), rather than previous analyses of about 10 select chromosomes by fluorescence *in situ* hybridization. The last of these improvements was blastocyst vitrification (83), which allowed good success rates in transfers that were delayed while genetic analyses were completed. The implications of improved outcomes after PGS, safer biopsy testing at the blastocyst stage, and improvements in vitrification technology support one argument for cycle segmentation.

Prior IVF failure

The potential causes of IVF failure are typically assumed to be embryonic or endometrial in nature. PGS, as described above, is available to address embryo aneuploidy. However, one study in patients aged 18–40 years estimated that 64.7% of failed fresh blastocyst transfers could be attributed to inferior endometrial receptivity following COS when compared to an artificially prepared uterine environment (9). A retrospective study compared patients who, following a failed fresh blastocyst transfer, opted for either another fresh transfer or else a segmented cycle (84). The latter group had cohort cryopreservation at the bi-pronuclear stage, followed by thaw of the entire cohort in a subsequent cycle and transfer of the best

blastocyst(s) from that cohort. The live birth rates per retrieval were 21.5% in the former group and 46.2% in the latter group, a difference that was statistically significant. After adjusting for confounding factors, logistic regression analysis yielded an odds ratio for cumulative live birth per retrieval of 1.9 in favor of the group opting for cycle segmentation. Cycle segmentation may therefore be an effective strategy in patients with a prior history of failed fresh blastocyst transfer.

Fertility preservation

Some women elect to cryopreserve their oocytes so that, should their ovarian reserve significantly decline in coming years, they can still have the option of IVF using frozen eggs. In many cases, this choice is motivated by pending oncotherapy that might significantly reduce ovarian reserve. Stimulation protocols for cancer patients may be modified to keep estradiol levels moderate, such as with aromatase inhibitors, and may be started in the follicular or secretory phases because the endometrium developed in that cycle will not be used to achieve pregnancy.

Availability of male gametes

Another limitation of traditional IVF is that sperm must be available by oocyte retrieval. This may not be possible for women seeking to preserve their fertility before selecting a male partner or while their known male partner is away for extended periods. In some cases, an available male partner may be unable to provide a semen sample or might provide an inadequate sample, precluding a planned fresh embryo transfer. Alternatively, additional surgical procedures may be required to procure adequate numbers of sperm for insemination. In such cases, the center may be compelled to cryopreserve the retrieved oocytes while awaiting sperm availability.

Donor egg banks

Another common application of cycle segmentation is donor egg banking. By temporally separating the oocyte donation cycle from the embryo transfer into a recipient, the banked oocytes may be shared among multiple recipients. This typically reduces costs per recipient and might also allow recruitment of more ideal donors by certain measures (85). Success rates using fresh versus cryopreserved oocytes are comparable (86,87).

Prerequisites to cycle segmentation

Elective cycle segmentation is a more practical consideration when the success rates with thawed embryos are at least as good as those with fresh embryos. This clinic-specific comparison should be made using success rates that are calculated per retrieval. Success rates that are calculated per transfer or per transferred embryo are useful measures of the quality of transferred embryos, but do not measure overall method efficacy because such rates exclude cases of embryo non-survival. In effect, the decision for elective cycle segmentation rests on the balance between embryonic damage via cryopreservation and embryonic wastage via transfer into a uterine environment impaired by COS exposure. This balance will depend on cryopreservation methodology and perhaps also on COS protocols.

The current standard for embryo cryopreservation is a set of techniques for rapid embryo freezing, collectively called vitrification. The recent trend has been toward vitrification of blastocysts, even at centers that perform cleavage-stage fresh transfers. Thawed vitrified blastocysts can have survival rates exceeding 90% and excellent implantation potential. Ideally, thawed embryos from young patients should implant as readily as fresh embryos derived from young oocyte donors (88,89). Failure to achieve this criterion might reflect the transfer of cryodamaged thawed embryos.

Centers not planning to perform elective cryopreservation will still benefit from an excellent cryopreservation program. These benefits include improved success rates with cryopreserved supernumerary embryos and embryo cohorts that were cryopreserved for non-elective reasons (e.g., OHSS or fertility preservation). Furthermore, an excellent cryopreservation program supports elective single fresh embryo transfer, thus reducing the many risks associated with multiple pregnancy.

National trends in cycle segmentation and success rates

The reported improving techniques for cryopreservation, increasing use of preimplantation genetic testing, and published findings regarding endometrial receptivity after COS exposure might be expected to have some effect on clinical behaviors. The results reported by the U.S. Centers for Disease Control and Prevention may be summarized graphically to reveal national trends within the U.S.A. Figure 45.1 shows the rapidly increasing usage of banking cycles and frozen embryo transfers, and the recently decreasing use of fresh embryo transfer. Figure 45.2 shows that the pregnancy rate, live birth rate, and singleton birth rate are each greater in autologous frozen embryo transfers than in fresh autologous transfers. However, these trends are reversed in the donor cycles shown in Figure 45.3, where there is typically no endometrial receptivity benefit from cryopreservation (donor recipients are typically not exposed to COS).

Figure 45.4 reveals that the implantation rates are greater in five of the six age groups used in national reporting. Only the youngest age group, those typically having the least COS exposure, did not have a greater implantation rate with frozen embryos.

Figure 45.5 shows the risk ratio of implantation with frozen embryo transfer to that of fresh embryo transfer by age group. The age-related increasing advantage of frozen embryo transfer is apparent, as the risk ratio consistently and dramatically increases from 0.99 in patients aged <35 years to 5.7 in patients aged >44 years. The cause of this dramatic increase cannot be discerned from the national averages alone, and might include multiple factors, such as increased use of PGS in FET cycles in older age groups, a patient selection effect (e.g., only those patients with the

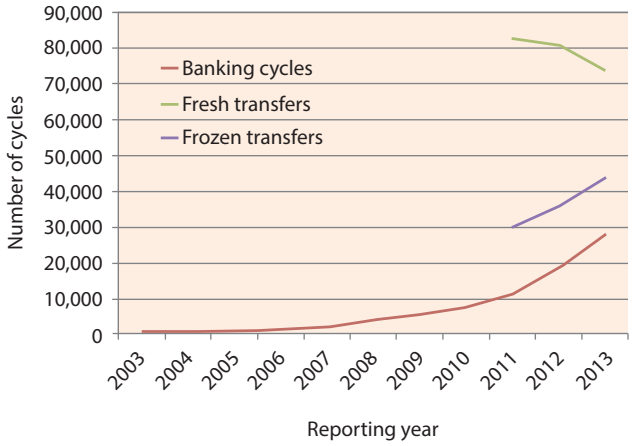

Figure 45.1 Number of banking cycles (red line), fresh embryo transfers (green line), and frozen embryo transfers (purple line) versus reporting year. Note the increasing use of embryo banking and frozen embryo transfers, and the recent decreasing use of fresh embryo transfers. Note: the U.S. Centers for Disease Control and Prevention report format has shown the number of transfers only since 2011.

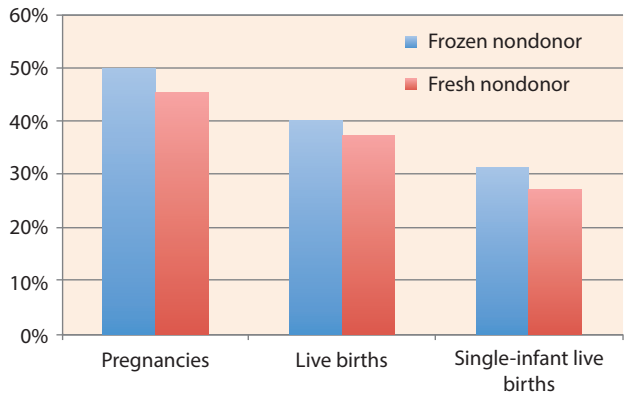

Figure 45.2 Nationally reported pregnancy rates, live birth rates, and singleton birth rates according to the transfer of autologous fresh or frozen embryos. Note the greater success rates with frozen embryos in all cases.

best cohorts have embryos to freeze) that becomes increasingly selective as age increases, accrual of more embryos through multiple retrieval cycles preparatory to FET in older patients, and increasing endometrial impairment in older patients as COS protocols become more prolonged and intensive with increasing age.

Who can benefit from cycle segmentation?

Young patients have the least benefit in terms of improved implantation rate from cycle segmentation (Figure 45.5). This may be because the younger patients are those most likely to have a large embryo cohort that is, in turn, most likely to include at least one rapidly developing day-5 blastocyst, potentially improving embryo–endometrium synchrony. Another possible cause is that young patients may require shorter stimulation cycles, perhaps with reduced

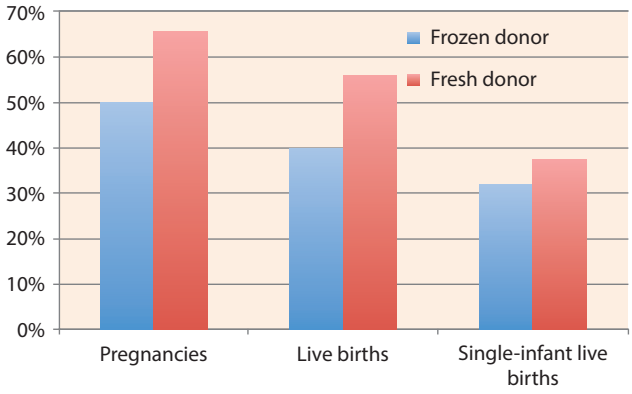

Figure 45.3 Nationally reported pregnancy rates, live birth rates, and singleton birth rates according to the transfer of fresh or frozen embryos derived from donor oocytes. Note the reduced success rates with frozen embryos in all cases.

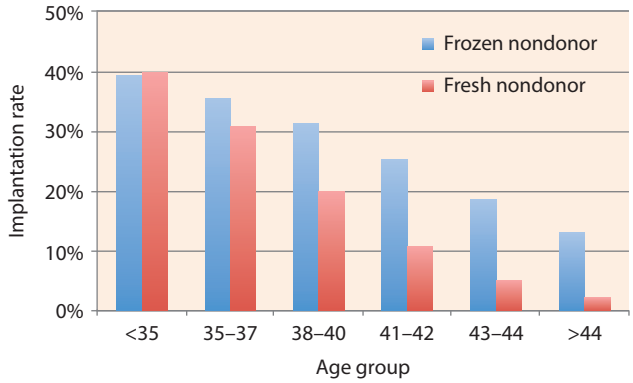

Figure 45.4 Nationally reported implantation rates according to age group and whether the transferred embryos are fresh or frozen. Note the greater implantation rates with frozen embryos in five of the six age groups.

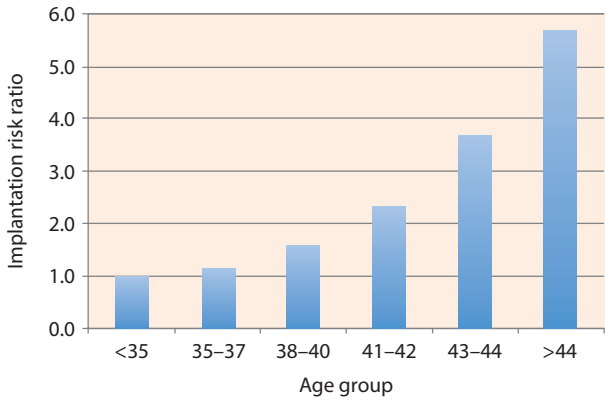

Figure 45.5 Risk ratios of nationally reported implantation rates with frozen embryos compared to those with fresh embryos. A ratio greater than 1.0 indicates a greater implantation rate with frozen embryos. Note the steady and dramatic increase in the implantation risk ratio with increasing age.

risk of premature progesterone elevation, and therefore have a reduced risk of impaired endometrial receptivity. However, young patients are also those most at risk for OHSS. Therefore, cycle segmentation in young patients may be motivated primarily through safety concerns, and perhaps also by efficacy following agonist trigger.

In contrast, the older autologous patient has less risk of OHSS, and therefore little benefit in terms of reduced OHSS risk through cycle segmentation. However, the older patient seems to have a greater benefit in terms of improved implantation rate through cycle segmentation.

Any patient using PGS can benefit from cycle segmentation if there is significant delay while the results are obtained.

Alternatives to elective cycle segmentation

Alternatives to elective cohort cryopreservation in order to avoid a potentially non-receptive uterine environment include fresh embryo transfer after assessing the probability of implantation. Certain parameters that are indicative of issues with embryo–endometrium asynchrony may be routinely observed. These include pre-trigger serum progesterone level and the embryo's developmental pace, as can be measured by the day of blastulation (8,76). Fresh transfers of rapidly developing embryos (day-5 blastocysts) in cycles without premature progesterone elevation are associated with excellent success rates, and might not benefit from elective cycle segmentation.

Another alternative to elective cycle segmentation might be to adjust the COS protocol to eliminate or reduce untoward uterine effects, although further research is needed to prove feasibility.

Needed research

Research continues in improving our understanding of endometrial receptivity (90), improving cryopreservation methodology (91,92), and optimizing luteal support for thaw cycles (93).

Other research might investigate the new vistas that arise for banking cycles, without consideration of sustaining endometrial receptivity. For example, random-start or luteal-phase stimulation protocols have proved successful in the absence of fresh transfer (94,95). In addition, it might be possible to increase the numbers of mature follicles and collected oocytes if the duration of stimulation is extended, despite detrimental effects on endometrial receptivity (96), and to take advantage of the positive correlation between follicle size and oocyte quality (97). Also, the means of pituitary suppression during COS might be made simpler and less expensive if there is no fresh autologous transfer, such as by using oral progesterone in lieu of the usual GnRH agonist or antagonist injections (98).

REFERENCES

1. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. *2013 Assisted Reproductive Technology Fertility Clinic Success Rates Report*. Atlanta, GA: U.S. Department of Health and Human Services, 2015.
2. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, Scott RT. Aneuploidy across individual chromosomes at the embryonic level in trophectoderm biopsies: Changes with patient age and chromosome structure. *J Assist Reprod Genet* 2014; 31: 1501–9.
3. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases *in vitro* fertilization implantation and delivery rates: A randomized controlled trial. *Fertil Steril* 2013; 100: 697–703.
4. Nikas G. Pinopodes as markers of endometrial receptivity in clinical practice. *Hum Reprod* 1999; 14(Suppl 2): 99–106.
5. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Ross R. Contrasting patterns in *in vitro* fertilization pregnancy rates among fresh autologous, fresh oocyte donor, and cryopreserved cycles with the use of day 5 or day 6 blastocysts may reflect differences in embryo–endometrium synchrony. *Fertil Steril* 2008; 89: 20–6.
6. Murata Y, Oku H, Morimoto Y, Tokuda M, Murata T, Sugihara K, Nagata F, Nakaoka Y, Fukuda A. Freeze–thaw programmes rescue the implantation of day 6 blastocysts. *Reprod Biomed Online* 2005; 11: 428–33.
7. Richter KS, Shipley SK, McVearry I, Tucker MJ, Widra EA. Cryopreserved embryo transfers suggest that endometrial receptivity may contribute to reduced success rates of later developing embryos. *Fertil Steril* 2006; 86: 862–6.
8. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Thomas S. Large blastocyst diameter, early blastulation, and low preovulatory serum progesterone are dominant predictors of clinical pregnancy in fresh autologous cycles. *Fertil Steril* 2008; 90: 302–9.
9. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for *in vitro* fertilization: A prospective randomized trial comparing fresh and frozen–thawed embryo transfer in normal responders. *Fertil Steril* 2011; 96: 344–8.
10. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, Checa MA. Fresh embryo transfer versus frozen embryo transfer in *in vitro* fertilization cycles: A systematic review and meta-analysis. *Fertil Steril* 2013; 99: 156–62.
11. Shapiro BS, Daneshmand ST, Restrepo H, Garner FC, Aguirre M, Hudson C. Matched-cohort comparison of single-embryo transfers in fresh and frozen-thawed embryo transfer cycles. *Fertil Steril* 2013; 99: 389–92.
12. Ozgur K, Berkkanoglu M, Bulut H, Isikli A, Coetzee K. Higher clinical pregnancy rates from

frozen-thawed blastocyst transfers compared to fresh blastocyst transfers: A retrospective matched-cohort study. *J Assist Reprod Genet* 2015; 32: 1483–90.
13. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: A systematic review and meta-analysis of over 60 000 cycles. *Hum Reprod Update* 2013; 19: 433–57.
14. Hofmann GE1, Bentzien F, Bergh PA, Garrisi GJ, Williams MC, Guzman I, Navot D. Premature luteinization in controlled ovarian hyperstimulation has no adverse effect on oocyte and embryo quality. *Fertil Steril* 1993; 60: 675–9.
15. Labarta E, Martinez-Conejero JA, Alama P, Horcajadas JA, Pellicer A, Simon C, Bosch E. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: A functional genomics analysis. *Hum Reprod* 2011; 26: 1813–25.
16. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Embryo cryopreservation rescues cycles with premature luteinization. *Fertil Steril* 2010; 93: 636–41.
17. Polotsky AJ, Daif JL, Jindal S, Lieman HJ, Santoro N, Pal L. Serum progesterone on the day of human chorionic gonadotropin administration predicts clinical pregnancy of sibling frozen embryos. *Fertil Steril* 2009; 92: 1880–5.
18. Hill MJ, Royster GD 4th, Healy MW, Richter KS, Levy G, DeCherney AH, Levens ED, Suthar G, Widra E, Levy MJ. Are good patient and embryo characteristics protective against the negative effect of elevated progesterone level on the day of oocyte maturation? *Fertil Steril* 2015; 103: 1477–84.e1–5.
19. Practice Committee of American Society for Reproductive Medicine. Ovarian hyperstimulation syndrome. *Fertil Steril* 2008; 90: S188–93.
20. Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing *in vitro* fertilization prevents the risk of ovarian hyperstimulation syndrome: A prospective randomized controlled study. *Fertil Steril* 2008; 89: 84–91.
21. Kol S, Dor J. Symposium: Update on prediction and management of OHSS. Prevention of OHSS: GnRH agonist versus HCG to trigger ovulation. *Reprod Biomed Online* 2009; 19: 59–60.
22. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Ross R. Comparison of human chorionic gonadotropin and gonadotropin-releasing hormone agonist for final oocyte maturation in oocyte donor cycles. *Fertil Steril* 2007; 88: 237–9.
23. Kolibianakis EM, Schultze-Mosgau A, Schroer A, van Steirteghem A, Devroey P, Diedrich K, Griesinger G. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of hCG in patients undergoing IVF with GnRH antagonists. *Hum Reprod* 2005; 20: 2887–92.
24. Engmann L, Benadiva C. Agonist trigger: What is the best approach? Agonist trigger with aggressive luteal support. *Fertil Steril* 2012; 97: 531–3.
25. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Comparison of "triggers" using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. *Fertil Steril* 2011; 95: 2715–7.
26. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Thomas S. Gonadotropin-releasing hormone agonist combined with a reduced dose of human chorionic gonadotropin for final oocyte maturation in fresh autologous cycles of *in vitro* fertilization. *Fertil Steril* 2008; 90: 231–3.
27. Griffin D, Benadiva C, Kummer N, Budinetz T, Nulsen J, Engmann L. Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders. *Fertil Steril* 2012; 97: 1316–20.
28. Humaidan P. Luteal phase rescue in high-risk OHSS patients by GnRHa triggering in combination with low-dose hCG: A pilot study. *Reprod Biomed Online* 2009; 18: 630–4.
29. Humaidan P, Ejdrup Bredkjaer H, Westergaard LG, Yding Andersen C. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: A prospective, randomized, controlled study. *Fertil Steril* 2010; 93: 847–54.
30. Griesinger G, Schultz L, Bauer T, Broessner A, Frambach T, Kissler S. Ovarian hyperstimulation syndrome prevention by gonadotropin-releasing hormone agonist triggering of final oocyte maturation in a gonadotropin-releasing hormone antagonist protocol in combination with a "freeze-all" strategy: A prospective multicentric study. *Fertil Steril* 2011; 95: 2029–33.
31. Grady R, Alavi N, Vale R, Khandwala M, McDonald SD. Elective single embryo transfer and perinatal outcomes: A systematic review and meta-analysis. *Fertil Steril* 2012; 97: 324–31.
32. Killick SR. Ultrasound and the receptivity of the endometrium. *Reprod Biomed Online* 2007; 15: 63–7.
33. Paltieli Y, Eibschitz I, Ziskind G, Ohel G, Silbermann M, Weichselbaum A. High progesterone levels and ciliary dysfunction—A possible cause of ectopic pregnancy. *J Assist Reprod Genet* 2000; 17: 103–6.
34. Rombauts L, McMaster R, Motteram C, Fernando S. Risk of ectopic pregnancy is linked to endometrial thickness in a retrospective cohort study of 8120 assisted reproduction technology cycles. *Hum Reprod* 2015; 30: 2846–52.
35. Acharya KS, Acharya CR, Provost MP, Yeh JS, Steward RG, Eaton JL, Muasher SJ. Ectopic pregnancy

rate increases with the number of retrieved oocytes in autologous *in vitro* fertilization with non-tubal infertility but not donor/recipient cycles: An analysis of 109,140 clinical pregnancies from the Society for Assisted Reproductive Technology registry. *Fertil Steril* 2015; 104: 873–8.
36. Ng EH, Yeung WS, So WW, Ho PC. An analysis of ectopic pregnancies following *in vitro* fertilisation treatment in a 10-year period. *J Obstet Gynaecol* 1998; 18: 359–64.
37. Ishihara O, Kuwahara A, Saitoh H. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: An analysis of single embryo transfer cycles in Japan. *Fertil Steril* 2011; 95: 1966–9.
38. Shapiro BS, Daneshmand ST, De Leon L, Garner FC, Aguirre M, Hudson C. Frozen–thawed embryo transfer is associated with a significantly reduced incidence of ectopic pregnancy. *Fertil Steril* 2012; 98: 1490–4.
39. Li Z, Sullivan EA, Chapman M, Farquhar C, Wang YA. Risk of ectopic pregnancy lowest with transfer of single frozen blastocyst. *Hum Reprod* 2015; 30: 2048–54.
40. Londra L, Moreau C, Strobino D, Garcia J, Zacur H, Zhao Y. Ectopic pregnancy after *in vitro* fertilization: Differences between fresh and frozen–thawed cycles. *Fertil Steril* 2015; 104: 110–8.
41. Perkins KM, Boulet SL, Kissin DM, Jamieson DJ, National ART Surveillance (NASS) Group. Risk of ectopic pregnancy associated with assisted reproductive technology in the United States, 2001–2011. *Obstet Gynecol* 2015; 125: 70–8.
42. Fang C, Huang R, Wei LN, Jia L. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. *Fertil Steril* 2015; 103: 655–61.
43. Ozgur K, Berkkanoglu M, Bulut H, Humaidan P, Coetzee K. Perinatal outcomes after fresh versus vitrified-warmed blastocyst transfer: Retrospective analysis. *Fertil Steril* 2015; 104: 899–907.
44. Huang B, Hu D, Qian K, Ai J, Li Y, Jin L, Zhu G, Zhang H. Is frozen embryo transfer cycle associated with a significantly lower incidence of ectopic pregnancy? An analysis of more than 30,000 cycles. *Fertil Steril* 2014; 102: 1345–9.
45. Decleer W, Osmanagaoglu K, Meganck G, Devroey P. Slightly lower incidence of ectopic pregnancies in frozen embryo transfer cycles versus fresh *in vitro* fertilization-embryo transfer cycles: A retrospective cohort study. *Fertil Steril* 2014; 101: 162–5.
46. Clayton HB, Schieve LA, Peterson HB, Jamieson DJ, Reynolds MA, Wright VC. Ectopic pregnancy risk with assisted reproductive technology procedures. *Obstet Gynecol* 2006; 107: 595–604.
47. Jun SH, Milki AA. Ectopic pregnancy rates with frozen compared with fresh blastocyst transfer. *Fertil Steril* 2007; 88: 629–31.
48. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through *in vitro* fertilization treatment: A systematic review and meta-analysis. *Fertil Steril* 2012; 98: 368–77.
49. Ishihara O, Araki R, Kuwahara A, Itakura A, Saito H, Adamson GD. Impact of frozen-thawed single-blastocyst transfer on maternal and neonatal outcome: An analysis of 277,042 single-embryo transfer cycles from 2008 to 2010 in Japan. *Fertil Steril* 2014; 101: 128–33.
50. Wennerholm UB, Henningsen AK, Romundstad LB et al. Perinatal outcomes of children born after frozen-thawed embryo transfer: A Nordic cohort study from the CoNARTaS group. *Hum Reprod* 2013; 28: 2545–53.
51. Wennerholm UB, Soderstrom-Anttila V, Bergh C et al. Children born after cryopreservation of embryos or oocytes: A systematic review of outcome data. *Hum Reprod* 2009; 24: 2158–72.
52. Shih W, Rushford DD, Bourne H et al. Factors affecting low birthweight after assisted reproduction technology: Difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. *Hum Reprod* 2008; 23: 1644–53.
53. Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: The Danish National Cohort Study 1995–2006. *Fertil Steril* 2010; 94: 1320–7.
54. Nakashima A, Araki R, Tani H et al. Implications of assisted reproductive technologies on term singleton birth weight: An analysis of 25,777 children in the national assisted reproduction registry of Japan. *Fertil Steril* 2013; 99: 450–5.
55. Pelkonen S, Koivunen R, Gissler M et al. Perinatal outcome of children born after frozen and fresh embryo transfer: The Finnish cohort study 1995–2006. *Hum Reprod* 2010; 25: 914–23.
56. Sullivan EA, Zegers-Hochschild F, Mansour R et al. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) world report: Assisted reproductive technology 2004. *Hum Reprod* 2013; 28: 1375–90.
57. Kalra SK, Ratcliffe SJ, Coutifaris C, Molinaro T, Barnhart KT. Ovarian stimulation and low birth weight in newborns conceived through *in vitro* fertilization. *Obstet Gynecol* 2011; 118: 863–71.
58. Davies MJ, Moore VM, Willson KJ et al. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012; 366: 1803–13.
59. Halliday JL, Ukoumunne OC, Baker HW et al. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. *Hum Reprod* 2010; 25: 59–65.
60. Kato O, Kawasaki N, Bodri D et al. Neonatal outcome and birth defects in 6623 singletons born following minimal ovarian stimulation and vitrified

versus fresh single embryo transfer. *Eur J Obstet Gynecol Reprod Biol* 2012; 161: 46–50.
61. Moster D, Lie RT, Markestad T. Long-term medical and social consequences of preterm birth. *N Engl J Med* 2008; 359: 262–73.
62. Lin CM, Chen CW, Chen PT, Lu TH, Li CY. Risks and causes of mortality among low-birthweight infants in childhood and adolescence. *Paediatr Perinat Epidemiol* 2007; 21: 465–72.
63. Olivennes F, Fanchin R, Lédée N, Righini C, Kadoch IJ, Frydman R. Perinatal outcome and developmental studies on children born after IVF. *Hum Reprod Update* 2002; 8: 117–28.
64. Pinborg A, Wennerholm UB, Romundstad LB et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013; 19: 87–104.
65. Imudia AN, Awonuga AO, Kaimal AJ, Wright DL, Styer AK, Toth TL. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: A preliminary study. *Fertil Steril* 2013; 99: 168–73.
66. Kallen B, Finnstrom O, Nygren KG, Otterblad Olausson P, Wennerholm UB. *In vitro* fertilisation in Sweden: Obstetric characteristics, maternal morbidity and mortality. *BJOG* 2005; 112: 1529–35.
67. Mainigi MA1, Olalere D, Burd I, Sapienza C, Bartolomei M, Coutifaris C. Peri-implantation hormonal milieu: Elucidating mechanisms of abnormal placentation and fetal growth. *Biol Reprod* 2014; 90: 26.
68. Dahdouh EM, Balayla J, García-Velasco JA. Comprehensive chromosome screening improves embryo selection: A meta-analysis. *Fertil Steril* 2015; 104: 1503–12.
69. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Warner L, Barfield WD, Centers for Disease Control and Prevention (CDC). Assisted reproductive technology surveillance—United States, 2012. *MMWR Surveill Summ* 2015; 64(6): 1–29.
70. Kissin DM, Kulkarni AD, Mneimneh A, Warner L, Boulet SL, Crawford S, Jamieson DJ, National ART Surveillance System (NASS) group. Embryo transfer practices and multiple births resulting from assisted reproductive technology: An opportunity for prevention. *Fertil Steril* 2015; 103: 954–61.
71. Qin J, Wang H, Sheng X, Liang D, Tan H, Xia J. Pregnancy-related complications and adverse pregnancy outcomes in multiple pregnancies resulting from assisted reproductive technology: A meta-analysis of cohort studies. *Fertil Steril* 2015; 103: 1492–508.
72. Crawford S, Boulet SL, Mneimneh AS, Perkins KM, Jamieson DJ, Zhang Y, Kissin DM. Costs of achieving live birth from assisted reproductive technology: A comparison of sequential single and double embryo transfer approaches, 2012. *Fertil Steril* 2016; 105: 444–50.
73. Allen BD, Adashi EY, Jones HW. On the cost and prevention of iatrogenic multiple pregnancies. *Reprod Biomed Online* 2014; 29: 281–5.
74. Gurbuz AS, Gode F, Ozcimen N, Isik AZ. Gonadotrophin-releasing hormone agonist trigger and freeze-all strategy does not prevent severe ovarian hyperstimulation syndrome: A report of three cases. *Reprod Biomed Online* 2014; 29: 541–4.
75. D'Angelo A, Brown J, Amso NN. Coasting (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev* 2011; 6: CD002811.
76. Huang CC, Lien YR, Chen HF, Chen MJ, Shieh CJ, Yao YL, Chang CH, Chen SU, Yang YS. The duration of pre-ovulatory serum progesterone elevation before hCG administration affects the outcome of IVF/ICSI cycles. *Hum Reprod* 2012; 27: 2036–45.
77. Kyrou D, Al-Azemi M, Papanikolaou EG, Donoso P, Tziomalos K, Devroey P, Fatemi HM. The relationship of premature progesterone rise with serum estradiol levels and number of follicles in GnRH antagonist/recombinant FSH-stimulated cycles. *Eur J Obstet Gynecol Reprod Biol* 2012; 162: 165–8.
78. Parmegiani L, Garello C, Granella F, Guidetti D, Bernardi S, Cognigni GE, Revelli A, Filicori M. Long-term cryostorage does not adversely affect the outcome of oocyte thawing cycles. *Reprod Biomed Online* 2009; 19: 374–9.
79. Aflatoonian N, Pourmasumi S, Aflatoonian A, Eftekhar M. Duration of storage does not influence pregnancy outcome in cryopreserved human embryos. *Iran J Reprod Med* 2013; 11: 843–6.
80. Riggs R, Mayer J, Dowling-Lacey D, Chi TF, Jones E, Oehninger S. Does storage time influence post-thaw survival and pregnancy outcome? An analysis of 11,768 cryopreserved human embryos. *Fertil Steril* 2010; 93: 109–15.
81. Scott RT, Jr., Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: A randomized and paired clinical trial. *Fertil Steril* 2013; 100: 624–30.
82. Schoolcraft WB, Katz-Jaffe MG. Comprehensive chromosome screening of trophectoderm with vitrification facilitates elective single-embryo transfer for infertile women with advanced maternal age. *Fertil Steril* 2013; 100: 615–9.
83. Balaban B, Urman B, Ata B, Isiklar A, Larman MG, Hamilton R, Gardner DK. A randomized controlled study of human day 3 embryo cryopreservation by slow freezing or vitrification: Vitrification is associated with higher survival, metabolism and blastocyst formation. *Hum Reprod* 2008; 23: 1976–82.

84. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Freeze-all can be a superior therapy to another fresh cycle in patients with prior fresh blastocyst implantation failure. *Reprod Biomed Online* 2014; 29: 286–90.
85. Cobo A, Remohí J, Chang CC, Nagy ZP. Oocyte cryopreservation for donor egg banking. *Reprod Biomed Online* 2011; 23: 341–6.
86. Cobo A, Meseguer M, Remohí J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: A prospective, randomized, controlled, clinical trial. *Hum Reprod* 2010; 25: 2239–46.
87. Nagy ZP, Chang CC, Shapiro DB, Bernal DP, Elsner CW, Mitchell-Leef D, Toledo AA, Kort HI. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertil Steril* 2009; 92: 520–6.
88. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, Salamonsen LA, Rombauts LJ. Fresh versus frozen embryo transfer: Backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update* 2014; 20: 808–21.
89. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Similar ongoing pregnancy rates after blastocyst transfer in fresh donor cycles and autologous cycles using cryopreserved bipronuclear oocytes suggest similar viability of transferred blastocysts. *Fertil Steril* 2010; 93: 319–21.
90. Garrido-Gómez T, Quiñonero A, Antúnez O, Díaz-Gimeno P, Bellver J, Simón C, Domínguez F. Deciphering the proteomic signature of human endometrial receptivity. *Hum Reprod* 2014; 29: 1957–67.
91. Sparks AE. Human embryo cryopreservation-methods, timing, and other considerations for optimizing an embryo cryopreservation program. *Semin Reprod Med* 2015; 33: 128–44.
92. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Freeze-all at the blastocyst or bipronuclear stage: A randomized clinical trial. *Fertil Steril* 2015; 104: 1138–44.
93. Haas J, Lantsberg D, Feldman N, Manela D, Machtinger R, Dar S, Rabinovici J, Orvieto R. Modifying the luteal phase support in natural cycle frozen–thawed embryo transfer improves cycle outcome. *Gynecol Endocrinol* 2015; 31: 891–3.
94. Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. *Curr Opin Obstet Gynecol* 2015; 27: 215–21.
95. Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. *Fertil Steril* 2011; 96: e51–4.
96. Kolibianakis EM, Bourgain C, Papanikolaou EG, Camus M, Tournaye H, Van Steirteghem AC, Devroey P. Prolongation of follicular phase by delaying hCG administration results in a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antagonist cycles. *Hum Reprod* 2005; 20: 2453–6.
97. Rosen MP, Shen S, Dobson AT, Rinaudo PF, McCulloch CE, Cedars MI. A quantitative assessment of follicle size on oocyte developmental competence. *Fertil Steril* 2008; 90: 684–90.
98. Zhu X, Zhang X, Fu Y. Utrogestan as an effective oral alternative for preventing premature luteinizing hormone surges in women undergoing controlled ovarian hyperstimulation for *in vitro* fertilization. *Medicine (Baltimore)* 2015; 94: e909.

The use of ovarian reserve biomarkers to tailor ovarian stimulation for *in vitro* fertilization

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INTRODUCTION

Stratified medicine is recognized as a key global priority for healthcare providers, patients, and pharmaceutical and diagnostic industries. Achieving personalized care with provision of the "right treatment, for the right person, at the right time" should be an inevitable progression as we gain greater understanding of the etiology and pathophysiology of disease, but requires critical assessment of all aspects of care. Advances in understanding have enabled us to predict disease reliably at population levels, with existing and novel biomarkers now being evaluated for incorporation into composite models (1). Reproductive medicine has taken a notable lead in the use of prognostic models for stratification of individuals to different likelihoods of success, in the utilization of novel biomarkers for predicting ovarian response and assigning risk, and for developing novel therapeutic algorithms to allow streamlining of individuals to the appropriate intervention.

Although a wide variety of biomarkers have been proposed as predictors of ovarian response, it is now clear that anti-Mullerian hormone (AMH) and antral follicle count (AFC) demonstrate the most favorable analytical and performance characteristics (2–4). Several recent large systematic reviews of cohort studies and individual patient data (IPD) meta-analyses have demonstrated consistent positive association with oocyte yield, poor and excess response, and live birth in *in vitro* fertilization (IVF) cycles (4–8). As these two markers both physiologically reflect the number of small antral follicles and are thus strongly correlated (9), they have sometimes been considered interchangeable (10–13). However, recent trial data where both AMH and AFC were determined have questioned this postulation (14,15). The aim of this chapter is to present a comprehensive update of the strengths and weaknesses of both AMH and AFC, including the development and analytical characteristics of the new automated AMH immunoassays, to evaluate the data underlying their performance characteristics as biomarkers of ovarian response, particularly drawing on recent randomized controlled trials (RCTs), and to provide a summary of the data on their use for tailoring ovarian stimulation.

The physiology of follicle growth determining availability for exogenous gonadotropin recruitment

The molecular mechanisms regulating the recruitment of non-growing follicles and selection for continued growth versus atresia continue to be elucidated. Several key concepts are relevant to the present analysis (16). Firstly, recruitment of primordial follicles occurs across the reproductive lifespan (17). This dynamic process with differential rates of follicular activation at different ages is necessary in order to have a continuous supply of growing follicles to support the selection processes that precede ovulation (18) and is probably influenced by health status. Secondly, follicles undergo atresia at all stages of development (19). Thirdly, the number of activated follicles reflects the total pool of primordial follicles in a variable manner, with markedly different correlation coefficients in childhood and adult life (20). Lastly, ovarian reserve depletion will depend on the initial quantity of primordial follicles and the rate of primordial follicle recruitment. Collectively, this means that although in adult life biomarkers of activated follicles such as AMH and AFC can potentially reflect the primordial follicle pool (21), their greatest strength and value will be in indicating the number of follicles that are at late stages of follicular development and capable of responding to exogenous gonadotropins. Thus, AMH and AFC are of greatest value in reflecting what has been termed the functional rather than the true ovarian reserve (22).

ANTI-MULLERIAN HORMONE

Factors that can influence AMH values

Assay: The move to automation

Since the original reports of measurement of serum AMH in 1990 (23–25), there has been continual development of the immunoassay by a variety of companies utilizing different antibody pairs. Four manual enzyme-linked immunosorbent assays (ELISAs) are still available, and the performance characteristics of all of these assays are variable. These manual assays have, however, now been complemented by two fully automated assays (26), with evaluation suggesting they are significantly more robust and sensitive. That both these automated assays exhibit superior performance characteristics with lower intra- and inter-laboratory variation means that there is no longer a role for manual AMH ELISA assays.

Stable automated assays are an initial critical step in the path to standardization. Agreement of an international human standard developed in accordance with the International Federation of Clinical Chemistry will

inevitably now follow, allowing external calibration of AMH assays and standardized reporting and clinical interpretation.

Inter-individual variation

Concomitant with the decline in the rate of follicular recruitment observed with age in adult women, circulating AMH concentrations progressively decline with advancing age, reaching undetectable levels approximately five years prior to the cessation of menses (27,28). Several groups have modeled the age-related decline of AMH in large population cohorts, but all exhibit wide confidence intervals suggesting that, for a given age, AMH levels in both normal and infertile populations can vary substantially (29–34). This is not surprising, as primordial follicle counts and follicular activity similarly vary substantially between individuals, with 100-fold differences in primordial follicle numbers observed in healthy women of the same age (18).

Ethnicity has been associated with altered age-specific levels of AMH, with women of Chinese, black African, Hispanic, and South Asian descent reported as having a lower AMH at a given age compared with Caucasian women (35,36). Whether this ethnic disparity reflects accelerated ovarian aging, inherent differences in follicular endowment or recruitment, and/or differences in AMH secretion per follicle are unclear. Clarification may be achieved by analyses of histological ovarian specimens from different ethnic groups combined with assessment of follicular AMH secretion. The significance of altered AMH secretion is potentially substantial and, if proven, ethnicity-specific cutoff points may be required in defining expected poor (37) and high responders (38–40). At present, however, there is little evidence for this, with the AMH to ovarian response relationship being similar across multiple different ethnicities.

Obesity is not thought to be associated with AMH levels. Initial cross-sectional data in 1896 non-polycystic ovary syndrome (PCOS) infertile women demonstrated a weak negative correlation between AMH levels and body mass index (BMI; $r = -0.064$); however, the authors did not adjust for age, which would be expected to confound this association (41). This negative association was not replicated in a population-based study of 2320 premenopausal women in terms of BMI, central adiposity, and age-specific AMH percentiles (42). In an analysis of 1308 adolescent 15-year-olds with detailed dual energy X-ray absorptiometry-determined fat mass, there was no association of AMH with fat mass or BMI (43), and longitudinal analysis of women during weight loss did not demonstrate an alteration in AMH concentrations (44,45). With respect to other lifestyle determinants, cross-sectional data suggest that current smoking is independently associated with lower age-adjusted aggregated levels of AMH (42,46). Increasing body weight does, however, reduce circulating concentrations of exogenous follicle-stimulating hormone (FSH) after gonadotropin injection, and thus bodyweight needs to be taken into account for dosing of gonadotropins, but this is independent of any effect on circulating AMH.

Comorbidities are increasingly recognized as being associated with altered AMH concentrations. The most established of these is PCOS, which is associated with substantially greater levels of AMH, to the extent that AMH cutoffs with optimal sensitivity and specificity have been suggested for the diagnosis of the syndrome (47–49). For other medical disorders, the data are more limited. Adolescent girls with type 1 diabetes have higher AMH levels than controls (50), but in adult life they are lower, suggesting altered follicular dynamics—certainly, women with type 1 diabetes go through the menopause earlier than healthy controls (51). In contrast, in a study of 72 women with recent-onset rheumatoid arthritis, no alteration of AMH was observed (52). More recently, it has been suggested that AMH production and/or follicular dynamics are acutely altered in response to being unwell. Young girls diagnosed with hematological or other childhood cancers exhibited decreased concentrations of AMH compared with their healthy peers at the time of initial diagnosis (53). In this cohort of 208 girls with newly diagnosed cancer, AMH was also negatively associated with markers of general health, including body temperature, C-reactive protein, and anemia. Similarly, lower AMH levels have been reported in adults with breast cancer, lymphoma, and acute-onset Crohn's disease than in healthy (though infertile) controls (54,55). These data thus indicate the importance of general health in ovarian function, and consequently in interpreting tests of ovarian follicular activity; however, they do not alter the AMH to ovarian response relationship, and dosing can be performed accordingly.

In contrast to initial conclusions from cross-sectional studies, it is now clear from prospective longitudinal studies that the endocrine environment, which influences follicular activation and development, also impacts on AMH concentrations. Pregnancy, gonadotropin-releasing hormone (GnRH) analogs, and combined hormonal contraceptives (irrespective of whether they are oral, transdermal, or vaginal) are now all known to reduce AMH concentrations (56–60). This is likely to reflect suppression of endogenous gonadotropin secretion and altered antral follicular development. Women would still be expected to respond to exogenous gonadotropins as predicted by their AMH value; although there are limited data regarding this, it is consistent with the finding that fewer oocytes are obtained from women with a range of malignancies undergoing IVF prior to specific treatment (61).

Although all of the above factors may contribute to large age-specific variation in AMH, they do not seem to interfere substantially with the consistent robust associations with oocyte yield (7). This reflects that while AMH is expressed by granulosa cells from the initiation of follicle growth, expression is near-absent in the final preovulatory stages of development. In normal women, it has been estimated that 60% of serum AMH is derived from follicles that are 5–8 mm in diameter (62), with an abrupt decline coincident with selection for dominance. The above factors should, however, be considered as potential confounders

when research studies and trials are designed, particularly with respect to studies of future reproductive health outcomes.

Intra-individual variation

In an influential paper, La Marca and colleagues found no evidence for significant fluctuation of AMH across the menstrual cycle, in contrast to the well-established marked variation in both inhibin B and FSH (63). Similar results were found by others (64,65), although secondary analysis revealed that younger women (thus being more likely to have higher AMH) had significantly larger intra-individual variation in AMH levels than older women, with 17 out of 22 women under 38 years of age showing a variation in AMH concentration greater than 0.5 ng/mL within one cycle (66). Evidence of statistically significant differences in mean values across the cycle can be misleading in studies aiming to assess the variation of AMH at the individual level. Analysis of the true intra-individual cycle variation indicated that the intra-class correlation coefficient (ICC) was 0.96, indicating that the between-subject variation was responsible for the larger proportion of the observed cyclical variation and only 4% of the variation was true within-subject variation related to the phase of the cycle (67).

Studies have confirmed cyclic variation with higher AMH in the late follicular phase (68), which is more evident in younger women with higher mean AMH levels (69). This cyclical variation in AMH, however, has minimal impact on clinical performance and was not large enough to warrant a shift in clinical practice towards timing AMH measurement (69).

To date, one study has addressed the circadian variation in AMH in a cohort of 19 women (70). AMH was lowest in the early morning hours (4 and 6 a.m.), with a maximum mean difference from its zenith values of 1.9 pmol/L (10.6%). AMH was relatively stable during daytime, when venepuncture is routinely performed; therefore, this result, though of interest, is not of clinical relevance. In contrast, the same study demonstrated that FSH, which is still used as a marker of ovarian response in some clinics, and other ovarian-derived hormones (estradiol and progesterone) exhibited substantial circadian fluctuation even during daytime (70). Collectively, the data suggest that although AMH can vary across the menstrual cycle, this variability may be primarily of potential value in detailed analysis of follicle growth patterns (71) and is not large enough to warrant restricting AMH measurements to a specific day or phase of the menstrual cycle.

The between-cycle variability of AMH has been evaluated in several studies and the results do not indicate assay-specific variation. AMH, measured over three consecutive cycles, had an ICC of 0.89, which was significantly higher than that of FSH, inhibin B, or AFC (0.55, 0.76, and 0.73, respectively) (72), indicating lower variability. Age-adjusted ICC for AMH across four consecutive cycles was found to be 0.89 (95% confidence interval [CI] 0.84–0.94), indicating that only 11% of the inter-cycle variability was attributable to intra-individual fluctuation (73); the ICC of AFC determined at the same time points was significantly lower (0.71, 95% CI 0.63–0.77). These analyses suggest that repeat measurements of AMH during subsequent cycles are not necessary for accurate patient assessment.

Antral follicle count

The developmental pathway from primordial follicle to ovulation is associated with an approximately 500-fold increase in follicular diameter (74). Primordial follicles have a diameter of approximately 30 μm, and thus cannot be visualized; the development of the fluid-filled antrum provides the necessary physical structure to give a change in ultrasound reflectivity and thus allow potential detection, although this occurs at sub-millimeter diameters (75); thus, the smallest antral follicles cannot be visualized by current technology. Antral follicles in the range 2–10 mm can readily be counted on transvaginal ultrasound to quantify an AFC and thereby predict ovarian response (2), although some clinics use a more limited range due to the increased variability in number of the larger follicles (67).

Factors that can influence AFC

Technical issues

The theoretical advantage of AFC over a biochemical marker is that transvaginal sonography is available in any reproductive clinic, hence AFC can be readily performed and provides immediate results. That the wide availability of ultrasound may have compounded the technical issues has not been fully appreciated. Inter-observer and intra-observer variability in AFC determination have been robustly analyzed (76,77), illustrating the key limitation of this biomarker as currently performed. The commonly used two-dimensional (2D) technique in estimating the AFC has wide limits of agreement varying from +8 to −7 follicles when two consecutive measures are performed by the same operator, or +7 to −5 follicles with two different experienced operators (77). This is sufficient to alter clinical management at an individual level and can introduce significant measure bias when pooling data in clinical research (14). The reproducibility of the test improves only modestly when 3D techniques and offline analysis of the stored images are performed (77–80), with additional analysis time expense, increased workload, and loss of the benefit of immediacy. The limits of agreement between consecutive measurements of AFC become significantly narrower when automatic analysis and counting or post-processing are implemented (77); however, the drawbacks of longer offline analysis persist, and this approach has not been widely adopted. In addition, the validity of automated analysis is questionable given that it only counts approximately a third of the antral follicles measured with manual or the post-processing techniques (77).

A consensus statement was expected to resolve the issues of the large variability in AFC measurement by describing in detail the optimal technique with the use of the appropriate ultrasound probe in carefully selected patients (81). However, this report excluded women with

previous ovarian surgery, ovarian endometriosis, and a single ovary or irregular cycles, thereby excluding a significant proportion of the patients seen in the fertility clinic. Furthermore, the technical settings of ultrasound, such as depth, gain, and focus, were not discussed (81). This contrasts with the use of ultrasound-based biomarkers such as nuchal translucency in prenatal diagnosis, which continuously undergo rigorous external quality assessment to ensure homogeneity and high accuracy in measurement by certified clinicians. The lack of this standardization for AFC measurement may underlie its limited transportability across different operators, sites, and settings. The degree of variation observed even in the research setting when the technique is as standardized as possible between centers (14) illustrates the difficulties that need to be overcome.

The steady improvement in ultrasound resolution over the last decade has led to a recent re-evaluation of the AFC threshold for diagnosing polycystic ovarian morphology, which is now suggested to increase from ≥12 to ≥25 follicles (82). That the upper limit for a normal AFC has more than doubled within such a short timeframe is not dissimilar to the issues that faced the AMH assay and its lack of standardization. The previous threshold of 12 follicles with currently available high-resolution ultrasound would classify an expected "normal" responder as a "high" responder with inappropriate selection of AFC-stratified hyperstimulation protocols, and suboptimal stimulation for that patient. Likewise, the cutoff values suggested in studies for predicting poor response have evolved substantially from AFC <3 in 1998 (83) to <12 in 2009 (84). Thus, the current suggested AFC thresholds of expected poor or normal ovarian response (37,38,40,85) may not be transferable to future ultrasound machines, which will inevitably have higher resolution. This issue is also illustrated by AFC determination by magnetic resonance imaging, which gives significantly higher values than when measured by ultrasound (86). Inevitably, AFC thresholds for clinical practice will always be subject to lagging behind the resolution of the available technology, thereby adversely impacting on its potential role as a globally applicable biomarker.

With regard to patient acceptability of transvaginal ultrasound, we are not aware of work to date specifically targeting the views of infertile patients. Studies have reported that patients regard transvaginal scans as uncomfortable procedures, but they are willing to undergo the procedure if recommended, while others have reported that only 10% of patients find the procedure embarrassing, stressful, or uncomfortable (87,88). Additionally, transvaginal ultrasound provides a wealth of useful clinical data above that of just AFC measurement, justifying its place as a pivotal investigation for infertile patients (89).

Inter-individual variation

Factors affecting AFC have been understudied relative to AMH, but are likely to be similar given that they both reflect similar stages in the highly dynamic processes of follicular activity. In contrast to the large population cohorts for analysis of AMH, assessment of the relationship of AFC with age has previously only been examined in relatively small sample sizes (90–93). More recent analysis of >10,000 infertile women and 5000 oocyte donors has demonstrated that infertile women have a reduced AFC relative to oocyte donors and an increased prevalence of women with low ovarian reserve (Figure 46.1) (94). Although all confirm an age-related decline in AFC with age, they also recognize the substantial variation present at a given age. A single cross-sectional study suggested ethnic differences, with the average age-specific AFC in Indian women being lower than in Caucasians. However, the study did not provide 95% CIs for each ethnicity-specific regression line and was limited by its sample size (n = 229 Caucasians, n = 236 Indian women) (36). Smokers were reported as having a lower AFC compared with non-smokers of similar age (95). It is unclear whether this is a result of accelerated depletion of the primordial pool or modified follicular recruitment among smokers. The former mechanism of the effect of smoking on the ovaries has been suggested in animal models (96,97) and may also be relevant in human fetal ovaries (98) and linked to the increased risk of earlier onset of menopause among current smokers (99). As with AMH, AFC is reduced in cancer patients at the time of diagnosis (100). It is also now clear that AFC behaves similarly to AMH in response to exogenous hormones (76). Gonadotropin suppression caused by the contraceptive pill decreased the number of antral follicles, particularly those measuring greater than 6 mm in diameter (101). In line with this, a cross-sectional study showed that women taking the contraceptive pill had persistently lower AFCs compared with women of the same age with natural cycles (33).

Confirmation of the effect size of these factors on AFC would be useful, but is unlikely to have an impact on the clinical application of AFC for the prediction of ovarian response, given that these factors are not modified prior to ovarian stimulation.

Intra-individual variation

AFC exhibits significant variation within and across consecutive cycles (67,73,76,102). Assessment of the ICC of AFC showed that it had a modest ICC of 0.71 between two cycles and of 0.69 within one cycle, which are substantially worse than for AMH; 0.89 and 0.87, respectively (73). The source of this intra-cycle variability appears to be the variation in the number of the larger follicles (6–10 mm in diameter) (67). Given these concerns, the consensus statement suggested that AFC should be performed from day 2 to day 4 of an index cycle (81). This recommendation is a significant limitation and inconvenience to both patient and clinic, and does not apply to women with irregular cycles.

In conclusion, despite its ready availability in every reproductive clinic, AFC is most accurately applied in well-selected patients, has limited flexibility in relation to the phase of the cycle, and exhibits substantial operator- and

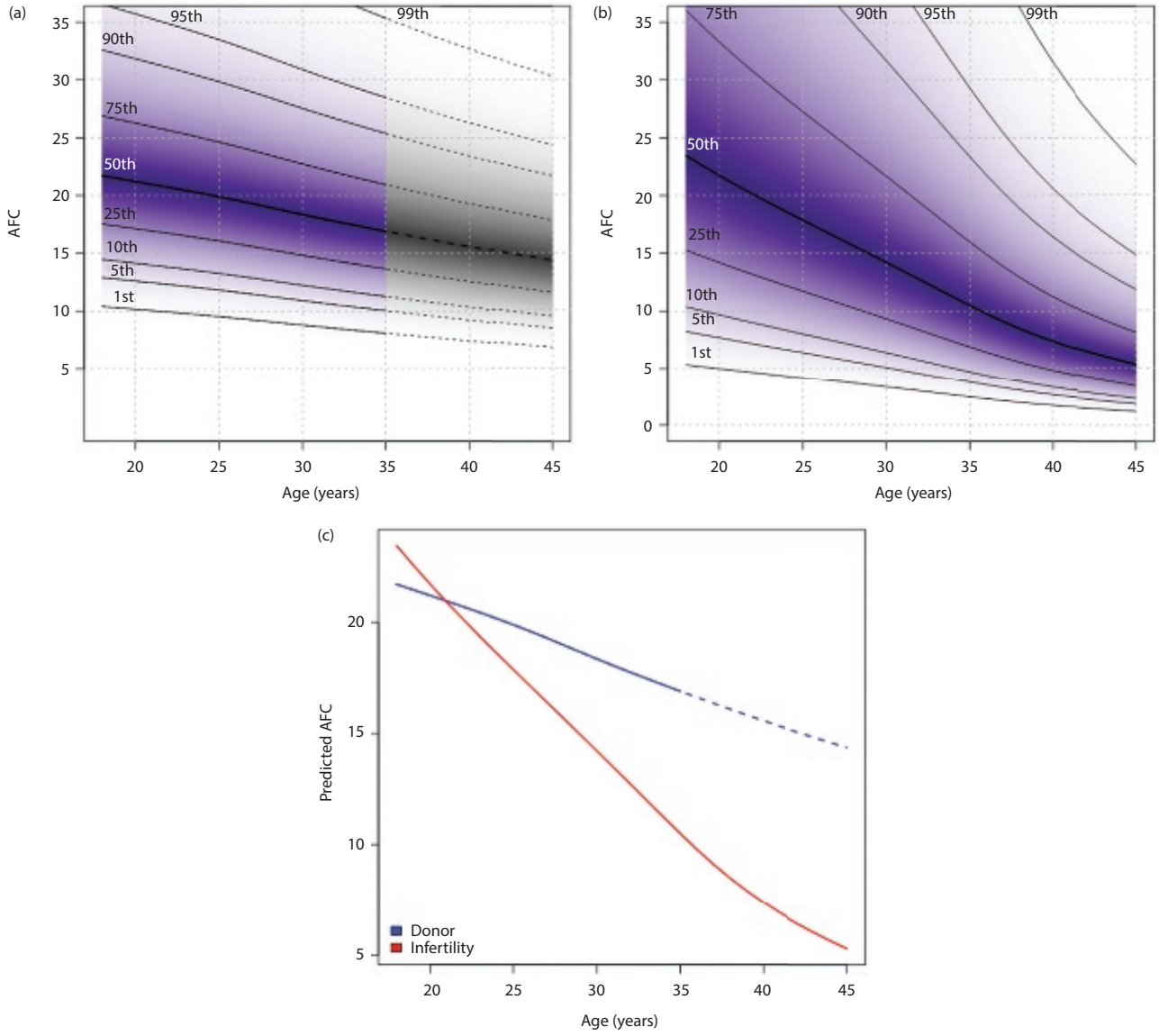

Figure 46.1 Comparison of age-specific declines in AFCs from oocyte donors and infertile patients. (a) Age-specific ranges of AFCs in potential oocyte donors. Black lines represent percentiles as labeled. The grey shaded area is for donors over the age of 35 years and have resulted from extrapolation of the spline prediction model. (b) Age-specific ranges of AFCs in women seeking fertility treatment. (c) Spline prediction declines of AFCs with aging in potential donors (blue line) and infertile patients (red line). *Abbreviation*: AFC, antral follicle count. (Data from Iliodromiti S et al. *J Clin Endocrinol Metab* 2016; 101(9): 1548–54.)

instrument-dependent performance. This would be anticipated to limit its clinical and research applicability.

Comparison of AMH and AFC performance in predicting ovarian response

AMH and AFC have often been considered as interchangeable biomarkers for the prediction of ovarian response prior to commencement of ovarian stimulation. Acceptance of this is highlighted by their recent inclusion as alternative independent markers, combined with age, in the consensus statement on the definition of expected poor response (37). At the other end of the ovarian response spectrum, despite excessive ovarian response not having an equivalent consensus statement definition, stratified stimulation algorithms with indicative starting dosing of gonadotropins have been developed based on specific cutoff points of either single biomarker in order to avoid oocyte yields in excess of 15–20 oocytes (40,103,104). Given that many centers may have had limited access to both biomarkers or exhibit a preference for one or the other, there has been limited consideration of their potential overlap and their relative strengths.

Observational cohort data assessing predictive performance of AMH and AFC

In excess of 40 cohort studies and an IPD meta-analyses have examined the performance of AMH in the prediction of poor ovarian response (7). Widely ranging threshold

values for apparent optimal trade-off between sensitivity and specificity have been proposed. That the threshold values for AMH range from 0.1 to 2.97 ng/mL primarily arises from the considerable heterogeneity introduced by the use of three different AMH assays, the inconsistencies in the definition of poor response, and the variable baseline characteristics and fertility potentials of the participants across the studies. Despite these limitations, the majority of the pooled studies reported that AMH has a sensitivity greater than 70% and a specificity of over 70% in predicting poor response in women undergoing fertility treatment (7). Similarly, 22 cohort studies and one IPD meta-analysis have assessed the performance of AFC (7); the performance characteristics of AFC also varied substantially among the studies, with threshold values ranging from 3 to 12, but the body of evidence suggests equivalent sensitivity and specificity to AMH in poor response prediction.

Single-center studies evaluating both biomarkers for the prediction of poor response have also generally not revealed significant differences in their performance (73,99–108), although a minority of studies demonstrated significant superiority of one marker over the other; either AFC over AMH (109) or AMH over AFC (110,111). A systematic review assessing the performance of each biomarker echoed the above findings, summarizing that both biomarkers have equivalent receiver-operating curves (ROCs) in the prediction of poor response (3). This finding was replicated in a recent IPD analysis that demonstrated that the area under the curve (AUC) of the age-adjusted ROC for AMH in predicting poor response was 0.77 (95% CI: 0.70–0.83), practically identical to that of AFC (AUC 0.79, 95% CI: 0.73–0.85) (6).

For excessive response, the same issues have been observed; there have been in excess of 16 cohort studies and one IPD meta-analysis for AMH with a diverse range of threshold values and associated performance characteristics reported. For AFC, there have been seven cohort studies and one IPD meta-analysis, but again no consensus on an overall threshold and anticipated performance. Cumulative analyses suggest comparable accuracy of AMH and AFC in predicting excessive ovarian response (4,5,7).

Collectively, the above data provide apparent confirmation that both markers are equally effective at predicting poor and excessive ovarian response. However, observational cohort studies from individual clinics may have potentially inflated the performance of the association between exposure (ovarian reserve test) and outcome (response), particularly because the value of the test may have influenced the allocation of treatment and thus the outcome of interest (ovarian response) or through confounding, a known major limitation of observational studies. It is possible to reduce confounding in observational studies by restriction or matching, and in the statistical analysis by techniques such as stratification or multivariable analyses. These methods, however, require that the confounding variables are known and measured. Notably, few of the single-center studies have undertaken this level of detailed analysis. In contrast, a key strength of RCTs is that the randomization process allows the investigator to assume that not only known but also unknown potential confounders are distributed evenly among the treatment arms. Although the generalizability of RCTs can be limited due to the often stricter inclusion criteria and rigid protocols, RCTs are specifically designed to overcome the issues of differential confounding and selection bias between the treatment groups, making them strong candidates for examining the strength of association between exposures and outcomes of interest, and hence their widespread recognition as providing high-level evidence. The marked heterogeneity in reported threshold values and performance characteristics from the single-center studies implies that each individual center would be required to develop its own thresholds. This does not have biological plausibility: there should not be marked heterogeneity in ovarian response of two biologically identical women treated in two different centers using an identical protocol. In the absence of such biological identity, we can assess how these models have performed in RCTs. Only one RCT has been specifically designed to compare AMH and AFC (with other markers) as predictive biomarkers (15). However, both have been included in four studies of protocols of ovarian stimulation for IVF (15,112–114). The comparison of AMH and AFC in these studies is thus a secondary or *post-hoc* analysis, and therefore potentially not as robust as if it were the primary analysis, as it was one for one trial. Issues of study design are also relevant, particularly if AMH is measured centrally while AFC is derived locally, which will inherently favor AMH. However, the relative ease of standardization of hormone assays compared to ultrasound analysis, with established quality control systems such as the U.K. National External Quality Assessment Service (NEQUAS), is an inherent potential advantage of AMH in determining clinically relevant cutoff values for widespread use, as well as in multicenter research.

RCTs assessing predictive performance of AMH and AFC

Initial doubts about the equivalence of AFC and AMH in response prediction started appearing when the pharmaceutically sponsored international multicenter Xpect trial failed to show an independent association between AFC and the number of retrieved oocytes (15). In contrast, AMH was the only robust predictor of ovarian response in univariate and multivariate models. Similar findings were observed in both treatment arms (i.e., whether patients were randomly assigned to receive treatment with oral contraceptives prior to controlled ovarian stimulation or no pretreatment) (15). When poor or excessive response were assessed as dichotomous outcomes, AMH remained a significant predictive variable in each treatment arm, with no independent association observed for AFC. Notably, the specific purpose of this trial was to identify factors capable of predicting ovarian response in patients undergoing their first treatment cycle with a daily dose of 200 IU recombinant FSH in a GnRH antagonist protocol, further strengthening the conclusion that AMH was the superior biomarker.

A subsequent pharmaceutically sponsored international multicenter RCT of two gonadotropin preparations (MEGASET trial) added significant weight to the above findings. This study demonstrated a significant association between AMH and oocyte yield, number of blastocysts, and cumulative live birth, but surprisingly, it did not detect a significant univariate association between AFC and any of these outcomes (14); consequently, only AMH was associated with oocyte yield in multivariate models. Although the findings of the trial were initially criticized as potentially being attributable to marked operator variability across centers, secondary analysis demonstrated that there were only weak associations between AFC and oocyte yield within individual centers (112). Retrospective

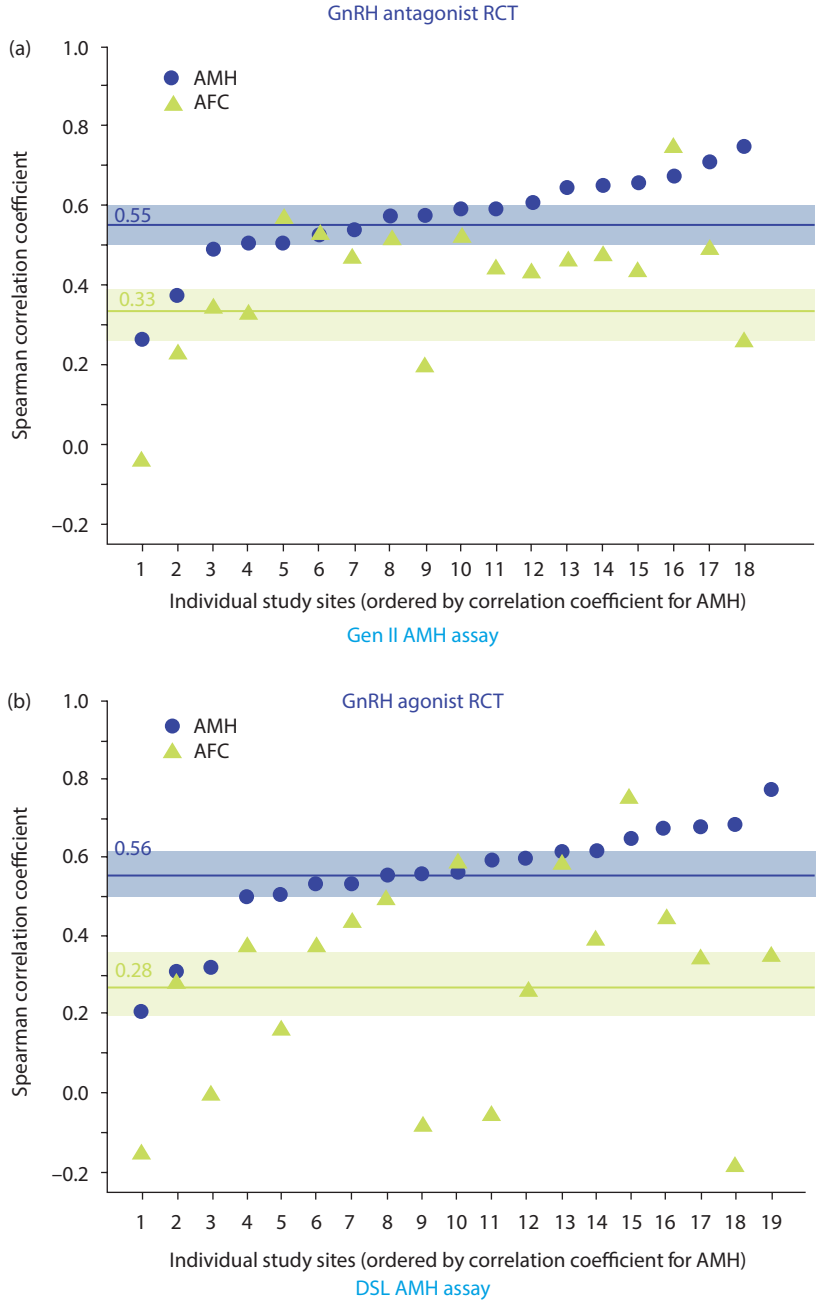

Figure 46.2 Correlations between basal values of AMH and AFC, respectively, and number of oocytes retrieved in patients treated in (a) a GnRH antagonist trial and (b) a long GnRH agonist trial at individual *in vitro* fertilization clinics. The lines show the overall mean ± 95% confidence interval correlation coefficients of AMH and AFC for the whole cohorts. AMH was a stronger predictor of oocyte yield (i.e., a difference in correlation coefficient of >0.10) in 13 of 19 study centers in the long GnRH agonist trial and in 12 of 18 study centers in the GnRH antagonist trial. Only study center no. 15 in the long GnRH agonist trial exhibited a stronger association with AFC than the remainder of the study centers in both trials. *Abbreviations*: AFC, antral follicle count; AMH, anti-Mullerian hormone; DSL, Diagnostic System Laboratories; Gen II, Generation II; GnRH, gonadotropin-releasing hormone; RCT, randomized controlled trial. (Data from Nelson SM et al. *Fertil Steril* 2015; 103(4); 923–30.e1.)

analysis of another multicenter RCT (MERIT trial) comparing two different gonadotropins in a long GnRH agonist protocol has also demonstrated that AMH had a consistently greater association with ovarian response compared with AFC across different sites (113). This site-specific analysis of the correlation of AFC and AMH with oocyte yield overcomes objections that the superior performance of AMH over AFC in these two multicenter trials may have been attributed to marked sonographer-dependent variability across the study sites and integrated data evaluation rather than by the actual performance at each study center (Figure 46.2) (113). Multivariate analysis confirmed that knowledge of AFC did not enhance the predictive power of AMH in these two trials. Apparent conflicting findings resulted from the retrospective analysis of the Engage trial, a double-blind RCT assessing the ongoing clinical pregnancy rate after a bolus dose of corifollitropin-α versus daily recombinant FSH injections, which supported the inclusion of AFC in prognostic models for high and low response (115); however, AMH was not measured. Retrospective analysis of the Pursue trial, which was similar to Engage in design, has shown that models incorporating only age and AMH have optimal characteristics for predicting high and low responders, whereas inclusion of AFC in the models only minimally improved the performance of the models (114). Table 46.1 summarizes the performance characteristics of the univariate or composite models, which include AMH or AFC as predictor variables from the above trials.

In a Phase II trial of a novel recombinant FSH (follitropin-δ), a range of biomarkers were considered for prediction of ovarian response (NCT01426386). Of those examined (age, AMH, AFC, FSH, and inhibin B), AMH best predicted the ovarian response with no or negligible explanation of the variation in oocytes retrieved by the addition of the other markers (116). By utilizing this information, a novel dosing algorithm that incorporated AMH and bodyweight in order to individualize follitropin dose was associated with a reduction in iatrogenic complications, while maintaining pregnancy and live birth rates as compared to conventional ovarian stimulation (117).

These consistent data from several large-scale RCTs assessing biomarker performance in the prediction of ovarian response indicate the inherent limitations of AFC for predicting ovarian response in a multicenter context, whereas AMH, when centrally analyzed, is the more accurate biomarker under those conditions.

Tailoring treatment based on ovarian biomarkers

The main objective of individualization of treatment based on ovarian biomarkers is to offer the best treatment tailored to a patient's unique characteristics, thus maximizing success, eliminating iatrogenic risks such as OHSS, and minimizing the risk of cycle cancellation (Figure 46.3). Although personalization of IVF treatment may lead to an improvement in patient compliance and better clinical practice, clinicians have largely struggled to achieve this. The difficulty derives from the

Table 46.1 Performance characteristics of prognostic models for ovarian response resulting from analyses of randomized controlled trial data

	Low ovarian response		High ovarian response	
Trial	**Predictor variables**	**Performance characteristics**	**Predictor variables**	**Performance characteristics**
Xpect	AMH	AUC: 0.84	AMH	AUC: 0.77
	AMH and smoking	AUC: 0.85	AMH and AFC	AUC: 0.80
MEGASET	AMH	AUC: 0.78/0.90[a]	AMH	AUC: 0.77/0.81[a]
	AFC	AUC: 0.67/0.74[a]	AFC	AUC: 0.64/0.65[a]
Engage[b]	Age	AUC: 0.63	Age	AUC: 0.64
	Age and AFC	AUC: 0.75	Age and AFC	AUC: 0.75
Pursue	Age	AUC: 0.61	Age	AUC: 0.61
	Age and AMH	AUC: 0.87	Age and AMH	AUC: 0.86
	Age, AMH, and AFC	AUC: 0.88	Age, AMH, and AFC	AUC: 0.88
Oocyte yield				
MERIT	AMH	R^2: 0.29		
	AFC	R^2: 0.07		
	AMH and AFC	R^2: 0.30		
MEGASET	AMH	R^2: 0.23		
	AFC	R^2: 0.07		
	AMH and AFC	R^2: 0.23		

Abbreviations: AMH, anti-Mullerian hormone; AFC, antral follicle count; AUC, area under the curve of the receiver-operating curve.

[a] Performance in each treatment arm.

[b] AMH was not measured.

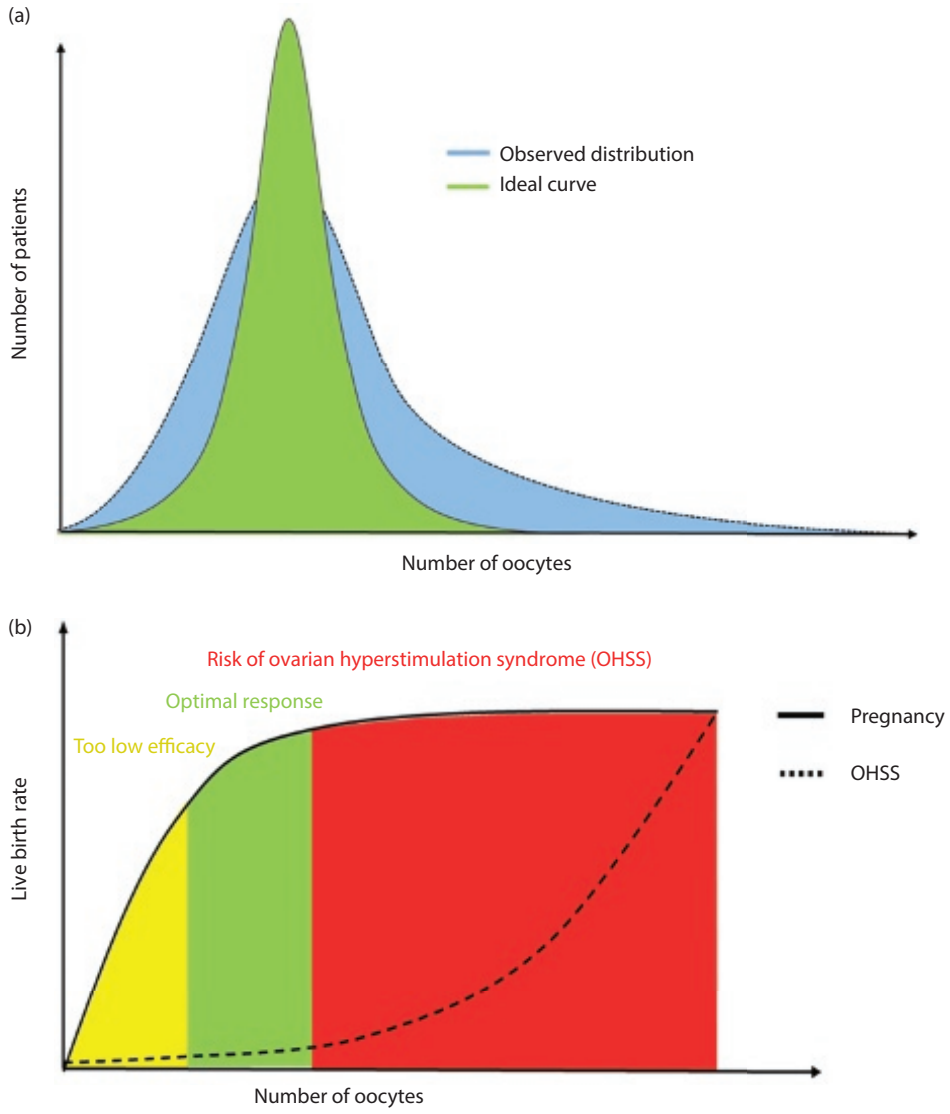

Figure 46.3 Optimization of ovarian response in order to facilitate a reduction in iatrogenic complications. (a) shows blue the anticipated spread of oocytes if the patient population were all treated with the same dose. The green reflects the aim of individualized dosing, with a higher percentage of the population attaining an optimal dose, with fewer poor responders and fewer excessive response. (b) illustrates the steep increase in live birth rates with increasing oocyte yield up to 15 oocytes. Beyond that point there is no further benefit in live birth rates, just increasing risk of OHSS.

vast number of drugs and choices available for ovarian stimulation, such as the GnRH analogs, the gonadotropin preparations, and other adjuvant therapies, and from the lack of a clear evidence-based therapeutic approach for different subgroups of patients. To date, a variety of protocols of approaches have been proposed based on pretreatment AMH and/or AFC, but these have broadly classified patients based on absolute thresholds of AMH or AFC, or have been small, single-center studies with no external validation (85,103,118).

Despite these limitations, there is now widespread agreement that for those identified with a high functional ovarian reserve, GnRH antagonist-controlled strategies are preferable. This reflects their altered follicular recruitment patterns, lower risk of OHSS, and ability to trigger with a GnRH agonist rather than human chorionic gonadotropin if required. There is, however, still considerable debate regarding the optimal protocol for those with a low ovarian reserve, or the value of GnRH agonist- versus GnRH antagonist-controlled cycles for those at low risk of developing an excessive ovarian response. Recognition that the starting dose of gonadotropin is critical, that there is a strong association of baseline AMH with ovarian response, and that bodyweight modifies the exposure to exogenous gonadotropins was the rational for the creation of a unique algorithm that encompassed AMH and bodyweight for individualization of the follitropin-δ dose in the ESTHER-1 study (116). This landmark trial confirmed that pretreatment AMH enables anticipation of the likely ovarian response and individualization of the FSH dose to modify oocyte yield and reduce iatrogenic complications while maintaining efficacy. This sets the new benchmark

for ovarian stimulation, with additional phenotyping of patients potentially improving identification of those who will benefit from alterations to this simple algorithm.

CONCLUSION

Significant changes have occurred in the measurement techniques for both AMH and AFC over the last decade, such that the appropriate reference values for both biomarkers have changed substantially, and indeed further change is expected. Both reflect a very similar ovarian follicle population, and thus, if perfectly measured, would be expected to have similar values; supported by single-site observational cohorts, this underpins the classical viewpoint that these biomarkers exhibit equivalent performance characteristics for the prediction of ovarian response. However, it appears likely that this equivalence has been overstated due to being inflated by study design, and emerging data from large-scale multicenter RCTs indicate substantially better performance of AMH. International standardization of AMH combined with a robust automated assay are likely to enhance its status as the biomarker of choice for assessing ovarian response. However, the advantages of ultrasound for structural assessment will mean that it will continue to have an important role in the pre-assessment of infertile woman. Clinicians will inevitably continue to assess and debate the value of individual ovarian reserve biomarkers, but we now have clear evidence that AMH can facilitate individualization of treatment and improve outcomes.

REFERENCES

1. Tunstall-Pedoe H. Cardiovascular risk and risk scores: ASSIGN, Framingham, QRISK and others: How to choose. *Heart* 2011; 97(6): 442–4.
2. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006; 12(6): 685–718.
3. Broer SL, Mol BWJ, Hendriks D, Broekmans FJM. The role of antimullerian hormone in prediction of outcome after IVF: Comparison with the antral follicle count. *Fertil Steril* 2009; 91(3): 705–14.
4. Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJM. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. *Hum Reprod Update* 2011; 17(1): 46–54.
5. Broer SL, Dolleman M, van Disseldorp J et al. Prediction of an excessive response in *in vitro* fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: An individual patient data meta-analysis. *Fertil Steril* 2013; 100(2): 420–9.e7.
6. Broer SL, van Disseldorp J, Broeze KA et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: An individual patient data approach. *Hum Reprod Update* 2013; 19(1): 26–36.
7. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: From theory to practice. *Hum Reprod Update* 2014; 20(1): 124–40.
8. Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Mullerian hormone for live birth after assisted conception: A systematic review and meta-analysis of the literature. *Hum Reprod Update* 2014; 20(4): 560–70.
9. Dewailly D, Andersen CY, Balen A et al. The physiology and clinical utility of anti-Mullerian hormone in women. *Hum Reprod Update* 2014; 20(3): 370–85.
10. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 2003; 18(2): 323–7.
11. Nardo LG, Christodoulou D, Gould D, Roberts SA, Fitzgerald CT, Laing I. Anti-Mullerian hormone levels and antral follicle count in women enrolled in *in vitro* fertilization cycles: Relationship to lifestyle factors, chronological age and reproductive history. *Gynecol Endocrinol* 2007; 23(8): 486–93.
12. Yang YS, Hur MH, Kim SY, Young K. Correlation between sonographic and endocrine markers of ovarian aging as predictors for late menopausal transition. *Menopause* 2011; 18(2): 138–45.
13. Leonhardt H, Hellstrom M, Gull B et al. Ovarian morphology assessed by magnetic resonance imaging in women with and without polycystic ovary syndrome and associations with antimullerian hormone, free testosterone, and glucose disposal rate. *Fertil Steril* 2014; 101(6): 1747–56.e1–3.
14. Arce JC, La Marca A, Mirner Klein B, Nyboe Andersen A, Fleming R. Antimullerian hormone in gonadotropin releasing-hormone antagonist cycles: Prediction of ovarian response and cumulative treatment outcome in good-prognosis patients. *Fertil Steril* 2013; 99(6): 1644–53.
15. Andersen AN, Witjes H, Gordon K, Mannaerts B. Predictive factors of ovarian response and clinical outcome after IVF/ICSI following a rFSH/GnRH antagonist protocol with or without oral contraceptive pre-treatment. *Hum Reprod* 2011; 26(12): 3413–23.
16. Nelson SM, Telfer EE, Anderson RA. The ageing ovary and uterus: New biological insights. *Hum Reprod Update* 2013; 19(1): 67–83.
17. Peters H, Byskov AG, Grinsted J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. *Clin Endocrinol Metab* 1978; 7(3): 469–85.
18. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. *PLoS One* 2010; 5(1): e8772.
19. Zuccotti M, Merico V, Cecconi S, Redi CA, Garagna S. What does it take to make a developmentally competent mammalian egg? *Hum Reprod Update* 2011; 17(4): 525–40.

20. Kelsey TW, Anderson RA, Wright P, Nelson SM, Wallace WH. Data-driven assessment of the human ovarian reserve. *Mol Hum Reprod* 2012; 18(2): 79–87.
21. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertil Steril* 2011; 95(1): 170–5.
22. Anderson RA, Nelson SM, Wallace WH. Measuring anti-Mullerian hormone for the assessment of ovarian reserve: When and for whom is it indicated? *Maturitas* 2012; 71(1): 28–33.
23. Baker ML, Metcalfe SA, Hutson JM. Serum levels of mullerian inhibiting substance in boys from birth to 18 years, as determined by enzyme immunoassay. *J Clin Endocrinol Metab* 1990; 70(1): 11–5.
24. Hudson PL, Dougas I, Donahoe PK et al. An immunoassay to detect human mullerian inhibiting substance in males and females during normal development. *J Clin Endocrinol Metab* 1990; 70(1): 16–22.
25. Josso N, Legeai L, Forest MG, Chaussain JL, Brauner R. An enzyme linked immunoassay for antimullerian hormone: A new tool for the evaluation of testicular function in infants and children. *J Clin Endocrinol Metab* 1990; 70(1): 23–7.
26. Gassner D, Jung R. First fully automated immunoassay for anti-Mullerian hormone. *Clin Chem Lab Med* 2014; 52(8): 1143–52.
27. Sowers MR, Eyvazzadeh AD, McConnell D et al. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. *J Clin Endocrinol Metab* 2008; 93(9): 3478–83.
28. Freeman EW, Sammel MD, Lin H, Gracia CR. Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. *J Clin Endocrinol Metab* 2012; 97(5): 1673–80.
29. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB. A validated model of serum anti-mullerian hormone from conception to menopause. *PLoS One* 2011; 6(7): e22024.
30. Nelson SM, Messow MC, Wallace AM, Fleming R, McConnachie A. Nomogram for the decline in serum antimullerian hormone: A population study of 9,601 infertility patients. *Fertil Steril* 2011; 95(2): 736–U813.
31. Nelson SM, Messow MC, McConnachie A et al. External validation of nomogram for the decline in serum anti-Mullerian hormone in women: A population study of 15,834 infertility patients. *Reprod Biomed Online* 2011; 23(2): 204–6.
32. Almog B, Shehata F, Suissa S et al. Age-related normograms of serum antimullerian hormone levels in a population of infertile women: A multicenter study. *Fertil Steril* 2011; 95(7): 2359–63.
33. Bentzen JG, Forman JL, Johannsen TH, Pinborg A, Larsen EC, Andersen AN. Ovarian antral follicle subclasses and anti-mullerian hormone during normal reproductive aging. *J Clin Endocrinol Metab* 2013; 98(4): 1602–11.
34. Nelson SM, Iliodromiti S, Fleming R, Anderson R, McConnachie A, Messow CM. Reference range for the antimullerian hormone Generation II assay: A population study of 10,984 women, with comparison to the established Diagnostics Systems Laboratory nomogram. *Fertil Steril* 2014; 101(2): 523–9.
35. Bleil ME, Gregorich SE, Adler NE, Sternfeld B, Rosen MP, Cedars MI. Race/ethnic disparities in reproductive age: An examination of ovarian reserve estimates across four race/ethnic groups of healthy, regularly cycling women. *Fertil Steril* 2014; 101(1): 199–207.
36. Iglesias C, Banker M, Mahajan N, Herrero L, Meseguer M, Garcia-Velasco JA. Ethnicity as a determinant of ovarian reserve: Differences in ovarian aging between Spanish and Indian women. *Fertil Steril* 2014; 102(1): 244–9.
37. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of "poor response" to ovarian stimulation for *in vitro* fertilization: The Bologna criteria. *Hum Reprod* 2011; 26(7): 1616–24.
38. Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: Prediction of live birth and extremes of response in stimulated cycles—Implications for individualization of therapy. *Hum Reprod* 2007; 22(9): 2414–21.
39. Dewailly D, Gronier H, Poncelet E et al. Diagnosis of polycystic ovary syndrome (PCOS): Revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. *Hum Reprod* 2011; 26(11): 3123–9.
40. Nelson SM. Biomarkers of ovarian response: Current and future applications. *Fertil Steril* 2013; 99(4): 963–9.
41. Cui Y, Shi Y, Cui L, Han T, Gao X, Chen ZJ. Age-specific serum antimullerian hormone levels in women with and without polycystic ovary syndrome. *Fertil Steril* 2014; 102(1): 230–6.e2.
42. Dolleman M, Verschuren WM, Eijkemans MJ et al. Reproductive and lifestyle determinants of anti-Mullerian hormone in a large population-based study. *J Clin Endocrinol Metab* 2013; 98(5): 2106–15.
43. Fraser A, McNally W, Sattar N et al. Prenatal exposures and anti-Mullerian hormone in female adolescents: The Avon Longitudinal Study of Parents and Children. *Am J Epidemiol* 2013; 178(9): 1414–23.
44. Thomson RL, Buckley JD, Moran LJ et al. The effect of weight loss on anti-Mullerian hormone levels in overweight and obese women with polycystic ovary syndrome and reproductive impairment. *Hum Reprod* 2009; 24(8): 1976–81.
45. Vosnakis C, Georgopoulos NA, Armeni AK et al. Sibutramine administration decreases serum anti-Mullerian hormone (AMH) levels in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* 2012; 163(2): 185–9.

46. Freour T, Masson D, Mirallie S et al. Active smoking compromises IVF outcome and affects ovarian reserve. *Reprod Biomed Online* 2008; 16(1): 96–102.
47. Dewailly D, Gronier H, Poncelet E et al. Revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of the polycystic ovary (PCO). *Hum Reprod* 2011; 26: i6.
48. Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Mullerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. *J Clin Endocrinol Metab* 2013; 98(8): 3332–40.
49. Lauritsen MP, Bentzen JG, Pinborg A et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Mullerian hormone. *Hum Reprod* 2014; 29(4): 791–801.
50. Codner E, Iniguez G, Hernandez IM et al. Elevated anti-Mullerian hormone (AMH) and inhibin B levels in prepubertal girls with type 1 diabetes mellitus. *Clin Endocrinol (Oxf)* 2011; 74(1): 73–8.
51. Soto N, Iniguez G, Lopez P et al. Anti-Mullerian hormone and inhibin B levels as markers of premature ovarian aging and transition to menopause in type 1 diabetes mellitus. *Hum Reprod* 2009; 24(11): 2838–44.
52. Brouwer J, Laven JS, Hazes JM, Schipper I, Dolhain RJ. Levels of serum anti-Mullerian hormone, a marker for ovarian reserve, in women with rheumatoid arthritis. *Arthritis Care Res (Hoboken)* 2013; 65(9): 1534–8.
53. van Dorp W, van den Heuvel-Eibrink MM, de Vries AC et al. Decreased serum anti-Mullerian hormone levels in girls with newly diagnosed cancer. *Hum Reprod* 2014; 29(2): 337–42.
54. Lawrenz B, Fehm T, von Wolff M et al. Reduced pretreatment ovarian reserve in premenopausal female patients with Hodgkin lymphoma or non-Hodgkin-lymphoma—Evaluation by using antimullerian hormone and retrieved oocytes. *Fertil Steril* 2012; 98(1): 141–4.
55. Su HI, Flatt SW, Natarajan L, DeMichele A, Steiner AZ. Impact of breast cancer on anti-mullerian hormone levels in young women. *Breast Cancer Res Treat* 2013; 137(2): 571–7.
56. Anderson RA, Themmen AP, Al-Qahtani A, Groome NP, Cameron DA. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. *Hum Reprod* 2006; 21(10): 2583–92.
57. Nelson SM, Stewart F, Fleming R, Freeman DJ. Longitudinal assessment of antimullerian hormone during pregnancy-relationship with maternal adiposity, insulin, and adiponectin. *Fertil Steril* 2010; 93(4): 1356–8.
58. Hagen CP, Sorensen K, Anderson RA, Juul A. Serum levels of antimullerian hormone in early maturing girls before, during, and after suppression with GnRH agonist. *Fertil Steril* 2012; 98(5): 1326–30.
59. Kallio S, Puurunen J, Ruokonen A, Vaskivuo T, Piltonen T, Tapanainen JS. Antimullerian hormone levels decrease in women using combined contraception independently of administration route. *Fertil Steril* 2013; 99(5): 1305–10.
60. Su HI, Maas K, Sluss PM, Chang RJ, Hall JE, Joffe H. The impact of depot GnRH agonist on AMH levels in healthy reproductive-aged women. *J Clin Endocrinol Metab* 2013; 98(12): E1961–6.
61. Friedler S, Koc O, Gidoni Y, Raziel A, Ron-El R. Ovarian response to stimulation for fertility preservation in women with malignant disease: A systematic review and meta-analysis. *Fertil Steril* 2012; 97(1): 125–33.
62. Jeppesen JV, Anderson RA, Kelsey TW et al. Which follicles make the most anti-Mullerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. *Mol Hum Reprod* 2013; 19(8): 519–27.
63. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. *Hum Reprod* 2006; 21(12): 3103–7.
64. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y. Stable serum levels of anti-Mullerian hormone during the menstrual cycle: A prospective study in normo-ovulatory women. *Hum Reprod* 2007; 22(7): 1837–40.
65. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER, Broekmans FJ. Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. *J Clin Endocrinol Metab* 2006; 91(10): 4057–63.
66. Overbeek A, Broekmans FJ, Hehenkamp WJ et al. Intra-cycle fluctuations of anti-Mullerian hormone in normal women with a regular cycle: A re-analysis. *Reprod Biomed Online* 2012; 24(6): 664–9.
67. Deb S, Campbell BK, Clewes JS, Pincott-Allen C, Raine-Fenning NJ. Intracycle variation in number of antral follicles stratified by size and in endocrine markers of ovarian reserve in women with normal ovulatory menstrual cycles. *Ultrasound Obstet Gynecol* 2013; 41(2): 216–22.
68. Hadlow N, Longhurst K, McClements A, Natalwala J, Brown SJ, Matson PL. Variation in antimullerian hormone concentration during the menstrual cycle may change the clinical classification of the ovarian response. *Fertil Steril* 2013; 99(6): 1791–7.
69. Kissell KA, Danaher MR, Schisterman EF et al. Biological variability in serum anti-Mullerian hormone throughout the menstrual cycle in ovulatory and sporadic anovulatory cycles in eumenorrheic women. *Hum Reprod* 2014; 29(8): 1764–72.
70. Bungum L, Jacobsson AK, Rosen F et al. Circadian variation in concentration of anti-Mullerian hormone in regularly menstruating females: Relation to age, gonadotrophin and sex steroid levels. *Hum Reprod* 2011; 26(3): 678–84.

71. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: A review. *Hum Reprod Update* 2012; 18(1): 73–91.
72. Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J. High reproducibility of serum anti-Mullerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. *Hum Reprod* 2005; 20(4): 923–7.
73. van Disseldorp J, Lambalk CB, Kwee J et al. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. *Hum Reprod* 2010; 25(1): 221–7.
74. Charleston JS, Hansen KR, Thyer AC et al. Estimating human ovarian non-growing follicle number: The application of modern stereology techniques to an old problem. *Hum Reprod* 2007; 22(8): 2103–10.
75. Gougeon A. Dynamics of follicular growth in the human: A model from preliminary results. *Hum Reprod* 1986; 1(2): 81–7.
76. Hansen KR, Morris JL, Thyer AC, Soules MR. Reproductive aging and variability in the ovarian antral follicle count: Application in the clinical setting. *Fertil Steril* 2003; 80(3): 577–83.
77. Deb S, Jayaprakasan K, Campbell BK, Clewes JS, Johnson IR, Raine-Fenning NJ. Intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC. *Ultrasound Obstet Gynecol* 2009; 33(4): 477–83.
78. Merce LT, Gomez B, Engels V, Bau S, Bajo JM. Intraobserver and interobserver reproducibility of ovarian volume, antral follicle count, and vascularity indices obtained with transvaginal 3-dimensional ultrasonography, power Doppler angiography, and the virtual organ computer-aided analysis imaging program. *J Ultrasound Med* 2005; 24(9): 1279–87.
79. Jayaprakasan K, Walker KF, Clewes JS, Johnson IR, Raine-Fenning NJ. The interobserver reliability of off-line antral follicle counts made from stored three-dimensional ultrasound data: A comparative study of different measurement techniques. *Ultrasound Obstet Gynecol* 2007; 29(3): 335–41.
80. Jayaprakasan K, Campbell BK, Clewes JS, Johnson IR, Raine-Fenning NJ. Three-dimensional ultrasound improves the interobserver reliability of antral follicle counts and facilitates increased clinical work flow. *Ultrasound Obstet Gynecol* 2008; 31(4): 439–44.
81. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: Practical recommendations for better standardization. *Fertil Steril* 2010; 94(3): 1044–51.
82. Dewailly D, Lujan ME, Carmina E et al. Definition and significance of polycystic ovarian morphology: A task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update* 2014; 20(3): 334–52.
83. Chang MY, Chiang CH, Hsieh TT, Soong YK, Hsu KH. Use of the antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil Steril* 1998; 69(3): 505–10.
84. Melo MA, Garrido N, Alvarez C et al. Antral follicle count (AFC) can be used in the prediction of ovarian response but cannot predict the oocyte/embryo quality or the *in vitro* fertilization outcome in an egg donation program. *Fertil Steril* 2009; 91(1): 148–56.
85. Nelson SM, Yates RW, Lyall H et al. Anti-Mullerian hormone-based approach to controlled ovarian stimulation for assisted conception. *Hum Reprod* 2009; 24(4): 867–75.
86. Leonhardt H, Gull B, Stener-Victorin E, Hellstrom M. Ovarian volume and antral follicle count assessed by MRI and transvaginal ultrasonography: A methodological study. *Acta Radiol* 2014; 55(2): 248–56.
87. Dutta RL, Economides DL. Patient acceptance of transvaginal sonography in the early pregnancy unit setting. *Ultrasound Obstet Gynecol* 2003; 22(5): 503–7.
88. Basama FM, Crosfill F, Price A. Women's perception of transvaginal sonography in the first trimester; in an early pregnancy assessment unit. *Arch Gynecol Obstet* 2004; 269(2): 117–20.
89. Kelly SM, Sladkevicius P, Campbell S, Nargund G. Investigation of the infertile couple: A one-stop ultrasound-based approach. *Hum Reprod* 2001; 16(12): 2481–4.
90. Broekmans FJ, Faddy MJ, Scheffer G, te Velde ER. Antral follicle counts are related to age at natural fertility loss and age at menopause. *Menopause* 2004; 11(6 Pt 1): 607–14.
91. Almog B, Shehata F, Shalom-Paz E, Tan SL, Tulandi T. Age-related normogram for antral follicle count: McGill reference guide. *Fertil Steril* 2011; 95(2): 663–6.
92. La Marca A, Spada E, Sighinolfi G et al. Age-specific nomogram for the decline in antral follicle count throughout the reproductive period. *Fertil Steril* 2011; 95(2): 684–8.
93. Wiweko B, Prawesti DM, Hestiantoro A, Sumapraja K, Natadisastra M, Baziad A. Chronological age vs biological age: An age-related normogram for antral follicle count, FSH and anti-Mullerian hormone. *J Assist Reprod Genet* 2013; 30(12): 1563–7.
94. Iliodromiti S, Sanchez CI, Messow CM, Cruz M, Garcia-Velasco JA. Excessive age-related decline in functional ovarian reserve in infertile women: Prospective cohort of 15,500 women. *J Clin Endocrinol Metab* 2016; 101(9): 3548–54.
95. Freour T, Masson D, Dessolle L et al. Ovarian reserve and *in vitro* fertilization cycles outcome according to women smoking status and stimulation regimen. *Arch Gynecol Obstet* 2012; 285(4): 1177–82.
96. Tuttle AM, Stampfli M, Foster WG. Cigarette smoke causes follicle loss in mice ovaries at concentrations representative of human exposure. *Hum Reprod* 2009; 24(6): 1452–9.

97. Gannon AM, Stampfli MR, Foster WG. Cigarette smoke exposure leads to follicle loss via an alternative ovarian cell death pathway in a mouse model. *Toxicol Sci* 2012; 125(1): 274–84.
98. Anderson RA, McIlwain L, Coutts S, Kinnell HL, Fowler PA, Childs AJ. Activation of the aryl hydrocarbon receptor by a component of cigarette smoke reduces germ cell proliferation in the human fetal ovary. *Mol Hum Reprod* 2014; 20(1): 42–8.
99. Wellons MF, Bates GW, Schreiner PJ, Siscovick DS, Sternfeld B, Lewis CE. Antral follicle count predicts natural menopause in a population-based sample: The Coronary Artery Risk Development in Young Adults Women's Study. *Menopause* 2013; 20(8): 825–30.
100. Ebbel E, Katz A, Kao CN, Cedars M. Reproductive aged women with cancer have a lower antral follicle count than expected. *Fertil Steril* 2011; 96: S199–S200.
101. Deb S, Campbell BK, Pincott-Allen C, Clewes JS, Cumberpatch G, Raine-Fenning NJ. Quantifying effect of combined oral contraceptive pill on functional ovarian reserve as measured by serum anti-Mullerian hormone and small antral follicle count using three-dimensional ultrasound. *Ultrasound Obstet Gynecol* 2012; 39(5): 574–80.
102. Elter K, Sismanoglu A, Durmusoglu F. Intercycle variabilities of basal antral follicle count and ovarian volume in subfertile women and their relationship to reproductive aging: A prospective study. *Gynecol Endocrinol* 2005; 20(3): 137–43.
103. La Marca A, Papaleo E, Grisendi V, Argento C, Giulini S, Volpe A. Development of a nomogram based on markers of ovarian reserve for the individualisation of the follicle-stimulating hormone starting dose in *in vitro* fertilisation cycles. *BJOG* 2012; 119(10): 1171–9.
104. La Marca A, Grisendi V, Giulini S et al. Individualization of the FSH starting dose in IVF/ICSI cycles using the antral follicle count. *J Ovarian Res* 2013; 6(1): 11.
105. Van Rooij IAJ, Broekmans FJM, Te Velde ER et al. Serum anti-Mullerian hormone levels: A novel measure of ovarian reserve. *Hum Reprod* 2002; 17(12): 3065–71.
106. Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P. Antral follicle count, anti-mullerian hormone and inhibin B: Predictors of ovarian response in assisted reproductive technology? *BJOG* 2005; 112(10): 1384–90.
107. Kwee J, Schats R, McDonnell J, Themmen A, de Jong F, Lambalk C. Evaluation of anti-Mullerian hormone as a test for the prediction of ovarian reserve. *Fertil Steril* 2008; 90(3): 737–43.
108. Jayaprakasan K, Campbell B, Hopkisson J, Johnson I, Raine-Fenning N. A prospective, comparative analysis of anti-Mullerian hormone, inhibin-B, and three-dimensional ultrasound determinants of ovarian reserve in the prediction of poor response to controlled ovarian stimulation. *Fertil Steril* 2010; 93(3): 855–64.
109. Mutlu MF, Erdem M, Erdem A et al. Antral follicle count determines poor ovarian response better than anti-mullerian hormone but age is the only predictor for live birth in *in vitro* fertilization cycles. *J Assist Reprod Genet* 2013; 30(5): 657–65.
110. Ficicioglu C, Kutlu T, Baglam E, Bakacak Z. Early follicular antimullerian hormone as an indicator of ovarian reserve. *Fertil Steril* 2006; 85(3): 592–6.
111. McIlveen M, Skull JD, Ledger WL. Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve in the prediction of cycle cancellation in a high-risk IVF population. *Hum Reprod* 2007; 22(3): 778–85.
112. Arce JC, la Marca A, Klein BM, Nyboe Andersen A, Fleming R. Reply of the authors. *Fertil Steril* 2013; 100(2): e10.
113. Nelson SM, Klein BM, Arce JC. Comparison of anti-mullerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis patients at individual fertility clinics in two multicenter trials. *Fertil Steril* 2015; 103(4): 923–30.e1.
114. Oehninger S, Nelson SM, Verweij P, Stegmann BJ. Predictive factors for ovarian response in a corifollitropin alfa/GnRH antagonist protocol for controlled ovarian stimulation in IVF/ICSI cycles. *Reprod Biol Endocrinol* 2015; 13: 117.
115. Broekmans FJ, Verweij PJ, Eijkemans MJ, Mannaerts BM, Witjes H. Prognostic models for high and low ovarian responses in controlled ovarian stimulation using a GnRH antagonist protocol. *Hum Reprod* 2014; 29(8): 1688–97.
116. Arce JC, Klein BM, Erichsen L. Using AMH for determining a stratified gonadotropin dosing regimen for IVF/ICSI and optimizing outcomes. In: *Anti-Müllerian Hormone: Biology, Role in Ovarian Function and Clinical Significance*. Seifer DB, Tal R (eds). Nova Science Publishers, Hauppauge, NY, pp. 83–102, 2016.
117. Nyboe Andersen A, Nelson SM, Fauser BC, García-Velasco JA, Klein BM, Arce JC; ESTHER-1 study group. Individualized versus conventional ovarian stimulation for in vitro fertilization: a multicenter, randomized, controlled, assessor-blinded, phase 3 noninferiority trial. *Fertil Steril* 2017; 107(2): 387–396.e4.
118. Yates AP, Rustamov O, Roberts SA et al. Anti-Müllerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. *Hum Reprod* 2011; 26(9): 2353–62.

Monitoring ovarian response in assisted reproduction (*in vitro* fertilization and intracytoplasmic sperm injection)

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INTRODUCTION

Historically, monitoring of ovarian response by means of measuring ovarian hormones came into use for ovulation induction due to the complications of gonadotropin therapy, such as multiple births and ovarian hyperstimulation syndrome (OHSS). In ovulation induction cycles with gonadotropins, Klopper and coworkers showed that success and complication rates were not dependent on monitoring as such, but on the treatment protocol used. Monitoring merely gives us the possibility to decide how far we want to go (1). This may be true for ovulation induction cycles, but not for assisted reproduction technology (ART; i.e., *in vitro* fertilization [IVF] and intracytoplasmic sperm injection) cycles, where the number of transferred embryos has to be restricted, thereby minimizing the risk for multiple births as well as OHSS.

Two different main methods for evaluating utero-ovarian response have clinically been used for the last 30 years: hormone analysis (estradiol [E2] and progesterone [P]) and two-dimensional ultrasound (2D-US) imaging of ovarian follicle growth, endometrial growth, and characteristics. Due to the dramatic increase in the number of ART cycles worldwide and the various ovarian stimulation protocols used, different monitoring protocols by either one of these methods or a combination of both have been utilized. Due to the actual status of the ovarian response that ultrasound imaging provides, this method has become a very useful clinical tool. Furthermore, the development of the ultrasound equipment with better imaging and the possibility of performing complicated image processing with resulting three-dimensional ultrasound (3D-US) provide us with new interesting tools.

The 2D-US method was first evaluated in the natural cycle, but it was soon realized that it was in stimulated cycles where it could be really useful (2,3). One problem, though, was that the size (mean diameter as well as volume) of the mature follicle seems to vary greatly (4–7). To overcome this problem, several studies have been performed in order to determine the value of combining E2 as well as P measurements and ultrasound monitoring of follicular maturation in stimulated cycles (8–12). However, this combination of ultrasound and hormonal monitoring seemed to be particularly important in protocols with clomiphene citrate and gonadotropins alone, where the endogenous luteinizing hormone (LH) peak could not be controlled. With the introduction of gonadotropin-releasing hormone (GnRH) analogs combined with gonadotropins, the risk for high tonic levels of LH or premature LH peaks disappeared (13). Thus, with the use of GnRH agonist as well as GnRH antagonist protocols, there seems to be less need for extensive hormonal monitoring of IVF cycles (14). Ultrasound alone or, in certain cases, combined with one or two serum E2 measurements seems to be sufficient in the majority of IVF cycles (15). Thus, in most ART programs today, ultrasound imaging of follicular and endometrial growth has become a major method for monitoring ovarian stimulation. However, one has to be aware of the limitation of follicular diameter as measured by 2D-US for the prediction of oocyte maturity due to the wide range in diameter (16–22 mm) that is associated with a mature oocyte (6).

WHY MONITOR THE CYCLE?

Today, there are several reasons for E2 and/or US monitoring in connection to ART:

1. Predict the ovarian response to gonadotropins
   a. Identify poor responders
   b. Identify those at risk for OHSS
2. Monitoring the effect of pituitary down-regulation
3. Dosage adjustment of gonadotropins
   a. Avoid OHSS
   b. Achieve optimal response
4. Optimal time for administration of human chorionic gonadotropin (hCG)
5. Identify optimal time for transfer of frozen–thawed embryos

Monitoring a cycle before starting controlled ovarian hyperstimulation (COH) may identify poor responders as well as women at risk for ovarian OHSS (16,17). Thus, the pre-stimulation monitoring can be useful for predicting the response to gonadotropins. Furthermore, if a protocol with a GnRH agonist has been used, pituitary down-regulation has to be verified before starting with gonadotropins, and this can be performed by E2 measurement and/or US in order to exclude any follicular development in the ovary and to ensure a thin endometrium.

Since multiple follicular development plays a major role in the success of ART, ovarian stimulation with gonadotropins is nowadays routine for COH. Adjustment of gonadotropin dosage to secure adequate follicular development thus requires monitoring. Ideally, the monitoring method

should be noninvasive and indicate when the oocytes are mature. Unfortunately, there is no such method. All methods are indirect with regard to assessment of oocyte maturity. With regard to the huge number of ART cycles performed today, monitoring also has to be simple, safe, and preferably inexpensive. In this respect, 2D-US of follicular growth based on mean diameter during COH has proved to be a very practical way of monitoring ART cycles (18). However, E2 measurement alone or combination with US can also be used and may, in certain patients, be the method of choice (see below).

Prediction of ovarian response prior to stimulation is valuable since it can help us to choose a safe and adequate starting dose of gonadotropins. To estimate the ovarian reserve in order to predict the response to gonadotropins, there are two methods available: antral follicle count (AFC) and serum anti-Mullerian hormone (AMH) levels (19). AFC is determined by transvaginal ultrasound scanning (TVUS) and AMH by enzyme-linked immunosorbent assay. Both methods have been shown to predict oocyte yield and, in the case of AMH, to predict pregnancy and live birth rates (20). AFC is simple but operator dependent and shows more inter-cycle variability than AMH (20).

The purpose with monitoring a frozen embryo transfer cycle is to identify the moment when the endometrium is in synchrony with embryo development. Thus, the endometrium has to be prepared for the embryo (21). However, there is still no consensus concerning the preparation of the endometrium in ovulatory women. The simplest method of endometrium preparation is represented by natural cycle frozen embryo transfer, in which the endocrine preparation of the endometrium is achieved by endogenous sex hormones from a developing follicle/corpus luteum. Thus, in women with a regular menstrual cycle, the time of ovulation is monitored by urinary LH, often combined with ultrasound, for identifying the dominant follicle and the growing endometrium and its pattern. Ultrasound is thus scheduled in the mid to late follicular phase (cycle days 10–12). Timing of embryo transfer is determined by detecting the spontaneous LH surge (by urinary dipstick) or by administering hCG in order to initiate luteinization. The latter approach requires regular ultrasound monitoring of the dominant follicle to ensure appropriate timing of hCG administration (follicular diameter 16–20 mm). The transfer time depends on whether there are cleavage-stage embryos (transfer two or three days after ovulation) or blastocysts (transfer five days after ovulation).

In women with irregular cycles and in those with polycystic ovary syndrome, there is usually no spontaneous ovulation. Endometrial preparation can then be performed by hormone-replacement therapy (HRT). The most common stimulation is with estradiol valerate 6 mg/day starting on cycle day 1 (22). Ultrasound is scheduled for between treatment days 10 and 14. When the endometrium has reached a thickness of at least 9 mm and displays a triple-line pattern, micronized progesterone is given intravaginally two to three times a day and the time of transfer can be determined. Occasionally, a higher dosage of estradiol valerate may be required. In anovulatory women, alternative ways to stimulate ovulation and endometrial receptivity can be applied, such as stimulation by a low dose of follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG), by letrozole, or by clomiphene citrate. However, these cycles often require more monitoring than the HRT cycle.

METHODS FOR MONITORING OVARIAN RESPONSE

As mentioned above, there are principally two methods for monitoring ovarian response in ART cycles: hormone analysis (E2 and P) and US imaging of the ovary and endometrium. The methods can be used separately as well as combined. Furthermore, during recent years, US imaging techniques have been refined.

Thus, over recent years, five different ways of monitoring the ovarian response to COH have been evaluated and clinically used:

1. Serum hormones (E2 and/or P)
2. 2D-US monitoring of follicular diameter, endometrial thickness, and pattern
3. Combining serum hormone analysis and 2D-US
4. 3D-US monitoring of follicle volume
5. Perifollicular blood flow by means of power Doppler imaging

There is today an extensive literature regarding the use of all of the above methods for monitoring the ovarian response in ART.

It has been claimed that ultrasound should be used for timing of hCG administration and E2 to avoid complications (23). Which of the two methods is more reliable for the clinician's decision to increase, decrease, or stop gonadotropin administration seems to be very much dependent on experience and/or the routines used at the clinic. Furthermore, there is no consensus on how often the monitoring has to be done during ovarian stimulation. The frequency of monitoring seems to be arbitrarily chosen and thus varies considerably between different clinics. Thus, there are simple as well as complicated methods on how to monitor ART cycles by means of serum E2 and/or US. However, irrespective of the method chosen, there seems to be no difference in the outcome of the ART cycle as measured by clinical pregnancy rate and the incidence of OHSS (12).

SERUM E2 ALONE

Serum E2, the only method of monitoring ART cycles stimulated with gonadotropins, was mainly used in the early days of ART. The method for monitoring was based on the experience from monitoring of ovulation induction cycles. Some groups have tried to identify a certain serum E2 level that should be reached before hCG is given (24). Others have claimed that the number of days E2 increased was important, and thus administered hCG accordingly (25). Even though some groups still continue to use E2

measurements as the sole monitoring modality, some of them also use US.

US ALONE

2D-US measurement of follicular diameter and endometrial thickness is a noninvasive method. It can be performed by the clinician or specially trained sonographer, and gives the actual status of the number and size of growing follicles. Endometrial thickness as measured by ultrasound can be used as a bioassay of the total follicular E2 production. Vaginal ultrasound scanning of the uteroovarian response to gonadotropin stimulation is a simple and reliable method that is a clinically practical way of monitoring ART cycles.

Even though TVUS have been used for many years for monitoring ovarian response to gonadotropins, few studies assessing the efficacy and safety of monitoring with ultrasound alone have been performed. However, studies have showed similar pregnancy and OHSS rates for patients monitored by 2D-US only, compared to women monitored by 2D-US and E2 (7,8,12). An updated Cochrane review based on six randomized controlled trials including 781 women monitored by 2D-TVUS alone or in combination with E2 during ART use found no evidence that any of the methods were superior to the others with regard to clinical pregnancy rates or the incidence of OHSS. The study concluded that both methods are safe and reliable (12).

Over the years, US monitoring during the stimulated cycle has been performed in many different ways, very much dependent on the stimulation protocol used as well as the routine of the clinic. However, when utilizing US monitoring alone, certain recommendations need to be applied. In GnRH antagonist protocols, it has been recommended to start US monitoring on stimulation day 5, since this has been the day for starting with the GnRH antagonist in the majority of patients (26). Further US monitoring in those cycles is often scheduled for cycle days 8 or 9.

In patients at risk for OHSS, the first US is done on stimulation days 5–6, and the dosage of gonadotropin can be adjusted if necessary.

In cycles stimulated with corifollitropin-α (Elonva), a long-acting FSH preparation, the hormone is designed to be effective for seven days. The first US can therefore be performed on stimulation days 7 or 8. The need for additional days of stimulation with conventional FSH can then be determined (27).

SERUM E2 AND US

As mentioned above, to date, randomized trials do not support the notion that cycle monitoring by US plus serum E2 is more efficacious than cycle monitoring by ultrasound only in terms of clinical pregnancy rate and incidence of OHSS (12). However, combining ultrasound and serum E2 in women at risk for OHSS should be retained as precautionary good clinical practice in this group of patients. Furthermore, an economic evaluation of the costs of the two methods would be welcome.

SERUM P

It has been shown that in some patients there is a slight increase of P levels before the injection of hCG (28). This occurs mainly in high responders and despite concomitant agonist or antagonist. It is believed that the large number of follicles together produce P, which leaks into the circulation and affects the endometrium. The endometrium will be advanced and implantation and pregnancy rates drop. There has been speculation that this is more common in FSH stimulation than in the case of purified hMG, which contains LH activity. Therefore, it has been suggested to monitor serum P before hCG and, if P is elevated, to perform total freezing of the embryos/blastocysts. However, there is still controversy in this subject (29).

COLOR DOPPLER AND 3D-US

Doppler duplex systems combining pulsed Doppler and grayscale US made it possible to noninvasively study ovarian blood flow and to use that as a measurement of ovarian angiogenesis. From animal studies, it is well known that there is a correlation between follicular vascularity and oocyte maturation. In a classic clinical study by Nargrund and co-workers, significantly increased oocyte recovery from follicles was shown, with a high peak systolic velocity as measured by pulsed Doppler and grayscale US (30). Furthermore, they found that oocytes from poorly vascularized follicles produced morphologically poor embryos as compared to oocytes from highly vascularized follicles. Later, Van Blerkom and co-workers showed by means of color Doppler imaging (CDI) that follicles with normal perifollicular blood flow contained oocytes free of cytoplasmic or chromosomal/spindle defects (31). However, CDI is time consuming and cannot be used in daily clinical setting. Another color Doppler technique called power Doppler imaging (PDI) has advantages as compared to CDI, as it is more sensitive and enables flows with lower volumes and velocities to be displayed, and can thus display areas where the mean velocity is zero (32). PDI seemed to be simple enough to be used in the daily clinical setting for monitoring ovarian response. Chui and co-workers adopted the PDI technique in their IVF program and showed that high-grade follicular vascularity resulted in oocytes/embryos that had an increased potential for becoming a full-term pregnancies (33). Further studies using PDI for monitoring perifollicular blood flow have shown that the technique can be used clinically for identifying follicles with oocytes that seem to have a better chance of resulting in good-quality embryos (34). However, the technique seems not to have been used very much in the daily clinical work for monitoring ART cycles.

Over the last years, other interesting and promising US techniques such as power Doppler angiography and 3D-US have been used for monitoring ovarian response in ART cycles (34,35). However, since the techniques were introduced and evaluated in studies, they do not seem to have been clinically very useful in the daily monitoring of ART cycles. Thus, whether the techniques really improve the outcome of the cycle or not is still unclear.

Two other and perhaps clinically more promising US techniques have recently been introduced and evaluated in ART cycles. The techniques are based on 3D-US and are called virtual organ computer-aided analysis (VOCAL) and sonography automated volume count (SonoAVC) (36). VOCAL and SonoAVC are accurate ways of measuring ovarian follicle volume (36–38). The advantage particularly with SonoAVC is that it enables follicle measurements automatically and thus more quickly and with very low inter-observer variability. The technique thus makes it possible to overcome the lack of standardization that represents 2D-US measurement of follicular diameter. One study has compared results between 2D-US and SonoAVC, showing similar outcomes with regard to number of mature oocytes, fertilization rate, and pregnancy rate. However, in that particular study, 2D-US mean diameter of the leading follicle was used for hCG administration (39). Thus, further studies are needed in order to evaluate the use of specific follicular volume for triggering oocyte maturation by administering hCG.

Recently, an interesting method for telemonitoring ovarian stimulation in ART cycles by means of self-operated TVUS has been tested (40). The method is called self-operated endovaginal telemonitoring, and is meant to be used by patients living long distances from the clinic.

CONCLUSION

Monitoring ovarian response during ovarian stimulation in ART cycles can be performed in two ways: US or E2 measurement, alone or in combination. However, US scanning seems to be sufficient in most cases and is probably the most cost-effective approach. Nevertheless, when dealing with poor responders or women at risk for OHSS, the combination is recommended.

REFERENCES

1. Klopper A, Aiman J, Besser M. Ovarian steroidogenesis resulting from treatment with menopausal gonadotropin. *Eur J Obstet Gynecol Reprod Biol* 1974; 4: 25–30.
2. Hackeloer BJ, Nitsche S, Daume E, Sturm G, Bucholz R. Ultraschaldarstellung von ovarveranderungen bei gonad-otropinstimulierung. *Geburtsh Fruenheilk* 1977; 37: 185–9.
3. Ylöstalo P, Lindgren P, Nillius SJ. Ultrasonic measurement of ovarian follicles, ovarian and uterine size during induction of ovulation with human gonadotrophins. *Acta Endocrinol* 1981; 98: 592–8.
4. Vargyas JM, Marrs R, Kletzky OA, Mishell DR. Correlation of ultrasonic measurement of ovarian follicle size and serum estradiol levels in ovulatory patients following clomiphene citrate for *in vitro* fertilization. *Am J Obstet Gynecol* 1982; 144: 569–73.
5. Wittmaack FM, Kreger DO, Blasco L et al. Effect of follicular size on oocyte retrieval, fertilization, cleavage, and embryo quality in *in vitro* fertilization cycles: A 6-year data collection. *Fertil Steril* 1994; 62: 1205–10.
6. Revelli A, Martiny G, Delle Piane L, Benedetto C, Rinaudo P, Tur-Kaspa I. A critical review of bi-dimensional and three-dimensional ultrasound techniques to monitor follicle growth: Do they help improving IVF outcome? *Reprod Biol Endocrinol* 2014; 12: 107–16.
7. Cabau A, Bessis R. Monotoring of ovulation induction with human menopausal gonadotropin and human chorionic gonadotropin by ultrasound. *Fertil Steril* 1981; 36: 178–82.
8. McArdle C, Seibel M, Hann LE, Weinstein F, Taymor M. The diagnosis of ovarian hyperstimulation (OHS): The impact of ultrasound. *Fertil Steril* 1983; 39: 464–7.
9. Salam MN, Marinho AO, Collins WP, Rodeck CH, Campbell S. Monitoring gonadotrophin therapy by real-time ultrasonic scanning of ovarian follicles. *Br J Obstet Gynaecol* 1982; 89: 155–9.
10. Venturoli S, Fabbri R, Paradisi R et al. Induction of ovulation with human urinary follicle stimulating hormone: Endocrine pattern and ultrasound monitoring. *Eur J Obstet Gynecol Reprod Biol* 1983; 16: 135–45.
11. Kwan I, Bhattacharya S, Kang A, Woolner A. Monitoring of stimulated cycles in assisted reproduction (IVF and ICSI). *Cochrane Database Syst Rev* 2014; 8: CD005289.
12. Messinis IE, Tempelton AA, Baird DT. Endogenous luteinizing hormone surge during superovulation induction with sequential use of clomiphene citrate and pulsatile human menopausal gonadotrophin. *J Clin Endocrin Metab* 1985; 61: 1076–81.
13. Wikland M, Borg J, Hamberger L, Svalander P. Simplification of IVF. Minimal monitoring and the use of sub-cutaneous highly purified FSH administration for ovulation induction. *Hum Reprod* 1994; 9: 1430–6.
14. Bergh C, Howles C, Borg K et al. Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F) versus highly purified urinary FSH (Metrodin HP): Results of a randomized comparative study in women undergoing assisted. *Hum Reprod* 1997; 12: 2133–9.
15. Forman R, Robinson J, Egan J et al. Follicular monitoring and outcome of *in vitro* fertilization in gonadotrophin-releasing hormone agonist-treated cycles. *Fertil Steril* 1991; 55: 567–73.
16. Shoham Z, Di Carlo C, Patel A, Conway G, Jacobs H. Is it possible to run a successful ovulation program based solely on ultrasound monitoring? The importance of endometrial measurements. *Fertil Steril* 1991; 56: 836–41.
17. Tomas C, Nuojua-Huttunen S, Martikainen H. Pretreatment transvaginal ultrasound examination predicts ovarian responsiveness to gonadotrophins in *in-vitro* fertilization. *Hum Reprod* 1997; 12: 220–3.
18. Wiser A, Gonen O, Ghetler Y, Shavit T, Berkovitz A, Shulman A. Monitoring stimulated cycles during *in vitro* fertilization treatment with ultrasound only: Preliminary results. *Gynecol Endocrinol* 2012; 28: 429–3.

19. Flemming R. Assessing ovarian response: Antral follicle count versus anti-Müllerian hormone. *Reprod Biomed Online* 2015; 31: 486–96.
20. Brodin T, Hadziosmanovic N, Berglund L, Olofsson M, Holte J. Antimüllerian hormone levels are strongly associated with live birth rates after assisted reproduction. *J Clin Endocrinol Metab* 2013; 98: 1107–14.
21. Groenewoud ER, Cantineau AE, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen–thawed embryo transfer cycles? A systematic review and meta-analysis. *Hum Reprod Update* 2013; 19: 458–70.
22. Simon A, Hurwitz A, Pharhat M, Revel A, Zentner BS, Laufer N. A flexible protocol for artificial preparation of the endometrium without prior gonadotropin-releasing hormone agonist suppression in women with functioning ovaries undergoing frozen-thawed embryo transfer cycles. *Fertil Steril* 1999; 71: 609–13.
23. Schoemaker J, Meer M, Weissenbruch M. Re-evaluation of the role of estrogens as a marker for ovulation induction. In: *FSH Alone in Ovulation Induction*. Lunenfelt B (ed.) New York, NY: The Parthenon Publishing Group, 1993, pp. 23–7.
24. Wramsby H, Sundstrom P, Liedholm P. Pregnancy rate in relation to number of cleaved eggs replaced after in-vitro fertilization in stimulated cycles monitored by serum levels of oestradiol and progesterone as sole index. *Hum Reprod* 1987; 2: 325–8.
25. Levran D, Lopata A, Nayudu PL et al. Analysis of the outcome of *in vitro* fertilization in relation to the timing of human chorionic gonadotropin administration by the duration of estradiol rise in stimulated cycles. *Fertil Steril* 1985; 44: 335–41.
26. A double-blind, randomized, dose-finding study to assess the efficacy of the GnRH-antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing controlled ovarian hyperstimulation with recombinant follicle stimulating hormone. Ganirelix dose-finding study group. *Hum Reprod* 1998; 13: 3023–31.
27. Devroey P, Boostanfar R, Koper NP, Mannerts B, Jzerman-Boon P, Fauser B, on behalf of the ENGAGE investigators. A double-blind, non-inferiority RCT comparing corifollitropin alfa and recombinant FSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol. *Hum Reprod* 2009; 24: 3062–72.
28. Bosch E, Labarta E, Crespo J, Simon C, Remohi J, Jenkins J, Pellicer A. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for *in vitro* fertilization: Analysis of over 4000 cycles. *Hum Reprod* 2010; 25: 2092–100.
29. Griesinger G, Mannaerts B, Andersen CY, Witjes H, Kolibianakos EM, Gordon K. Progesterone elevation does not compromise pregnancy rates in high responders: A pooled analysis of *in vitro* fertilization patients treated with recombinant follicle-stimulating hormone/gonadotropin releasing hormone antagonist in six trials. *Fertil Steril* 2013; 100: 1622–29.
30. Nargrund G, Bourne T, Doyle P et al. Association between ultrasound indices of follicular blood flow and oocyte recovery and preimplantation embryo quality. *Hum Reprod* 1996; 11: 109–13.
31. Van Blerkom J, Antczak M, Schrader R. The developmental potential of human oocytes is related to the dissolved oxygen content of follicular fluid: Association with endothelial growth factor levels and perifollicular blood flow characteristics. *Hum Reprod* 1997; 12: 1047–55.
32. Vlaisavljevic V, Reljic M, Gavric Lovrec V, Zazula D, Sergent N. Measurement of perifollicular blood flow of the dominant follicle using three-dimensional power Doppler. *Ultrasound Obstet Gynecol* 2003; 22: 520–6.
33. Chui DK, Pugh ND, Walker SM, Gregory M, Shaw RW. Follicular vascularity—The predictive value of transvaginal ultrasonography in an *in vitro*-fertilization program: A preliminary study. *Hum Reprod* 1997; 12: 191–6.
34. Mercé LT, Barco MJ, Bau S, Troyano JM. Prediction of ovarian response and IVF/ICSI outcome by three-dimensional ultrasonography and power Doppler angiography. *Eur J Obstet Gynecol Reprod Biol* 2007; 132: 93–100.
35. Ata B, Tulandi T. Ultrasound automated volume calculation in reproduction and pregnancy. *Fertil Steril* 2011; 95: 2163–70.
36. Deb S Campbell BK, Clewes JS, Raine-Fenning NJ. Quantitative analysis of antral follicle number and size: A comparison of two-dimensional and automated three-dimensional ultrasound techniques. *Ultrasound Obstet Gynecol* 2010; 35: 354–60.
37. Raine-Fenning N, Jayaprakasan K, Clewes J et al. Sono-AVC: A novel method of automatic volume calculation. *Ultrasound Obstet Gynecol* 2008; 31: 691–6.
38. Raine-Fenning N, Jayaprakasan K, Chamberlain S, Devlin L, Priddle H, Johnson I. Automated measurements of follicle diameter: A chance to standardize? *Fertil Steril* 2009; 91: 1469–72.
39. Raine-Fenning N, Deb S, Clewes J, Hopkisson J, Campbell B. Timing of oocyte maturation and egg collection during controlled ovarian stimulation: A randomized trial evaluating manual and automated measurements of follicle diameter. *Fertil Steril* 2010; 94: 184–8.
40. Gerris J, Delvinge A, Dhont N et al. Self-operated endovaginal telemonitoring versus traditional monitoring of ovarian stimulation in assisted reproduction: An RCT. *Hum Reprod* 2014; 29: 1941–8.

Oocyte collection

48

GAB KOVACS

HISTORY

The very first human pregnancy using *in vitro* fertilization (IVF) was achieved using laparotomy for obtaining the oocyte (1). Meanwhile, Morgenstern and Soupart (2) in 1972 had described an experimental procedure for both abdominal and vaginal approaches to oocyte recovery, using a special oocyte recovery unit, in conjunction with gynecological surgery. As laparotomy was very invasive and laparoscopy was just beginning to be applied to gynecology, the laparoscopic approach for oocyte collection became routine by the late 1970s (3,4).

It was the expertise of Patrick Steptoe with laparoscopy that resulted in his successful partnership with Robert Edwards, resulting in the birth of Louise Brown in 1978. It was the laparoscopic approach with modification of the collection needle (5) that was used in the stimulated/controlled cycles that resulted in the next eight births from the Monash team, which converted IVF from a research tool to clinical treatment. Laparoscopy was also used by the Jones's team when they used human menopausal gonadotrophins to achieve the first pregnancies in the U.S.A. (6).

During the early 1980s, IVF became used worldwide, using laparoscopic oocyte collection. It was the pioneering work of Susan Lenz in Copenhagen (7) and Wilfred Feichtinger (8) in Vienna that changed oocyte collection from laparoscopic to the far less invasive transvaginal ultrasound-guided technique. With its efficacy being proven to be as good as laparoscopy by a comparative study by Kovacs and colleagues (9), most of the world's IVF units abandoned laparoscopy for the transvaginal route.

ANESTHESIA/ANALGESIA

With the change to transvaginal ultrasound-guided oocyte collection, relaxant analgesia was no longer required. Currently, there is great variation in the type of analgesia used for oocyte collection. In many clinics, oocyte collection is undertaken without any analgesia, whereas in other places some intravenous sedation or even general anesthesia is administered. This depends on several factors, including cultural expectations, the facility used for the oocyte collection, and the medical financial rebate system. A balance has to be reached with minimal risk and cost, but without causing the women unacceptable discomfort. A survey of anesthetic practice employed for oocyte collection in the U.K. a decade ago (10) found that intravenous sedation was the preferred method of sedation, being used in 62.4% of units. General anesthesia was the primary method in 24.6% of units. Sedation was performed by non-anesthetic doctors in 46% of units, and by nurses in 8.2%.

Vlahos and colleagues in 2009 (11) undertook a survey that found that conscious sedation was the most popular method used. It has a relatively low risk of adverse events and no effects on oocyte and embryo quality and pregnancy rates. In 2013, Kwan and colleagues (12) carried out a Cochrane analysis to assess the effectiveness and safety of different methods of conscious sedation and analgesia on pain relief and pregnancy outcomes in women undergoing transvaginal oocyte retrieval. They compared randomized controlled trials comparing different methods of conscious sedation and analgesia for pain relief during oocyte recovery using various adjuncts such as para-cervical block, acupuncture, and various analgesic agents. They analyzed a total of 21 trials including 2974 women undergoing oocyte retrieval. Unfortunately, there was inconsistency between the trials and small numbers of cases reported, so it is no surprise that conflicting results were found. Their findings did not support the superiority of one particular method or technique over another. All the approaches appeared to be acceptable and were associated with a high degree of satisfaction in women. As women vary in their experience of pain and in coping strategies, the optimal method may be individualized depending on the preferences of both the women and the clinicians, as well as resource availability.

The unique use of electro-acupuncture (EA) analgesia has been explored as a method of pain relief. Gejervall and colleagues (13) compared the technique to conventional analgesia using opiates. They found that pain ratings on an analog score were significantly higher with the EA method than conventional analgesia. Consequently, they concluded that EA cannot generally be recommended as a pain-relieving method at oocyte aspiration; however, it might be an alternative for women desiring a non-pharmacological method.

Corson and colleagues (14) carried out research on the use of para-cervical block for transvaginal ultrasound-guided collection. In a prospective study of 101 patients, they concluded that para-cervical block was not significantly better than no injection at all for pain relief.

Pre-ovarian block (POB), where the local anesthetic is deposited in the vaginal wall and between the vaginal wall and the peritoneal surface near the ovary using ultrasound guidance, has also been suggested as a possible method of analgesia. A prospective, randomized, multicenter study of POB versus para-cervical block (15) in 183 patients found no difference in overall pain experienced during the oocyte retrieval procedure, whichever method was used.

CLEANSING/STERILIZING THE VAGINA

When the transvaginal route of oocyte collection was introduced in the mid-1980s, there was concern that entering the peritoneal cavity through a potentially

infected field (the vagina) may result in pelvic infection. There were therefore attempts to carry out routine pre-operative sterilization with antiseptic solutions. This then resulted in anxiety that the antiseptic may be toxic to the oocytes collected. van Os and colleagues (16) carried out a prospective randomized study that showed that using 1% povidon iodine and normal saline washout resulted in a lower pregnancy rate (30.3% vs. 17.2%) and therefore was not advisable. They simultaneously showed that there was no significant infection risk in the saline group, as it had no higher incidence of infection than the iodine group. Hannoun and colleagues (17) also studied whether washing out the vagina with saline after preparation by iodine affected outcomes. They found not washing out was associated with an increase in the rate of chemical pregnancy, and they recommended that it is advisable to cleanse after iodine before oocyte aspiration. Supportive evidence comes from a recent study from Osaka, Japan (18). These authors compared 956 infertile patients undergoing vaginal preparation with saline douching alone versus 1216 infertile patients undergoing a combination of povidone iodine disinfection and subsequent saline douching in an IVF program. They recorded four infections in the saline douching-alone group and none in the combination group, which was a statistically significant difference ($p = 0.016$). There were no significant differences in the rate of fertilization, morphologically good embryo development, and clinical and ongoing pregnancy rates between the two groups. They advocated the use of vaginal povidone iodine disinfection and subsequent saline douching to prevent infection, and concluded that the regime had no evidence of harming oocyte quality.

Today, there is still no consensus on what vaginal preparation is optimal. Many surgeons carry out no vaginal preparation and simply insert the needle through the vagina. Others wash out with saline, while some use betadine followed by saline lavage. The experience at Monash IVF with no vaginal preparation of over 100,000 transvaginal oocyte collections is that post-operative infection is rare, unless an endometrioma has been entered. Younis and colleagues (19) reported as early as 1997 that severe endometriosis with ovarian endometriomata seems to be a significant risk factor for pelvic abscess development following transvaginal oocyte pickup for IVF embryo transfer. They proposed that the presence of old blood in an endometrioma provides a culture medium in which bacteria can grow after transvaginal inoculation.

THE EQUIPMENT

The suction source

In the early days, manual suction was conducted using a needle, plastic tubing, and syringe (2). Berger and colleagues devised a special aspiration unit, with a 20-gauge, 10-inch needle connected by a polyethylene tube to a 10-mm Vacutainer, which then connected to a vacuum bottle with an adjustable pressure gauge. The suction was turned on or off by a thumb valve. The technique then was modified with the use of a suction pump operated by a foot pump (5). Today, sophisticated suction pumps with adjustable aspiration pressures are widely available commercially.

The suction

There has been surprisingly little study undertaken on the physical aspects of oocyte recovery. We published the findings of experiments on bovine eggs carried out in the laboratories of Cook Medical Technology in Brisbane, Australia (20). Some of the observations of these studies are outlined below. In this study, we measured the velocity and flow rates of oocytes through the collection system, and observed the damaging effect of non-laminar flow to the oocyte.

Application of vacuum to the follicle

Vacuum applied after needle entry into the follicle

After application of the vacuum, the pressure within the system equilibrates, resulting in a steady flow rate until the fluid volume decreases and the follicle collapses, so that the follicular wall blocks the lumen of the needle. The time for the system to equilibrate depended on the vacuum pressure, the diameter of the needle, and the volume of the follicle. Maximum flow was achieved when the pressure was at a steady state. Should air be sucked into the system by entering around where the needle pierced the follicle wall, frothing with non-laminar flow resulted, which I call the "cappuccino effect." This has a deleterious effect on the oocyte, as it is thrown around the collection system.

Vacuum deactivated before the needle was withdrawn from the follicle

If the pressure was deactivated whilst the needle was still in the follicle (and there were no leaks), the pressure within the needle and collecting tube drops, and there is often backflow towards the follicle. This can result in the oocyte being sucked back and possibly lost. The amount of backflow depends on how much air enters the system and how much higher the collection tube is above the patient's pelvis.

The vacuum profiles within the aspiration system

It was estimated that when using the system at 150 kPa it took five seconds for the system to stabilize. The pressure within the follicle before penetration varies depending on the size (maturity), shape, and position of the follicle. The internal pressure increases correlating with size. However, due to the pressure caused by the needle deforming the surface of the follicle at the time of puncture, the pressure within the follicle may be much higher (up to 60 mmHg). The more blunt the needle, the higher the resultant pressure. This may result in follicular fluid being lost as it spurts out during this process. If the pressure is already applied, some/most of this fluid will be aspirated as it escapes along the outer wall of the follicle.

There is a pressure gradient down the collection system, so that the pressure at the tip of the needle is only 5% of the pressure at the pump. The oocyte is therefore exposed

to ever-increasing pressures as it travels along the needle, the collection tube, and the collecting test tube. Excessive pressure can cause the ovum to swell and the zona to crack.

Follicle and needle volumes

Table 48.1 lists the respective volumes contained in follicles between 6 and 20 mm in diameter. A 6-mm follicle only contains 0.1 mL, so that 10–12 follicles need to be emptied before the "dead-space" of 1.0–1.2 mL in a standard needle and collecting tube is filled and fluid reaches the collection test tube.

Application of the vacuum

Following the penetration of the follicle by the needle and the application of suction, the pressure within the follicle, the needle, and the collecting tube equilibrates. If there is a tight seal around the needle (i.e., the needle was sharp and was introduced precisely through the follicular wall and the hand is kept still so that tearing does not result), when the suction pressure is reduced, there will be backflow of fluid into the follicle. This can result in the oocyte being lost. On the other hand, if the needle is withdrawn whilst the suction is still being applied, there is a sudden change of pressure at the needle tip from the high vacuum of the follicle to atmospheric pressure, with a rapid surge of fluid towards the collection tube. If the oocyte is contained in the terminal portion of the fluid, it is subjected to increased speeds of travel as well as turbulence, resulting in loss of the cumulus mass and even fracture of the zona pellucida.

Damage within the follicle

During aspiration, the oocyte has to accelerate from a resting state to the velocity of the fluid within the needle.

Table 48.1 The diameter to volume ratios of typical follicles

Follicle diameter (mm)	Follicle volume (mL)
6	0.1
7	0.2
8	0.3
9	0.4
10	0.5
11	0.7
12	0.9
13	1.1
14	1.4
15	1.8
16	2.1
17	2.6
18	3.0
19	3.6
20	4.2

Note: The typical dead-spaces of needles and collecting tubules are 1.0–1.2 mL.

If this is too rapid, the cumulus may be stripped off. The higher the aspiration pressure, the greater the risk, and the smaller the follicle, the higher the pressure that is needed. This may be particularly relevant in the collection of immature oocytes for *in vitro* maturation.

Damage to oocytes

It was noted that high velocities of flow may strip the cumulus from the oocyte. Even with laminar flow, there are significant differences in velocity of the follicular fluid within the center of the needle compared to the periphery. This can result in "drag" on the outer layers of the cumulus, resulting in potential damage. The longer the needle, the smaller its internal diameter, and the greater the pressure required to maintain the same velocity. It was found that when a 17-gauge collection needle was used, all oocytes lost their cumulus mass when the aspiration pressure reached 20 kPa (150 mmHg). It is therefore recommended that pressures be kept below 120 mmHg.

Apart from the speed of travel, turbulent non-laminar flow can also damage the oocyte, either stripping its cumulus mass or fracturing the zona. It is believed that an intact cumulus may be important in preventing damage to oocytes.

The needle

The initial aspiration system consisted of a single-lumen needle. This had to be disconnected at the hub from the suction tubing if follicular flushing was required. There was also always a dead-space of 1.0–1.2 mL and the oocyte would often be flushed up and down in the collection system. It would only be finally recovered when the needle was removed and flushed with fluid—the "needle wash." To allow simpler irrigation of the follicle "flushing," the concept of a double-channel needle was introduced. This required a channel used for oocyte aspiration, with a side channel where fluid could be injected into the follicle. It also allowed simultaneous flushing and aspiration. Scott and colleagues (21) compared single- and double-lumen needle aspirations, albeit with only 22 patients in each arm. Although there were no differences between the two needles in the number of oocytes provided for IVF, there were technical differences. The double-lumen needle was more flexible and frequently deviated from the projected path as observed by ultrasound. The single-lumen needle may be preferable because it is technically easier to use. Haydardedeoglu and colleagues (22) compared the retrieval efficiency of the single-lumen technique (only aspirating) with a double-lumen approach where flushing was also used. They found that there was no improvement in outcome with respect to oocyte numbers, clinical pregnancy rate, or live birth rate.

A unique quasi-double-lumen needle has been designed by Steiner (23). The needle is composed of three parts: a 7-cm-long, 21-gauge needle that penetrates the vagina and ovary; an adjacent, rigid, 17-gauge tube that carries aspirates and flush media back to a collection tube; and a plastic sheath surrounding this tube for carrying flush media,

which connects to a flush syringe on one end and extends down to the top of the 21-gauge needle on the other end. There are holes drilled in the 21-gauge needle so that flush media goes through it into the ovary. The suction on the longer 17-gauge tube pulls the aspirate and flush media away from the patient and into the media collection tube. A major difference between this needle and a standard 19-gauge single-lumen needle is the amount of dead-space. The dead-space in a 19-gauge needle will hold the fluid contained in more than four 6-mm follicles, whereas the dead-space in the Steiner needle will hold the fluid from only one follicle. This enables flushing with a single-lumen needle, with minimal time added to the procedure.

Does size matter?

The original Teflon-lined needle devised at Monash IVF in 1980 (5) was a 19-gauge needle. Attempts have been made to compare different needle diameters. A prospective comparative study by Kushnir and colleagues (24) in 2013 where they used a 17-gauge needle for one ovary and a 20-gauge needle for the other concluded that needle diameter did not affect oocyte yield, yet the smaller-diameter needle prolonged the operative time.

Technique

Flushing or rapid oocyte collection

When transvaginal oocyte collection was first undertaken, the technique of laparoscopic harvesting was transferred to the transvaginal approach. Follicles were initially aspirated, and then repeatedly flushed to try and recover as many oocytes as possible. This, however, is time consuming and also uses large quantities of culture medium. It was soon recognized that most oocytes can be recovered by just aspirating, and that the follicular fluid from the next follicle will often flush the oocyte into the collection tube. This was called the rapid oocyte recovery technique. Hill and Levens (25) reviewed the evidence regarding the effectiveness of ovarian follicular flushing in improving oocyte yield in 2010. They concluded that follicular flushing offers no substantive benefit to oocyte yield, fertilization rates, or pregnancy outcomes for normal and poor-responding patients. When undertaking natural cycle or minimal stimulation, follicular flushing may result in more mature embryos.

Wongtra-Ngan and colleagues (26) in Thailand undertook a Cochrane review of studies comparing flushing to simple aspiration. They found no difference in oocyte numbers, or other clinical outcomes, but did find that operative time was significantly increased (3–15 minutes) by flushing. However, a small trial from France (27) in women undergoing minimal stimulation found that flushing in this group resulted in better embryo morphology and implantation rates, but not increased clinical pregnancy rates. Thus, with poor responders, there is still a place for flushing. Consequently, it is the clinical protocol at Monash IVF that if four or fewer follicles are present, then a double-lumen needle should be used and follicles flushed. If more than four follicles are present, then a single-lumen needle is used and follicles are sequentially aspirated. Flushing of follicles requires the use of a double-lumen needle, as with a single-lumen needle with a dead-space of 1.0–1.2 mL, the oocyte is likely to be flushed up and down within the system.

Curetting the follicle

In the early days of IVF using laparoscopy, each oocyte collection lasted an hour. Follicles were visualized directly, aspirated, flushed, and, if still no oocyte was collected, they were "curetted" with the needle (5). With the change to ultrasound-guided oocyte collection, this practice has been abandoned. Nevertheless, Dahl and colleagues (28) retrospectively reviewing an unselected 275 cases of oocyte collection from 2003 to 2005 and concluded that patients undergoing follicle curetting had a 22% increase in oocyte yield, but not in live birth rates. This is not a practice that is widely used today.

Avoiding turbulent flow

When aspirating follicles, it is important to recognize that in order to fill the "dead-space" between the needle tip and the aspiration tube, somewhere between 1 and 2 mL of follicular fluid is needed.

As described above, it is desirable to avoid damage to the cumulus–oocyte mass during aspiration. The aim is to avoid non-laminar flow within the collection tube, which is likely to damage the oocyte. Attention should be paid to filling the tubing with fluid prior to aspiration, using gentle changes in aspiration pressure, limiting the suction pressure, and stopping aspiration whilst withdrawing the needle to avoid the aspiration of air causing turbulence (the "cappuccino effect").

Temperature control

Another important point is to deliver oocytes to the laboratory in the best condition, including minimizing the effect of cooling, and colleagues from New Zealand (29) investigated the effects of IVF aspiration on the temperature, pH, and dissolved oxygen of bovine follicular fluid. They found that the temperature of follicular fluid dropped by 7.7 ± 1.3°C upon aspiration. Dissolved oxygen levels rose by 5 ± 2 vol.%. The pH increased by 0.04 ± 0.01, and these authors concluded that these changes could be detrimental to oocyte health, and consequently, efforts should be made to minimize these changes. The collection tubes are therefore kept in a test tube warmer whilst they are waiting to be connected to the collection system.

The approach

Any ultrasound machine with the capacity to use a transvaginal probe with a needle guide can be used. The ovaries are visualized and ovarian follicles are then aspirated in a systematic fashion. It is my habit to always commence with the right ovary, and then to aspirate follicles sequentially. It is best to keep the needle within the ovary if possible, to minimize the amount of trauma to the ovarian capsule. When all follicles within the right ovary are aspirated, the needle is withdrawn from the vagina and the needle

is flushed with medium to clear any blood. The pressure is retested, and the left ovary is then aspirated.

COMPLICATIONS

Whilst these are discussed in detail in Chapter 62, a brief synopsis is provided here.

Transvaginal oocyte collection has become the method of choice during the last two decades. However, although complications are rare, several possible complications of transvaginal oocyte collection have been reported.

The most common operative complications are:

- Hemorrhage
- Trauma to pelvic structures
- Pelvic infection, tubo-ovarian, or pelvic abscess

Rarely reported complications include:

- Ovarian torsion
- Rupture of ovarian endometriosis
- Appendicitis
- Ureteral obstruction (30)
- Vertebral osteomyelitis (31)
- Anesthetic complications

Maxwell and colleagues (32) reported the rate of serious complications (ovarian hyperstimulation syndrome, ovarian torsion, infection, and ruptured ovarian cyst) as being 6 in 886 (0.7%) retrieval cycles in oocyte donors. The rate of minor complications severe enough to prompt the donor to seek medical attention after retrieval was 8.5%.

We surveyed 118 women being treated at Monash IVF undergoing oocyte collection in stimulated cycles. Women were asked to rate the inconvenience and pain associated with the blood tests, injections, and oocyte collection procedures using a numeric rating scale of 0–10. Data on analgesic usage and the time taken to return to work and normal activity were also recorded. The median number of oocytes collected was nine. The mean pain score immediately post-operation was 4.6, and at 24 hours was 3.9, on a scale of 1–10. Most women returned to normal activity and work within two days (mean 1.7, SD 1.5 days, and mean 1.8, SD 1.5, respectively). Immediate post-operative discomfort was not found to be correlated with the number of oocytes collected. However, the number of oocytes collected positively correlated with the quantity and duration of analgesics consumed and the time taken to resume work and normal activity.

The incidence of a post-operative acute abdomen was reported by Dicker and colleagues in 1993 from Israel (33). They reported 14 cases out of 3656 patients undergoing the procedure presenting with a clinical picture of an acute abdomen. In nine patients, tubo-ovarian and pelvic abscesses were diagnosed. In three cases, severe intra-abdominal bleeding occurred, with one requiring laparotomy for hemostasis. Ruptured endometriotic cysts caused acute abdomen in two patients.

Tureck et al. (34) from Philadelphia, PA, published a retrospective analysis in 1993 performed on 674 patients who underwent transvaginal retrieval of oocytes during a three-year period. Ten (1.5%) required hospital admission because of peri-operative complications. Nine of these patients needed intravenous antibiotics and one required admission and observation for an expanding broad-ligament hematoma.

Hemorrhage can result in vaginal bleeding at and after the oocyte collection (overt bleeding) or in intra-abdominal bleeding (covert bleeding). Bennet and colleagues (35) reported on a four-year prospective study carried out at King's College, London, of 2670 consecutive procedures, reporting that vaginal hemorrhage occurred in 229 (8.6%) of the cases, with a significant loss (classified as more than 100 mL) in 22 cases (0.8%). Hemorrhage from the ovary with hemoperitoneum formation was seen on two occasions and necessitated emergency laparotomy in one instance. A single case of pelvic hematoma formation from a punctured iliac vessel was also recorded; this settled without intervention.

Nouri and colleagues (36) reviewed published series of cases of post-operative bleeding requiring surgical intervention, and noted that evidence of severe bleeding was obvious within one hour in a third of cases.

As early as the 1990s, it was recognized that pre-existing endometrioma was a risk factor for pelvic infection after oocyte collection. Younis and colleagues from Israel in 1997 (19) reported on three infertile women with ovarian endometriomata who presented with late manifestation of severe pelvic abscess 40, 24, and 22 days after oocyte collection, respectively. Severe endometriosis with ovarian endometriomata seems to be a significant risk factor for pelvic abscess development. Late manifestation of pelvic abscess supports the notion that the presence of old blood in an endometrioma provides a culture medium for bacteria to grow after transvaginal inoculation. Moini and colleagues (37), working in Tehran, Iran, reported that during a six-year period, when 5958 transvaginal ultrasound-guided oocyte retrievals were carried out, 10 cases of acute pelvic inflammatory disease (0.12%) were observed. Eight of the 10 patients were diagnosed as infertile because of endometriosis. They concluded that this supports the previous reports that endometriosis can raise the risk of pelvic infection after oocyte retrieval. More vigorous antibiotic prophylaxis and better vaginal preparation were recommended when oocyte pickup is performed in patients with endometriosis.

Overall, the risk of significant pelvic infection is between 1:200 and 1:500. Consequently, prophylactic antibiotics are not indicated, unless an endometrioma is entered or there is a past history of pelvic infection, and then it is our policy to administer a single dose of intravenous antibiotic (e.g., gentamicin).

Very uncommon complications

Ureteric obstruction

There is a case report from Greenville, SC (30), of acute ureteral obstruction following seemingly uncomplicated oocyte retrieval. Prompt diagnosis and ureteral stenting

led to rapid patient recovery with no long-term urinary tract sequelae.

Jayakrishnan and colleagues (38) reported a case of pseudoaneurysm causing massive hematuria with hemodynamic instability occurring after oocyte retrieval. The patient required a blood transfusion, cystoscopy, and resection and cauterization of the pseudoaneurysm. They concluded that injury to surrounding structures should always be kept in mind during oocyte retrieval.

Vertebral osteomyelitis

The most bizarre complication reported after oocyte collection is vertebral osteomyelitis reported from Tel Aviv, Israel, by Almog and colleagues (31). They reported a case of vertebral osteomyelitis as a complication of transvaginal oocyte retrieval in a 41-year-old woman who underwent IVF embryo transfer treatment. After she returned with severe low back pain, vertebral osteomyelitis was diagnosed and treated with antibiotics.

Cullen's sign (periumbilical hematoma)

Bentov and colleagues (39) described two cases of periumbilical hematoma (Cullen's sign) following ultrasound-guided transvaginal oocyte retrieval. Spontaneous resolution of the symptoms occurred within two weeks. They concluded that the appearance of a periumbilical hematoma (Cullen's sign) following ultrasound-guided transvaginal oocyte retrieval reflects a retroperitoneal hematoma of a benign course.

Troubleshooting

It is important that before commencing oocyte collection the system is tested by aspirating some culture medium. This also provides a column of medium into which to collect the follicular fluid, thus encouraging laminar flow.

Should suction then subsequently decrease or stop, the following steps should be undertaken:

- Ensure that the suction pump is turned on and that the suction pedal is functioning (many aspiration pumps have a light that goes on, and some have audible signals when the pump is activated).
- Check that all connections of tubing between the aspiration tube and the pump are tightly connected.
- Exclude any cracks in the aspiration test tube.
- Ensure that the collection tubing is not kinked or damaged.
- Rotate the needle within the follicle to ensure that it is not blocked by follicular wall tissue.
- If there is still no suction, remove the needle and perform a "retrograde flush" to clear any blockage.
- Before re-inserting the needle, re-check by aspirating some culture medium.

Failure to get oocytes: Check human chorionic gonadotrophin has been given

Sometimes several follicles are aspirated and no oocytes are recovered. If the fluid collected is very clear and devoid of cells (granulosa and cumulus), suspicion may be raised that the patient has not had her trigger human chorionic gonadotrophin (hCG). It is suggested that before follicles from the second ovary are aspirated, some of the follicular fluid is tested with a urinary pregnancy test strip. As these turn blue (react positive) when the concentration exceeds 25 miU/mL of hCG, if it has been administered, there should be sufficient hCG in the follicle to give a positive result. If the test is negative, it is possible to abandon the collection, administer hCG, and defer the collection from the other ovary until about 36 hours later. Although the number of oocytes collected will be limited to one ovary, it is still possible to salvage the cycle.

Pre-treatment of pathology

It has long been suggested that tubal disease, and particularly hydrosalpinx, has a detrimental effect on the outcome of IVF. To determine whether surgical removal of hydrosalpinges improved outcome, Johnson and colleagues (40) undertook a Cochrane analysis of all trials comparing a surgical treatment for tubal disease with a control group generated by randomization. The studied outcomes were live birth (and ongoing pregnancy), pregnancy, ectopic pregnancy, miscarriage, multiple pregnancy, and complications. Three randomized controlled trials involving 295 couples were included in this review. The odds of ongoing pregnancy and live birth were increased with laparoscopic salpingectomy for hydrosalpinges prior to IVF. The odds of pregnancy were also increased, but there was no significant difference in the incidence of ectopic pregnancy. They recommended that laparoscopic salpingectomy should be considered for all women with hydrosalpinges prior to IVF treatment. They also concluded that the role of surgery for tubal disease in the absence of a hydrosalpinx is unclear and merits further evaluation.

Endometriosis

Al-Fadhli and colleagues (41) reported a study to evaluate the effects of different stages of endometriosis on the outcome of treatment in an IVF program. They found that the presence of endometriosis, including stages III and IV, does not affect IVF outcome. However, women with endometriosis required more gonadotropin than those with no endometriosis. Women with an obliterated cul-de-sac have fewer oocytes retrieved.

Assessing clinical competence

It is recommended that prior to clinicians being credentialed for undertaking oocyte collections, a structured training program should be carried out. One approach is that the instructor aspirates one side, and having collected some eggs, the trainee should do the other side under supervision. The number of supervised collections probably varies between 20 and 40 before the trainee should be allowed to perform collections on their own.

Ongoing assessment of clinical competence should then be regularly performed. Our clinical indicator is the

Table 48.2 Oocyte collection checklist

Check that operating list is in the correct order
See patient in preadmission room
Check name, date of birth, and ID number
Check for any allergies (e.g., latex)
Check most recent ultrasound
Check most recent hormone levels (if performed)
Check consent form signed
Check whether any limit on the number of oocytes inseminated
Check whether it is standard *in vitro* fertilization or intracytoplasmic sperm injection
Check whether cleavage stage, blastocyst transfer, or "freeze all"
Check equipment
Ensure ultrasound machine works and check orientation of image
Check tubes connected to needle and suction pump
Specify single- or double-lumen needle
Test that suction is working and adjust pressure
Fill collection tube with media
Procedure
Double-check that patient ID and names on collection tubes match—"time out"
Proceed with collection
Complete notes
Leave message for patient about the number of eggs collected
Contact partner with outcome

oocyte collection rate: the number of oocytes aspirated per follicle (>13 mm) on the pre-hCG scan. The collection rates are then compared between clinicians working within the unit. Other indicators that could be recorded are the time taken for oocyte collection and the complication rate, although the incidence of bleeding and infection is so low that this is probably meaningless unless there is a large number of cases that can be studied.

A checklist prior to oocyte collection, similar to that used by pilots flying airplanes, has also been designed. It is encouraged that clinicians tick off each step to ensure that routine procedures are followed. A copy of this checklist is shown in Table 48.2.

REFERENCES

1. De Kretzer D, Dennis P, Hudson B, Leeton J, Lopata A, Outch K, Talbot J, Wood C. Transfer of a human zygote. *Lancet* 1973; 2: 728–9.
2. Morgenstern LL, Soupart P. Oocyte recovery from the human ovary. *Fertil Steril* 1972; 23: 751–8.
3. Berger MJ Smith DM, Taymor ML, Thompson RS. Laparoscopic recovery of mature human oocytes. *Fertil Steril* 1975; 26: 513–22.
4. Steptoe PC, Edwards RG. Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins. *Lancet* 1970; 1: 683–6.
5. Renou P, Trounson AO, Wood C, Leeton JF. The collection of human oocytes for in vitro fertilization. I. An instrument for maximizing oocyte recovery. *Fertil Steril* 1981; 35: 409–12.
6. Jones HW, Acosta AA, Garcia J. A technique for the aspiration of oocytes from human ovarian follicles. *Fertil Steril* 1982; 37: 26–9.
7. Lenz S. Ultrasonic-guided follicle puncture under local anesthesia. *J In Vitro Fert Embryo Transf* 1984; 1: 239–43.
8. Feichtinger W, Kemeter P. Laparoscopic or ultrasonically guided follicle aspiration for *in vitro* fertilization? *J In Vitro Fert Embryo Transf* 1984; 1244–9.
9. Kovacs GT, King C, Cameron I, Cushnahan L, Wood EC, Leeton JF, Renou P, Shekleton P, Levy S, Baker G. A comparison of vaginal ultrasonic-guided and laparoscopic retrieval of oocytes for *in vitro* fertilization. *Asia-Oceanic J Obstet Gynaecol* 1990; 16: 39–43.
10. Yasmin E, Dresner M, Balen A. Sedation and anaesthesia for transvaginal oocyte collection: An evaluation of practice in the UK. *Hum Reprod* 2004; 19: 2942–5.
11. Vlahos NF, Giannakikou I, Vlachos A, Vitoratos N. Analgesia and anesthesia for assisted reproductive technologies. *Int J Gynaecol Obstet* 2009; 105: 201–5.
12. Kwan I, Bhattacharya S, Knox F, McNeil A. Pain relief for women undergoing oocyte retrieval for assisted reproduction. *Cochrane Database Syst Rev* 2013; 1: CD004829.
13. Gejervall AL, Stener-Victorin E, Möller A, Janson PO, Werner C, Bergh C. Electro-acupuncture versus conventional analgesia: a comparison of pain levels during oocyte aspiration and patients' experiences of well-being after surgery. *Hum Reprod* 2005; 20: 728–35.
14. Corson SL, Batzer FR, Gocial B, Kelly M, Gutmann JN, Go KJ, English ME. Is paracervical block anesthesia for oocyte retrieval effective? *Fertil Steril* 1994; 62: 133–6.
15. Cerne A, Bergh C, Borg K, Ek I, Gejervall AL, Hillensjö T, Olofsson JI, Stener-Victorin E, Wood M, Westlander G. Preovarian block versus paracervical block for oocyte retrieval. *Hum Reprod* 2006; 21: 2916–21.
16. van Os HC, Roozenburg BJ, Janssen-Caspers HA, Leerentveld RA, Scholtes MC, Zeilmaker GH. Alberda ATVaginal disinfection with povidon iodine and the outcome of *in-vitro* fertilization. *Hum Reprod* 1992; 7: 349–50.
17. Hannoun A, Awwad J, Zreik T, Ghaziri G, Abu-Musa A. Effect of betadine vaginal preparation during oocyte aspiration in *in vitro* fertilization cycles on pregnancy outcome. *Gynecol Obstet Invest* 2008; 66: 274–8.

18. Funabiki M, Taguchi S, Hayashi T, Tada Y, Kitaya K, Iwaki Y, Karita M, Nakamura Y. Vaginal preparation with povidone iodine disinfection and saline douching as a safe and effective method in prevention of oocyte pickup-associated pelvic inflammation without spoiling the reproductive outcome: Evidence from a large cohort study. *Clin Exp Obstet Gynecol* 2014; 41: 689–90.
19. Younis JS, Ezra Y, Laufer N, Ohel G. Late manifestation of pelvic abscess following oocyte retrieval, for *in vitro* fertilization, in patients with severe endometriosis and ovarian endometriomata. *J Assist Reprod Genet* 1997; 14: 343–6.
20. Horne R, Bishop CJ, Reeves G, Wood C, Kovacs GT. Aspiration of oocytes for *in-vitro* fertilization. *Hum Reprod Update* 1996; 2: 77–85.
21. Scott RT, Hofmann GE, Muasher SJ, Acosta AA, Kreiner DK, Rosenwaks ZA. Prospective randomized comparison of single- and double-lumen needles for transvaginal follicular aspiration. *J In Vitro Fert Embryo Transf* 1989; 6: 98–100.
22. Haydardedeoglu B, Cok T, Kilicdag EB, Parlakgumus AH, Simsek E, Bagis T. *In vitro* fertilization–intracytoplasmic sperm injection outcomes in single- versus double-lumen oocyte retrieval needles in normally responding patients: A randomized trial. *Fertil Steril* 2011; 95: 812–4.
23. Steiner HP. Optimising techniques in follicular aspiration and flushing. In: *Technique of Minimal Stimulation in IVF—Milder, Mildest or Back to Nature*. Chivas-badiola A, Allahbadia GN (eds). New Delhi, India: Jaypee Brothers Medical Publishers, 2011, pp. 98–102.
24. Kushnir VA, Kim A, Gleicher N, Barad DH. A pilot trial of large versus small diameter needles for oocyte retrieval. *Reprod Biol Endocrinol* 2013; 11: 22–6.
25. Hill MJ, Levens ED. Is there a benefit in follicular flushing in assisted reproductive technology? *Curr Opin Obstet Gynecol* 2010; 22: 208–12.
26. Wongtra-Ngan S, Vutyavanich T, Brown J. Follicular flushing during oocyte retrieval in assisted reproductive techniques. *Cochrane Database Syst Rev* 2010; 8: CD004634.
27. Mendez Lozano DH, Brum Scheffer J, Frydman N, Fay S, Fanchin R, Frydman R. Optimal reproductive competence of oocytes retrieved through follicular flushing in minimal stimulation IVF. *Reprod Biomed Online* 2008; 16: 119–23.
28. Dahl SK, Cannon S, Aubuchon M, Williams DB, Robins JC, Thomas MA. Follicle curetting at the time of oocyte retrieval increases the oocyte yield. *J Assist Reprod Genet* 2009; 26: 335–9.
29. Redding GP, Bronlund JE, Hart AL. The effects of IVF aspiration on the temperature, dissolved oxygen levels, and pH of follicular fluid. *J Assist Reprod Genet* 2006; 23: 37–40.
30. Miller PB, Price T, Nichols JE, Jr., Hill L. Acute ureteral obstruction following transvaginal oocyte retrieval for IVF. *Hum Reprod* 2002; 17: 137–8.
31. Almog B, Rimon E, Yovel I, Bar-Am A, Amit A, Azem F. Vertebral osteomyelitis: a rare complication of transvaginal ultrasound-guided oocyte retrieval. *Fertil Steril* 2000; 73: 1250–2.
32. Maxwell KN, Cholst IN, Rosenwaks Z. The incidence of both serious and minor complications in young women undergoing oocyte donation. *Fertil Steril* 2008; 90: 2165–71.
33. Dicker D, Ashkenazi J, Feldberg D, Levy T, Dekel A, Ben-Rafael Z. Severe abdominal complications after transvaginal ultrasonographically guided retrieval of oocytes for *in vitro* fertilization and embryo transfer. *Fertil Steril* 1993; 59: 1313–5.
34. Tureck RW, García CR, Blasco L, Mastroianni L, Jr. Perioperative complications arising after transvaginal oocyte retrieval. *Obstet Gynecol* 1993; 81: 590–3.
35. Bennett SJ, Waterstone JJ, Cheng WC, Parsons J. Complications of transvaginal ultrasound-directed follicle aspiration: A review of 2670 consecutive procedures. *J Assist Reprod Genet* 1993; 10: 72–7.
36. Nouri K, Walch K, Promberger R, Kurz C, Tempfer CB, Ott J. Severe haematoperitoneum caused by ovarian bleeding after transvaginal oocyte retrieval: A retrospective analysis and systematic literature review. *Reprod Biomed Online* 2014; 29: 699–707.
37. Moini A, Malekzadeh F, Amirchaghmaghi E, Kashfi F, Akhoond MR, Saei M, Mirbolok MH. Risk factors associated with endometriosis among infertile Iranian women. *Arch Med Sci* 2013; 9: 506–14.
38. Jayakrishnan K, Raman VK, Vijayalakshmi VK, Baheti S, Nambiar D. Massive hematuria with hemodynamic instability—Complication of oocyte retrieval. *Fertil Steril* 2011; 96: 22–4.
39. Bentov Y, Levitas E, Silberstein T, Potashnik G. Cullen's sign following ultrasound-guided transvaginal oocyte retrieval. *Fertil Steril* 2006; 85: 227.
40. Johnson NP, Mak W, Sowter MC. Surgical treatment for tubal disease in women due to undergo *in vitro* fertilisation. *Cochrane Database Syst Rev* 2004; 1: CD002125.
41. Al-Fadhli R, Kelly SM, Tulandi T, Tanr SL. Effects of different stages of endometriosis on the outcome of *in vitro* fertilization. *J Obstet Gynaecol Can* 2006; 28: 888–91.

Luteal-phase support in assisted reproduction technology

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INTRODUCTION

Controlled ovarian stimulation (COS) is the single most effective measure ever undertaken for increasing assisted reproduction technology (ART) outcomes. COS, however, disrupts the proper support of the corpus luteum (CL) at the level of the anterior pituitary by altering the pulsatile release of luteinizing hormone (LH) (1). There is now a general consensus professing that progesterone supplementation must be provided in ART, at least during the first weeks following oocyte retrieval (1).

LUTEAL FUNCTION IN ART

Physiology of CL function

After ovulation is induced by the mid-cycle LH surge or a triggering dose of human chorionic gonadotropin (hCG; 5000–10,000 IU), the luteinized granulosa cells collectively forming the CL start producing estradiol (E2) and progesterone. The hormonal activity of CL is tightly controlled by the pulsatile production of LH by the anterior pituitary. During the mid-luteal phase of the menstrual cycle, the daily production of progesterone is of approximately 25 mg/24 hours. In a seminal study, Filicori et al. reported the results of serial (every 10 minutes) blood sampling (2): pulsatile LH secretion (one pulse approximately every 3 hours) is tightly accompanied by a progesterone pulse. Based on these data, the through levels (in between pulses) are of approximately 5 ng/mL (2).

Disruption of CL in ART

In ART, numerous hormonal changes caused by COS interfere with the normal function of the anterior pituitary, causing a disruption of CL function. As a result, the pulsatile production of LH and, in turn, progesterone are seriously disrupted. In ART, the factors interfering with the normal support of CL function by the anterior pituitary include notably:

- Gonadotropin-releasing hormone (GnRH) analogs, agonists, and antagonists
- Excessive levels of E2 induced by COS
- The replacement of the LH surge by a triggering dose of hCG

The net result of these effects is an insufficient production of progesterone by the CL, which compromises embryo implantation and development (3).

There is now overwhelming evidence that ART outcomes—pregnancy rates and live birth rates—are improved by luteal-phase support (LPS) (4). LPS can be accomplished by either an intermittent administration of hCG or sustained (daily) progesterone replacement. The former has been definitively abandoned due a several-fold increase in the risk of ovarian hyperstimulation syndrome and a lack of demonstrated superiority over simple progesterone supplementation (4). The latter has therefore been universally adopted and is today a routine complement of COS prescribed in all ART cycles accompanied with fresh embryo transfers (ETs).

PROGESTERONE ADMINISTRATION

Injectable preparations

The classical options for delivering progesterone for LPS in ART are illustrated in Figure 49.1. Injectable progesterone preparations have existed since pre-ART times (5). Because progesterone is poorly soluble in water, all preparations available until recently were in an oil base, which mandates intramuscular (i.m.) administration. The latter are notoriously painful and a source of possible sterile abscesses. The oil base—sesame or peanut oil—preparations were put on the market and approved for treating threatened abortions, an indication that does not warrant such treatment anymore. Practically, therefore, all oil base injectable progesterone preparations available are used in ART off-label.

Injectable progesterone preparations have been validated in numerous investigator-initiated trials. Injectable progesterone was found to be effective for LPS in ART and, in case of complete absence of endogenous progesterone, in donor egg (5) and frozen ET (FET) models (6).

Impossible oral and transdermal progesterone

Progesterone cannot be administered orally in ART due to intense hepatic metabolism during the first liver pass (7). In micronized form (nowadays, all preparations are micronized) progesterone is readily and totally absorbed following oral ingestion, but is highly metabolized in the liver. Contrary to the situation prevailing with E2, liver metabolism effects cannot be overcome by simply increasing the doses of progesterone administered. In the case of E2, daily administration of doses 100-times higher than the daily production by the ovary reliably succeeds in duplicating the serum levels and peripheral effects encountered in the menstrual cycle. Oral E2, albeit at increased doses, is therefore usable for E2 administration in ART. This is not the case for progesterone, however. In prior work, oral doses of progesterone of up to 1 g/24 hours failed to reliably induce pre-decidual changes in the endometrium,

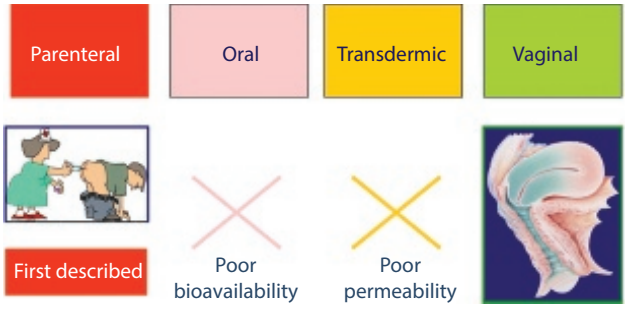


Figure 49.1 Treatment options.

as seen in the luteal phase of the menstrual cycle (8). Conversely, however, oral progesterone can be effectively used for preventing endometrial hyperplasia in hormonal treatments of menopause. Indeed, oral progesterone (or its metabolites) effectively exerts anti-proliferative effects on the endometrium. Hence, despite a lack of secretory transformation, oral progesterone effectively protects from the risk of endometrial cancer.

E2 can be successfully administered transdermally using either adhesive systems ("patches") or gel preparations. The advantages of this route of administration—it avoids hepatic metabolism—sparked interest for doing the same with progesterone. Unfortunately, progesterone cannot be administered transdermally and probably never will be. First, the doses that need to be administered for matching CL production (25 mg/24 hours in the mid-luteal phase) are several orders of magnitude larger than the daily production of E2 (from 0.05 to 0.5 mg/24 hours). This would require that skin systems be much too large for any practical application. Second, the skin is rich in 5α-reductase, an enzyme that is capable of inactivating progesterone, thus hampering any possible efficacy. While transdermal administration of synthetic progestins exists (for contraception), transdermal progesterone is not available for LPS in ART.

Vaginal progesterone

Starting from the early days of ART, progesterone has been administered vaginally primarily for avoiding the side effects of i.m. injections (9). With the oral and transdermal administration of progesterone not being possible, the vaginal route indeed appeared to be the only practical alternative remaining.

Early work with vaginal progesterone demonstrated the great efficacy of its endometrial effects. Despite relatively low plasma levels being achieved (8,9), biopsies reliably showed complete pre-decidual changes of the endometrial stroma (8). The high efficacy of vaginal progesterone led to its widespread use for LPS and FET preparation in ART.

Over the years, various vaginal progesterone preparations were developed and approved for use for LPS in ART, and for priming endometrial receptivity for FETs. Some women, however, report being distressed by the vaginal leakages encountered and/or wish to avoid vaginal administration for cultural or other personal reasons. In certain countries, vaginal progesterone is used almost exclusively, whereas in the U.S.A., for example, injectable progesterone is still used in nearly 50% of all ART cycles.

New subcutaneous progesterone preparation

The search for practical options that avoid the painful i.m. progesterone injections while retaining reliable efficacy led to the development of what was seemingly impossible: an aqueous progesterone preparation. Indeed, an aqueous progesterone preparation available for subcutaneous administration was developed by encapsulating progesterone in cyclodextrin (10). Cyclodextrin, a starch residue commonly utilized in the pharmaceutical and food industry, enhances the polarity—and hence water solubility—of substances. Upon entering the body, cyclodextrin is readily digested, liberating free progesterone (10). The product developed, Prolutex®, is now commercially available in numerous countries and constitutes a new therapeutic alternative to i.m. injections and vaginal administration (Figure 49.2).

Pharmacokinetics analyses of the aqueous progesterone preparation originally studied two doses: 25 and 50 mg/day. In both cases, steady state was achieved after a little over two days of repeated daily administration (11). The trough levels (minimum before next daily injection) are approximately 6 ng/mL in the 25-mg group. Peak levels after injections are, however, much higher (Figure 49.3) (11). This pattern therefore resembles the values seen in case of pulsatile production of LH and progesterone in the luteal phase of the menstrual cycle, as shown by Filicori et al. (2).

In a seminal study, the new aqueous progesterone preparation was tested in women deprived of endogenous progesterone production. This constitutes an extreme model—an "acid test"—for studying the effects of progesterone preparations (12). In volunteers whose endogenous ovarian function had been suppressed with GnRH agonist (GnRHa), the effects of daily injections of either 25 or 50 mg/24 hours of progesterone were studied by analyzing secretory changes in endometrial biopsies performed after 11 days of treatment. Pre-decidual changes of the endometrial stroma were seen in all interpretable biopsies, with no differences noted between the 25-mg and 50-mg dose groups (12). This led the parent pharmaceutical company, IBSA Pharmaceuticals, to develop and commercialize the 25-mg dose—Prolutex—for LPS in ART. As mentioned above, this dose corresponds to the actual amount of progesterone produced by the CL in the mid-luteal phase. Two Phase III trials documented that the aqueous progesterone preparation administered subcutaneously—Prolutex 25 mg—is as effective as existing vaginal progesterone preparations for LPS in ART (13,14).

DURATION OF PROGESTERONE TREATMENT

Onset of treatment

The impairment of progesterone production encountered in COS used in ART primarily affects the mid-to-late

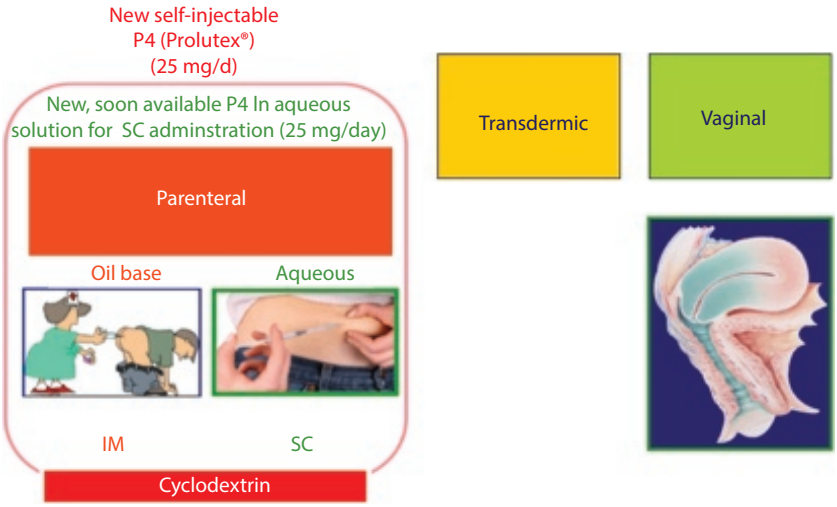


Figure 49.2 A new self-administered progesterone option.

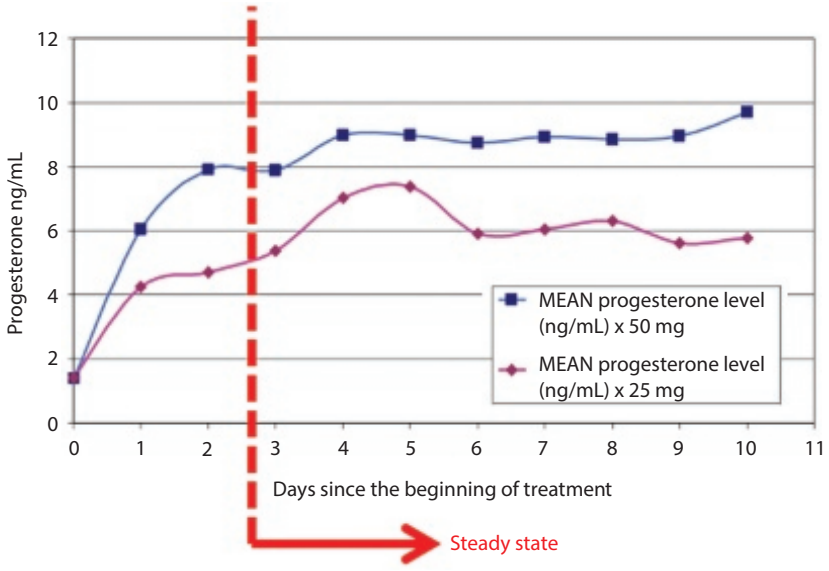


Figure 49.3 Pharmacokinetic data of subcutaneous progesterone administration. (Data derived from Sator M et al. *Gynecol Endocrinol* 2013; 29: 205–8.)

luteal phase. Early in the history of ART, certain authors had claimed that LPS could therefore be initiated only a few days after oocyte retrieval (15). The recommendation for late onset of LPS was also motivated by the fear in these authors' mind that early LPS might advance the secretory transformation of the endometrium, causing an early closure of the window of receptivity (15). Today, these fears—however intellectually founded—have been proven not to be realized practically in everyday ART.

There is now a general consensus for favoring an early onset of LPS in ART on the evening of oocyte retrieval or the day after. This is in part motivated by the fact that the uterus-relaxing properties of progesterone tend to reduce uterine contractions (UCs) at the time of ET (16,17). The latter is indeed a desirable effect considering the inverse correlation existing between UC frequency and ART outcome (16).

Termination of treatment

As stated above, the prevailing hypothesis for the pathophysiology of luteal-phase dysfunction and hence the need for LPS in ART contends that it is the normal pituitary support of CL that is disrupted. Following this principle, LPS would only need to be administered until the positive pregnancy test. Later, it is indeed the hCG produced by the developing embryo, not the anterior pituitary, that sustains proper CL function. In spite of this seemingly simple principle, it has been common practice for most ART centers to continue LPS until 10 weeks of pregnancy. This corresponds to the moment of luteo-placental transition

when hormone production (E2 and progesterone) is entirely taken over from the CL by the placenta.

The extended duration of LPS prescribed by many groups is not supported by evidence, however. In a prospective and randomized trial, Aboulghar's group has elegantly shown that LPS can be safely interrupted after the first positive ultrasound at six to seven weeks of pregnancy (18). Other prospective trials have even indicated that LPS can be discontinued upon obtaining a positive pregnancy test. In a prospective trial, 200 pregnant women interrupted vaginally administered LPS upon obtaining a positive pregnancy test (19), whereas 200 other women pursued LPS for an extra two weeks. Ultimately, there were no differences in ongoing pregnancy rates between these two groups.

The primary reason that leads many groups not to interrupt LPS upon a positive pregnancy test, which includes our own, is a clear lack of courage. What if miscarriages could possibly be blamed on early interruption of LPS (20)? A second, more honorable reason is the fact that prolonged hormonal treatment is warranted in FETs. Hence, early discontinuation of LPS in case of fresh ET would impose having two distinct regimens for fresh ETs and FETs. As these two regimens could be successively prescribed to the same women, it is feared that this could constitute a source of confusion.

E2 ADMINISTRATION

Some investigators have advocated supplementing E2 during the luteal phase of ART cycles. Subsequently, meta-analyses argued against this practice as being not justified based on results (21). The debate persists, however, and some groups like ours still prescribe transdermal E2 supplementation starting three days after ET.

E2 pretreatment is mandatory, however, for priming endometrial receptivity for FET (22). Decades of ART and donor egg ART activity have revealed that E2 administration is relatively simple, effective, and extremely forgiving. The daily oral administration of 4–8 mg of E2 reproduces the serum levels and peripheral effects of E2 as seen in the menstrual cycle. The liver, however, is exposed to markedly higher quantities of E2, as it sees the whole amount administered, which far exceeds what is normally produced in the menstrual cycle. The excess liver exposure due to the first liver pass effect inherent to oral administration may cause problems in certain individuals, notably in women at higher risk of veno-thromboembolism accidents. In these individuals, E2 should not be administered orally if at all possible and/or preventive medication (e.g., low-molecular-weight heparin) should be provided simultaneously.

The intense liver metabolism of orally administered E2 renders this route of administration vulnerable to the effects of enzymatic inductors, such as psychotropic medications. Women exposed to enzymatic inductors are notoriously resistant to the effects of oral E2. In these cases, it is preferable to revert to non-oral E2 administration.

E2 can be easily administered non-orally. Transdermal administration of E2 can be done using skin systems or gels. In ART, skin systems have been generally preferred because they allow us to more precisely adjust the administered dose. Basically, it suffices to choose the doses of E2 normally produced by the ovary (mean production rate of 0.2 mg/24 hours) for reproducing similar blood levels and peripheral effects of E2. During non-oral administration of E2, estrone (E1) levels remain in the physiological range: $E2/E1 > 1$. By contrast, E1 levels are nearly 10-times higher than E2 following oral administration. To this day, however, no detrimental effects have been recognized regarding the pharmacological levels of E1 induced by oral E2 administration. The transdermal systems have been found to be possibly weakly effective in overweight and/or obese women, in whom oral administration will be preferred. Finally, transdermal systems may become poorly effective in case of hot and humid weather. One practical element for enhancing the adhesion of transdermal skin systems consists of wiping off skin grease with a tissue soaked in nail polish remover before applying the E2 skin systems.

E2 can also be administered vaginally. While specially designed creams exist in the U.S.A., numerous investigators have simply given oral tablets vaginally. This, however, results in extremely high serum levels, but without further hepatic impact than is encountered with oral tablets (23). Vaginal E2 can be a valuable alternative, particularly for women whose endometrial response to E2 appears impaired.

FROZEN ETs

The donor egg lesson

ART—originally called *in vitro* fertilization (IVF)—was conceived for women whose tubes were absent or blocked. Yet ART readily opened new therapeutic avenues that could not have been envisioned before. One of these new possible options brought about by IVF is donor egg ART. Indeed, as fertilization could take place outside of the body, it was possible to obtain embryos from the oocytes of a donor and transfer them into the uterus of a woman whose ovaries had failed. This, however, required that endometrial receptivity could be primed in women whose ovaries had failed with the sole help of exogenous hormones—E2 and progesterone (24).

Today, we know that the endometrium primed by E2 and progesterone only is as receptive as it possibly gets, with implantation rates that can be equaled in the natural cycle, but never surpassed. This indicates that everything else produced by the ovaries during the menstrual cycle (peptides, androgens, etc.) either does nothing or possibly harms endometrial receptivity.

The donor egg model laid out the groundwork that allowed us to understand and apply practically the principles governing the hormonal control of endometrial receptivity. From the early days of donor egg ART, one has been struck by the fact that recipients of donor egg ART generally had better results than their counterparts undertaking regular ART. This has led us to suspect that COS used in ART exerts negative effects on endometrial receptivity not seen in donor egg ART.

The concerns about COS harming endometrial receptivity are now extended one step further. Indeed, we recently realized that the obstetrical outcomes of fresh ETs and FETs are likely to be different, with fewer complications encountered in FETs. This latter issue is addressed below in a section of this chapter on the novel concept of the "window of endometrial vulnerability."

Estrogen priming and progesterone-driven receptivity

The early days of donor egg ART have witnessed the writing of seminal papers that precisely described the hormonal control of endometrial receptivity. Amazingly, all of these early concepts originating from the heyday of donor egg ART remain valid today. Schematically, endometrial receptivity depends on two necessary hormonal effects:

- *Estrogen priming*: This is necessary for allowing the indispensable endometrial proliferation and the E2-dependent development of progesterone receptors.
- *Time-related, progesterone-induced secretory changes of the endometrium*: The secretory transformation of the endometrium is induced by progesterone. From the early days of reproductive endocrinology, we know that these changes—taking place in the endometrial glands and later stroma—are time dependent and relatively progesterone dose and serum level independent.

A wealth of data that accumulated through four decades of ART activity have by and large confirmed and expanded upon these early but still valid concepts (24).

No need for ovarian suppression

When E2 and progesterone replacement cycles were introduced for priming FETs (based on the strong results of donor egg ART), ovarian function was commonly suppressed using a GnRHa (25). Later, it became evident that E2 alone sufficed if initiated early enough (on cycle day 1 or, even better, a few days before menses) for suppressing the inter-cycle follicle-stimulating hormone elevation and preventing follicular recruitment (26,27). Today, most centers use E2 and progesterone treatment regimens for planning FETs, as these have been found to be equivalent yet simpler than timing FETs in the menstrual cycle. Generally, FET priming implies an E2 priming phase of two to three weeks followed by timed progesterone administration. In principle, a single clinical control is necessary at the end of the E2-only priming phase. This is done for asserting proper estrogenization (endometrial thickness $\geq$7 mm) and ensuring that no exposure to progesterone had taken place (plasma progesterone $\leq$1.5 ng/mL). The timing of ETs is scheduled on the third to fourth day and fifth to sixth day of progesterone exposure for cleaving-stage and blastocyst transfers, respectively. We personally prefer edging on the early side—the third and fifth days of progesterone exposure for cleavage-stage and blastocyst transfers, respectively—as this was found to be equally effective and possibly more forgiving.

The window of endometrial vulnerability

Possible negative effects of COS on the endometrium were first suggested by witnessing higher pregnancy rates in donor egg ART as compared to the fresh ET counterparts. Today, we realize that there is more to these effects of COS on the endometrium than merely a decrease in embryo implantation rates. Indeed, alterations of endometrial development may exert durable effects on the quality of placentation and, in turn, obstetrical development of the fetus. In rat models, poor placentation generated by transferring blastocysts in a hyperstimulated endometrium led to lower-weight pups and placentas (28).

In humans, a wealth of data suggest that obstetrical alterations associated with ART (earlier delivery, smaller birth weight, etc.) are not encountered in FETs (29). This indicates that the obstetrical alterations seen in ART might in part stem from endometrial effects of COS not seen in FETs when embryos are transferred in a more physiologically developed endometrium. The endometrium therefore appears to be highly sensitive—hence the concept of vulnerability—to hormonal imbalances that are encountered in COS, with consequences that could far exceed just pregnancy rates. The exact duration of this period of endometrial vulnerability has not been clarified yet. Everything indicates, however, that the period of endometrial vulnerability starts during the E2-only priming phase, when exposure to excessive E2 levels may ultimately lead to poor placentation and obstetrical complications. The latter include preterm birth, small for gestational age, and low birth weight. Further work should clarify the exact extent and practical consequences of endometrial vulnerability to hormonal imbalances and define the duration of the phenomenon.

CONCLUSION

LPS has been proven to be necessary in ART. LPS is ideally started early, on the day of oocyte retrieval or the day after, as this minimizes the risk that UCs adversely affect ART outcome. LPS consists of delivering supplemental doses of progesterone either by injectable preparation or vaginal administration. The recent availability of an aqueous progesterone preparation allowing self-administration by subcutaneous injections provides women who dislike vaginal administration with an alternative. It has been amply documented that LPS can be stopped after the first positive ultrasound finding or even positive pregnancy test. Yet many groups continue to prescribe LPS for longer than necessary, in part because this is necessary in FETs, as many fear that having two distinct regimens for fresh ART and FET might cause confusion.

REFERENCES

1. Yanushpolsky EH. Luteal phase support in *in vitro* fertilization. *Semin Reprod Med* 2015; 33: 118–27.
2. Filicori M, Butler JP, Crowley WF, Jr. Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. *J Clin Invest* 1984; 73: 1638–47.

3. Fatemi HM, Popovic-Todorovic B, Papanikolaou E, Donoso P, Devroey P. An update of luteal phase support in stimulated IVF cycles. *Hum Reprod Update* 2007; 13: 581–90.
4. van der Linden M, Buckingham K, Farquhar C, Kremer JA, Metwally M. Luteal phase support for assisted reproduction cycles. *Cochrane Database Syst Rev* 2015; 10: CD009154.
5. Navot D, Laufer N, Kopolovic J et al. Artificially induced endometrial cycles and establishment of pregnancies in the absence of ovaries. *N Engl J Med* 1986; 314: 806–11.
6. Casper RF, Yanushpolsky EH. Optimal endometrial preparation for frozen embryo transfer cycles: Window of implantation and progesterone support. *Fertil Steril* 2016; 105: 867–72.
7. de Ziegler D, Fanchin R. Progesterone and progestins: Applications in gynecology. *Steroids* 2000; 65: 671–9.
8. Fanchin R, De Ziegler D, Bergeron C, Righini C, Torrisi C, Frydman R. Transvaginal administration of progesterone. *Obstet Gynecol* 1997; 90: 396–401.
9. De Ziegler D, Fanchin R, Massonneau M, Bergeron C, Frydman R, Bouchard P. Hormonal control of endometrial receptivity. The egg donation model and controlled ovarian hyperstimulation. *Ann N Y Acad Sci* 1994; 734: 209–20.
10. Cometti B. Pharmaceutical and clinical development of a novel progesterone formulation. *Acta Obstet Gynecol Scand* 2015; 94(Suppl 161): 28–37.
11. Sator M, Radicioni M, Cometti B et al. Pharmacokinetics and safety profile of a novel progesterone aqueous formulation administered by the s.c. route. *Gynecol Endocrinol* 2013; 29: 205–8.
12. de Ziegler D, Sator M, Binelli D et al. A randomized trial comparing the endometrial effects of daily subcutaneous administration of 25 mg and 50 mg progesterone in aqueous preparation. *Fertil Steril* 2013; 100: 860–6.
13. Lockwood G, Griesinger G, Cometti B, European C. Subcutaneous progesterone versus vaginal progesterone gel for luteal phase support in *in vitro* fertilization: A noninferiority randomized controlled study. *Fertil Steril* 2014; 101: 112–9.e3.
14. Baker VL, Jones CA, Doody K et al. A randomized, controlled trial comparing the efficacy and safety of aqueous subcutaneous progesterone with vaginal progesterone for luteal phase support of *in vitro* fertilization. *Hum Reprod* 2014; 29: 2212–20.
15. Paulson RJ, Sauer MV, Lobo RA. Potential enhancement of endometrial receptivity in cycles using controlled ovarian hyperstimulation with antiprogestins: A hypothesis. *Fertil Steril* 1997; 67: 321–5.
16. Fanchin R, Ayoubi JM, Olivennes F, Righini C, de Ziegler D, Frydman R. Hormonal influence on the uterine contractility during ovarian stimulation. *Hum Reprod* 2000; 15(Suppl 1): 90–100.
17. Ayoubi JM, Epiney M, Brioschi PA, Fanchin R, Chardonnens D, de Ziegler D. Comparison of changes in uterine contraction frequency after ovulation in the menstrual cycle and in *in vitro* fertilization cycles. *Fertil Steril* 2003; 79: 1101–5.
18. Aboulghar MA, Amin YM, Al-Inany HG et al. Prospective randomized study comparing luteal phase support for ICSI patients up to the first ultrasound compared with an additional three weeks. *Hum Reprod* 2008; 23: 857–62.
19. Schmidt KL, Ziebe S, Popovic B, Lindhard A, Loft A, Andersen AN. Progesterone supplementation during early gestation after *in vitro* fertilization has no effect on the delivery rate. *Fertil Steril* 2001; 75: 337–41.
20. Zarutskie PW. Help me understand what you are saying about luteal phase support for *in vitro* fertilization. *Fertil Steril* 2009; 91: e1; author reply e2.
21. Serna J, Cholquevilque JL, Cela V, Martinez-Salazar J, Requena A, Garcia-Velasco JA. Estradiol supplementation during the luteal phase of IVF–ICSI patients: A randomized, controlled trial. *Fertil Steril* 2008; 90: 2190–5.
22. Hancke K, More S, Kreienberg R, Weiss JM. Patients undergoing frozen-thawed embryo transfer have similar live birth rates in spontaneous and artificial cycles. *J Assist Reprod Genet* 2012; 29: 403–7.
23. Tourgeman DE, Gentzchein E, Stanczyk FZ, Paulson RJ. Serum and tissue hormone levels of vaginally and orally administered estradiol. *Am J Obstet Gynecol* 1999; 180: 1480–3.
24. de Ziegler D, Fanchin R, de Moustier B, Bulletti C. The hormonal control of endometrial receptivity: Estrogen (E2) and progesterone. *J Reprod Immunol* 1998; 39: 149–66.
25. Schmidt CL, de Ziegler D, Gagliardi CL et al. Transfer of cryopreserved-thawed embryos: The natural cycle versus controlled preparation of the endometrium with gonadotropin-releasing hormone agonist and exogenous estradiol and progesterone (GEEP). *Fertil Steril* 1989; 52: 609–16.
26. de Ziegler D, Cornel C, Bergeron C, Hazout A, Bouchard P, Frydman R. Controlled preparation of the endometrium with exogenous estradiol and progesterone in women having functioning ovaries. *Fertil Steril* 1991; 56: 851–5.
27. Lelaidier C, de Ziegler D, Gaetano J, Hazout A, Fernandez H, Frydman R. Controlled preparation of the endometrium with exogenous oestradiol and progesterone: A novel regimen not using a gonadotrophin-releasing hormone agonist. *Hum Reprod* 1992; 7: 1353–6.
28. Weinerman R, Mainigi M. Why we should transfer frozen instead of fresh embryos: The translational rationale. *Fertil Steril* 2014; 102: 10–8.
29. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through *in vitro* fertilization treatment: A systematic review and meta-analysis. *Fertil Steril* 2012; 98: 368–77.e1–9.

Treatment strategies in assisted reproduction for the poor-responder patient

50

ARIEL WEISSMAN, COLIN M. HOWLES, and SESH K. SUNKARA

OVERVIEW

In a spontaneous menstrual cycle, only one follicle out of a cohort of 10–20 usually completes maturation and ovulates to release a mature oocyte. The aim of controlled ovarian stimulation (COS) in assisted reproductive technology (ART) protocols is to overcome the selection of a dominant follicle and to allow the growth of a cohort of follicles. This strategy leads to an increase in the number of oocytes and hence embryos available for transfer, thereby increasing the chance of transferring viable embryos. However, the chance of pregnancy and also live birth begins to dramatically decline after the age of 35 years, and successful treatment for these patients continues to be a major challenge in ART programs.

In this chapter, we describe the current strategies aimed at augmenting follicular recruitment, oocyte yield, and ultimate desired clinical outcomes following *in vitro* fertilization (IVF) treatment among women identified as poor responders.

INTRODUCTION

The human ovary has a finite number of non-growing follicles (NGFs) established before birth that decline with increasing age, culminating in menopause at age 50–51 years. For 95% of women, only 12% of their pre-birth NGF population is present by the age of 30 years, declining to only 3% by the age of 40 years (1). This provides the basis for decline in female fecundity with increasing age. This decline in fecundity can be based on a variety of age-related conditions, including an increase in gynecological disorders such as endometriosis or fibroids, an increase in ovulatory disorders due to effects on the hypothalamic–pituitary–ovarian axis, or a compromised uterine vascular supply that may impede implantation (2). Spontaneous conception is rare in women >45 years of age: a study carried out in orthodox Jewish sects that are proscribed from using contraceptives showed that natural pregnancies and deliveries after the age of 45 years constitute only 0.2% of total deliveries, and >80% of these are in grand multiparas (3). Similar findings have been described in Bedouin women as well (4). In infertile couples, IVF may be a reasonable option for such women of advanced maternal age (AMA) who are aged >40 years, but at the age of ≥45 years, deliveries are a rare event (5).

The peak number of oocytes present in the human ovary occurs during fetal gestation, and follicles are continually lost thereafter through the mechanism of apoptosis, a process known as atresia (6). A cohort of growing follicles is recruited each month, and the cohort enters the final stages of follicle maturation during the first half of the menstrual cycle. This maturation phase is gonadotropin dependent. Painstaking histological and *in vitro* studies carried out by Gougeon suggest that follicles require a period of approximately 70 days from the time they enter the preantral stage (0.15 mm) to reach a size of 2 mm (7). These 2-mm follicles have very low steroidogenic activity, and they are impervious to cyclic follicle-stimulating hormone (FSH) and luteinizing hormone (LH) changes in terms of granulosa cell (GC) proliferation. Over a four- to five-day period during the late luteal phase, follicles that are 2–5 mm in diameter enter a recruitment stage, and cyclic changes in FSH drive the development of the follicle and proliferation of GCs; GC aromatase activity is not affected during this stage. Thus, as the follicle develops, it becomes increasingly responsive to gonadotropins.

From the perspective of treatment management, this means that in order to influence the size of the recruitable pool of follicles, it would be necessary to "boost" continued healthy follicle development over a protracted period of time (≥70 days). However, gonadotropins play a role only during the phases of recruitment and final follicular maturation, which occur over the last 20 days or so of this 70-day period. Therefore, extrapolating from knowledge about basic physiology, different agents would be required at different times in order to successfully overcome the age-related decline in follicle numbers.

Women who postpone childbearing until their late 30s or early 40s are therefore frequently faced with the distressing realization that their chance of achieving a pregnancy is significantly reduced, and that they may require the help of ART, with further complex difficulties that can jeopardize their quest for successful conception. In Europe for the year 2010, women undergoing IVF or intracytoplasmic sperm injection (ICSI) procedures in the age group >40 years represented approximately 16.7% and 17.3%, respectively, of those attending IVF clinics (8). A number of different variables can affect success rates in ART, and the negative impact of increasing age is one feature that is well recognized. Not only does the response to stimulation steadily deteriorate, requiring larger amounts of gonadotropins, but also the cancelation rate is higher, and there is a significant increase in the rate of miscarriage.

Data from the U.S.A. (Center for Disease Control 2013 report on ART success rates) (9) clearly show that the potential for embryo implantation and successful delivery of a live birth decreases rapidly in women >35 years (Figure 50.1). This same report also documents the increased incidence of pregnancy loss that is related to increased maternal age, going from less than 15% in women ≤36 years of age, increasing rapidly among women in their late 30s to reach 29% at 40 years of age, and over 50% in women ≥44 years. These data suggest that the lower age limit to defining women of AMA should be considered as ≥35 years.

Although chronological age is the most important predictor of ovarian response to stimulation, the rate of reproductive aging and ovarian sensitivity to gonadotropins varies considerably among individuals (10). Biological and chronological age are not always equivalent, and biological age is more important in predicting the outcome of ART (10). Biological aging often renders the ovaries increasingly resistant to gonadotropin stimulation, with the result that the number of oocytes harvested may be very low.

Any strategy that might enhance the efficacy of treatment for these women would be of great benefit, and different areas of research have recently been explored, such as the use of pharmacogenomics to assess response to gonadotropin stimulation, manipulating the endocrinology of the treatment cycle, and screening of embryos for aneuploidy.

DEFINITIONS AND TERMINOLOGY

Over the years, a plethora of papers on different aspects of the pathogenesis and management of poor ovarian response (POR) have been published. One of the major problems in comparing these studies was the lack of a uniform definition of a poor response. The considerable heterogeneities in the definition of POR (inclusion criteria, outcome measures, etc.) made it almost impossible to develop or assess any protocol to improve the outcome (11,12). To this effect, the European Society of Human Reproduction and Embryology (ESHRE) working group attempted to standardize the definition of POR to stimulation in a simple and reproducible manner (the Bologna consensus) (13). POR to ovarian stimulation usually indicates a reduction in follicular response, resulting in a reduced number of retrieved oocytes. The consensus definition recommends that two of the following three features should be present for a diagnosis of POR: (1) AMA (≥40 years) or other risk factor for POR; (2) a previous POR (≤3 oocytes with a conventional stimulation protocol); and (3) an abnormal ovarian reserve test (ORT) (i.e., antral follicle count [AFC] <5–7 follicles or anti-Mullerian hormone [AMH] <0.5–1.1 ng/mL [3.57–7.85 pmol/L]). Two episodes of POR after maximal stimulation were considered sufficient to define a patient as a poor responder in the absence of AMA or abnormal ORT. Patients of AMA with an abnormal ORT may be more properly defined as "expected poor responders." Although subject to initial criticism about its validity in defining a homogenous population (14), subsequent studies have established its validity that the various subgroups of the Bologna criteria poor responders have a uniform poor prognosis (15,16).

ASSESSMENT AND PREDICTION OF OVARIAN RESPONSE TO STIMULATION

The ability to accurately assess and predict ovarian response would reduce the burdens imposed by failure because of inadequate response to stimulation. Unfortunately, the response to stimulation cannot be reliably predicted, even for young patients with no evidence of endocrine disorders. Parameters that have been identified as exerting an influence include age (17,18), cause of infertility (10), body weight (18), and body mass index (BMI) (18). Ovarian characteristics have also been assessed by ultrasound, such as the number and size of antral follicles,

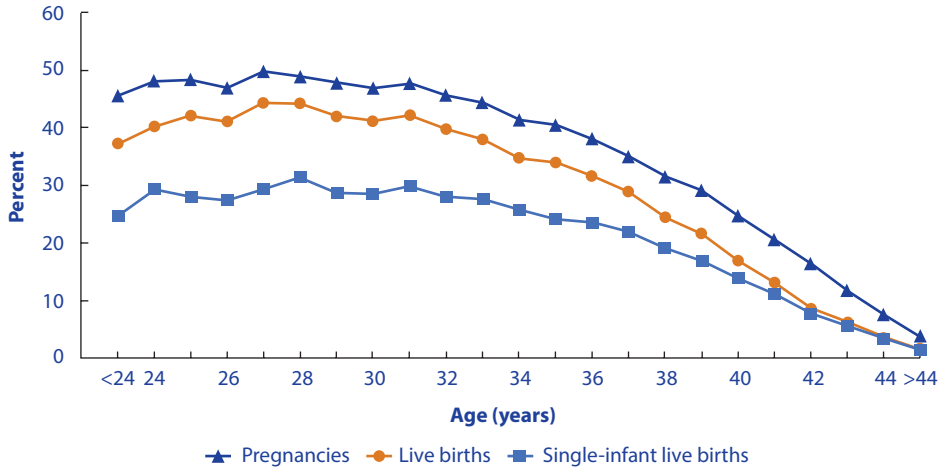


Figure 50.1 Percentages of assisted reproduction technology cycles using fresh non-donor eggs or embryos that resulted in pregnancies, live births, and single-infant live births, by age of woman, from U.S. Centers for Disease Control and Prevention data 2013. Over the age of 35, there is a further acceleration in the decline in pregnancy rate following assisted reproduction technology procedures (9).

ovarian volume, and ovarian vascular resistance measured by Doppler ultrasound.

There is a clear correlation between the number of antral follicles (defined as ≥2 mm to ≥10 mm) seen at the beginning of the follicular phase during a natural cycle (NC) and subsequent ovarian response to stimulation. However, there is as yet no consensus of agreement regarding the minimum number of antral follicles below which an influence can be seen (19–25); a minimum of fewer than five follicles of 2–5 mm in diameter has been suggested as a predictive parameter (24). One of the major reasons for this was a lack of standardized definition for assessment of the AFC, whose accuracy of measurement is highly operator dependent (23). Klinkert et al. (24) suggest that patients with an AFC of fewer than five follicles of 2–5 mm in diameter are expected to have a poor response, and in a randomized controlled trial (RCT), they demonstrated that doubling the starting doses of gonadotropins does not lead to an improvement in response for these patients during IVF treatment (25). In this study of 52 patients, more than half were aged >40 years, and 13 had basal FSH levels >15 IU/L.

Basal hormone assessment at the start of the follicular phase has been used to predict ovarian response, including FSH (26–32), estradiol (E2) (31,32), and inhibin-B (32–37). AMH is an accurate marker of ovarian reserve and oocyte yield (38–42). Circulating levels of AMH decline with increasing biological ovarian age, but remain relatively stable throughout each menstrual cycle (42,43), leading to it being measureable with accuracy at any time during the cycle. A comparison of AMH and FSH as predictors of retrieved oocyte numbers showed that AMH was clearly superior at predicting ovarian response (44). Moreover, a meta-analysis comparing AMH and AFC showed that AMH had the same level of accuracy and clinical value as AFC for the prediction of poor and hyper-response in IVF (45,46). A prospective cohort study of 538 patients undergoing their first ART cycle with differential COS strategies based on an AMH measurement showed that AMH was associated with oocyte yield and that a low AMH (1 to <5 pmol/L) was associated with a reduced clinical pregnancy rate (47). Similarly, other investigators showed that AMH-based prediction of ovarian response was independent of age and polycystic ovary syndrome (PCOS) in 165 patients undergoing a first COS cycle for ART (48). AMH was a significantly better predictor of poor response compared with FSH but not AFC. Various AMH cutoff values to predict a poor response have been explored. It has been suggested that an AMH cutoff level of <1.0 ng/mL (7.14 pmol/L) may have modest sensitivity and specificity in predicting a poor response to COS (48). For further details, see Figure 50.2.

Individualized, AMH-guided treatment protocols were shown to significantly improve IVF outcomes whilst reducing adverse effects and costs compared with conventional treatment in a retrospective study of 796 women (49). The incidence of ovarian hyperstimulation syndrome (OHSS) was also significantly lower with AMH-tailored versus conventional treatment. An age-related AMH nomogram is available for pretreatment patient counseling (42).

There have been attempts to develop models for ovarian response based upon algorithms made up of multiple predictive factors. For instance, Popovic-Todorovic and colleagues developed a scoring system for calculating the FSH starting dose, based on four predictors: the total number of antral follicles, total Doppler score, serum testosterone (T) levels, and smoking habit (21). This model was tested prospectively in a two-site clinical study, in which an ongoing pregnancy rate of 36.6% was reported using the algorithm to assign starting FSH doses of between 100 and 250 IU, compared with an ongoing pregnancy rate of 24.4% with a standard protocol using 150 IU FSH (50). Another algorithm to predict the recombinant human FSH (r-hFSH; follitropin-α) starting dose has been described but is only applicable to young (<35 years of age), normogonadotropic women (51). The four factors identified as significantly predictive of ovarian response were baseline serum FSH levels, BMI, age, and AFC. The basal and dynamic tests for

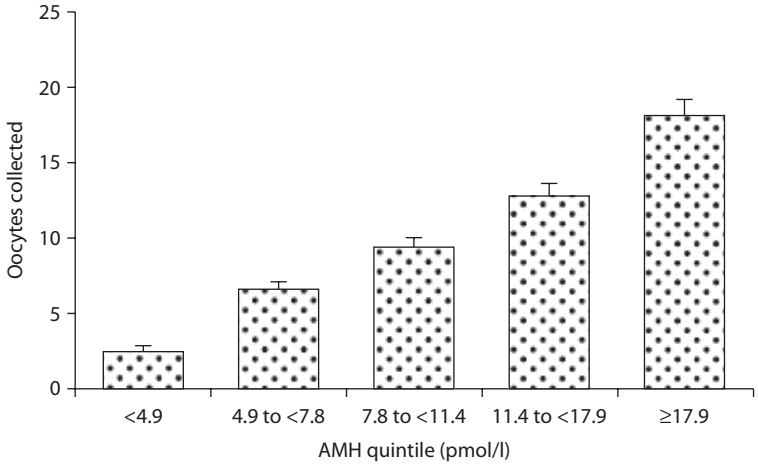


Figure 50.2 Mean oocyte yield per AMH quintile. Values are mean ± standard error of the mean (SEM) (38). *Abbreviation*: AMH, anti-Müllerian hormone.

prediction of ovarian reserve and response to stimulation are described in further detail in Chapter 38.

Given that AFC and AMH have the best predictive accuracy among other ovarian reserve markers, current therapeutic strategies have been proposed using either of these tests to choose the ideal protocol. Such tailored treatment protocols maximize IVF outcomes whilst reducing avoidable risks such as cycle cancelation and OHSS (52).

This chapter will focus on classic and specialized protocols designed for poor-responder patients, as well as on hormonal and pharmacologic manipulations that are expected to improve ovarian response. A large variety of strategies have been developed to improve outcome in patients with diminished ovarian reserve. There is no established intervention or treatment protocol for poor responders (53,54). Indeed, all of the currently available COS protocols have been used, with or without modifications, for the treatment of poor responders. Unfortunately, each of these approaches has achieved only limited success (53–58).

HIGH-DOSE GONADOTROPINS

It is generally believed that the dose of gonadotropins should be adjusted upwards in an attempt to overcome the age-related decline in ovarian response to FSH stimulation. Patients who responded poorly to conventional doses (150–225 IU of FSH) may produce more follicles when given 300–450 IU or even 600 IU per day. It is expected that an enhanced response would lead to an increased number of oocytes retrieved, number of available embryos, and, ultimately, higher pregnancy and live birth rates (59,60). These expectations, however, are not always met, and this strategy is often of limited effectiveness.

Although higher circulating levels may be achieved by increasing the quantity of gonadotropins being administered, at some point saturation kinetics are attained (59,61) and the ovarian response is determined more by the number of follicles available for recruitment than by circulating gonadotropin levels. This is of particular importance, since poor responders generally have markedly diminished numbers of follicles available for recruitment, as reflected in their low AFC.

Very few studies have been conducted on the effects of increasing the dose of gonadotropins in poor responders. There are currently two published RCTs evaluating the efficacy of high gonadotropin starting doses in presumed poor responders (62,63). The first RCT randomized women undergoing IVF with an AFC <12 to receiving daily fixed doses of 300 IU versus 375 IU versus 450 IU of recombinant FSH using a micro-dose flare protocol (62). There was no significant difference in the number of mature oocytes retrieved, cycle cancelation, number of embryos transferred, and clinical pregnancy rates between the three arms. In the more recent RCT involving 356 women categorized as being at risk of POR based on age <41 years with basal FSH >10 IU/L, AMH <1 ng/mL, AFC ≤8, or a previous IVF cycle with ≥300 IU/day gonadotrophin that resulted in a cycle cancelation, fewer than eight follicles, or fewer than five oocytes, these women were randomized to 450 IU versus 600 IU of gonadotropin daily in a micro-dose agonist flare-up protocol (63). The study showed no significant differences in the number of mature oocytes, fertilization rate, implantation rate, and clinical pregnancy rate between the two groups. The commencement of both of these studies predated the ESHRE consensus and hence POR was not defined according to the Bologna criteria. However, evidence from these studies suggests that very high gonadotropin doses of >300 IU daily are unlikely to be beneficial.

An interesting question is whether it is possible to rescue a cycle with initial poor response by doubling the gonadotropin dose after stimulation has already started. An RCT (64) evaluated the effect of doubling the human menopausal gonadotropin (hMG) dose in the current cycle in which the ovarian response after five days of ovarian stimulation with 225 IU/day was considered "low." No effect of doubling the hMG dose was noted with regard to the length of ovarian stimulation, peak E2 values, number of follicles >11, and >14 mm in diameter on the day of human chorionic gonadotropin (hCG) administration, number of canceled cycles, number of oocytes retrieved, and the number of patients with three or fewer oocytes retrieved. It was concluded that doubling the hMG dose in the course of an IVF cycle is not effective at enhancing ovarian response. This is in accordance with current understanding of follicular growth dynamics, which states that follicular recruitment occurs only in the late luteal phase of the previous and early follicular phase of the current menstrual cycle.

In summary, increasing the starting dose of gonadotropins in poor responders is a rational approach that is widely practiced. A common starting dose would be at least 300 IU/day. Nevertheless, further dose increments are of limited effectiveness, and clinically meaningful improvements are only rarely obtained with doses >300 IU/day.

GONADOTROPIN-RELEASING HORMONE AGONISTS IN THE TREATMENT OF POOR RESPONDERS

Long gonadotropin-releasing hormone agonist protocols

The use of gonadotropin-releasing hormone agonists (GnRHas) has gained widespread popularity and most ART programs frequently use this approach for COS. A meta-analysis of RCTs and quasi-RCTs showed that use of GnRHas reduced cancelation rates, increased the number of oocytes retrieved, and improved clinical pregnancy rates per cycle commenced and per embryo transfer (ET), compared with conventional stimulation regimens without the use of GnRH analogs (65). The aim of the long protocol is to achieve pituitary down-regulation with suppression of endogenous gonadotropin secretion before stimulation with exogenous gonadotropins. Once pituitary down-regulation and ovarian suppression are achieved, ovarian stimulation with exogenous gonadotropins is commenced, while GnRHa administration is continued concomitantly

until the day of hCG administration. In the general IVF population, the long protocol has been found to be superior in terms of efficacy compared with the short protocol (66) and is therefore used most frequently. However, the matter of which GnRHa protocol is preferable in poor responders remains controversial.

Down-regulation of the hypothalamic–pituitary–ovarian axis prior to gonadotropin therapy is often associated with prolongation of the follicular phase and a significant increase in the dosage of gonadotropins required to achieve adequate follicular development. The extent of this increase is far greater than what could be attributed to simply delaying hCG administration to the point where a larger cohort of homogeneously well-synchronized large follicles are present. Moreover, in some relatively young patients with normal ovarian reserve, it was difficult to induce any ovarian response in the presence of pituitary down-regulation, even with very large doses of exogenous gonadotropins (67–71). Normal ovarian function was restored in these patients after withdrawal of the GnRHa, with subsequent normal response to hMG (69,70). These early observations indicated that GnRHa may induce a state of ovarian hyporesponsiveness and raised doubt on the efficacy of the long protocol for poor responders.

Several theories have been suggested in an attempt to explain the dramatic (often two-fold) increase in exogenous gonadotropin requirements during pituitary down-regulation:

1. Diminished circulating endogenous gonadotropin levels (67,68)
2. Altered biologic activity of endogenous gonadotropins (72–74)
3. Interference with follicular recruitment (75)
4. Direct ovarian inhibition effects by GnRHas (76,77)

It has been well established that there is a dose-dependent duration of ovarian suppression after single implant injections of GnRHa, and that in a suppressed pituitary gland the dose needed to maintain suppression gradually decreases with the length of treatment (78). This supports the concept of step-down GnRHa protocols, where the dose of the agonist is decreased once the criteria for ovarian suppression have been achieved. Furthermore, the minimal effective dose for sufficient pituitary suppression with GnRHas has not been thoroughly studied before their actual introduction to clinical practice. Regarding triptorelin, for example, Janssens et al. (79), in a prospective, placebo-controlled, double-blind study, demonstrated that daily administration of 15 μg of triptorelin is sufficient to prevent a premature LH surge, and that 50 μg is equivalent to 100 μg in terms of IVF results.

In an attempt to maximize ovarian response without losing the benefits of GnRHa down-regulation, Feldberg et al. (80) introduced the use of the mini-dose GnRHa protocol in poor responders. They found that patients with elevated basal FSH levels who received daily triptorelin 100 μg subcutaneously (s.c.) from the mid-luteal phase until menstruation and 50 μg thereafter had higher peak E2 levels, more oocytes recovered, and more embryos transferred. They also noted a trend toward improved pregnancy and implantation rates and a lower spontaneous abortion rate.

Olivennes et al. (81) studied 98 IVF patients with a high basal FSH concentration who were previously treated by the long protocol with a GnRHa in a depot formulation. The same patients received s.c. leuprolide acetate (LA) 0.1 mg/day from cycle day 21, reducing it to 0.05 mg/day upon down-regulation. The comparison was made using the previous IVF cycle of the same patient as a control. The use of a low-dose agonist protocol resulted in significantly reduced gonadotropin requirements, a shorter duration of stimulation, a higher E2 concentration on stimulation day 8, a higher number of mature oocytes, and a higher number of good-quality embryos. The cancelation rate was lower (11% vs. 24%). Kowalik et al. (76) have demonstrated that lowering the dose of LA resulted in a faster E2 rise and higher mean peak E2 level. The higher E2 levels were obtained with a lower total gonadotropin dose. The oocyte yield was not affected. It was concluded that lowering the dosage of LA allows higher E2 response, which suggests an inhibitory *in vivo* effect of LA on ovarian steroidogenesis. Davis and Rosenwaks (71) reported similar results using a low-dose LA protocol.

Weissman et al. (82) prospectively compared two stimulation protocols specifically designed for poor-responder patients. Sixty poor responders who were recruited on the basis of response in previous cycles received either a modified flare-up protocol in which a high dose of triptorelin (500 μg) was administered for the first four days followed by a standard dose (100 μg), or a mini-dose long protocol in which 100 μg triptorelin was used until pituitary down-regulation, after which the triptorelin dose was halved during stimulation. Twenty-nine cycles were performed with the modified flare-up protocol and 31 were performed with the mini-dose long protocol. Significantly more oocytes were obtained with the modified long protocol than the modified flare protocol. The number and quality of embryos available for transfer were similar in both groups. One clinical pregnancy (3.4%) was achieved with the modified flare protocol, and seven pregnancies (22.5%) were achieved using the mini-dose long protocol.

Ovarian cyst formation is a common complication of the long GnRHa protocol. It has been suggested as being typical for poor responders and as being a reliable predictor of poor stimulation and low pregnancy rates in a given cycle (83,84). Although the pathophysiology of ovarian cyst formation following GnRHa administration has not been completely elucidated, the higher the serum progesterone level at the time of commencing GnRHa administration, the lower the incidence of cyst formation (85). Progestagen pretreatment directly inhibits endogenous gonadotropin secretion and influences the pattern of gonadotropin and hypothalamic GnRH secretion. Three RCTs have demonstrated the successful use of progestins to prevent ovarian

cyst formation during pituitary suppression in IVF cycles (86–88). We have also successfully included progestagen pretreatment in the long mini-dose protocol (82).

It has to be recognized that the above studies varied in their definitions of POR, and as they were conducted well before the ESHRE consensus definition, none of them fulfilled the Bologna criteria for POR. An RCT comparing the efficacy of the long GnRHa protocol versus the short GnRHa protocol versus the GnRH antagonist (GnRH-ant) protocol among women with a previous POR demonstrated the long GnRHa and the GnRH-ant protocols to be superior in terms of the numbers of oocytes retrieved. Women who had the short GnRHa protocol had significantly lower numbers of retrieved oocytes (2.71 ± 1.60) compared to the long protocol (4.42 ± 3.06) (89). This study used stringent inclusion criteria and POR was defined as a previous canceled IVF cycle or three of fewer oocytes retrieved following stimulation with gonadotrophin $\geq$300 IU/day. Summarizing the above evidence, the long GnRHa protocol seems to be a suitable option for poor responders (see Figure 50.3).

GnRHa "stop" protocols

Pituitary recovery and resumption of gonadotropin secretion following GnRHa treatment may take up to several weeks, depending on the dose and route of administration of the agonist. For example, with intranasal buserelin acetate (BA), suppression of endogenous gonadotropin secretion seems to continue for at least 12 days after the discontinuation of the agonist (90), as was also reported for s.c. BA (91). Interestingly, using the "ultrashort protocol," suppression of endogenous LH secretion was more profound when LA administration was stopped after five days of administration, compared with continuous LA administration, and no premature LH peak was recorded (92). This forms the basis for a variety of discontinuous or "stop" GnRHa protocols.

The above observations prompted several studies in which GnRHas were administered in the long protocol, but agonist administration was withheld once gonadotropin stimulation had started (93–97). The majority of studies have shown favorable results in terms of both clinical outcome and cost-effectiveness, but studies showing discouraging results were also reported (97). Corson et al. (93) prospectively evaluated the effect of stopping GnRHa (s.c. LA) therapy upon initiation of ovarian stimulation versus simultaneous GnRHa and gonadotropin therapy. Both groups were found to be comparable in terms of the duration of stimulation and amount of exogenous gonadotropins required, as well as for any other stimulation or outcome parameter studied. Stopping LA upon initiation of ovarian stimulation did not reduce its efficacy at suppressing LH secretion, as in neither group was a premature LH surge detected.

Similar results were obtained in a prospective study that compared two protocols with variable duration of BA

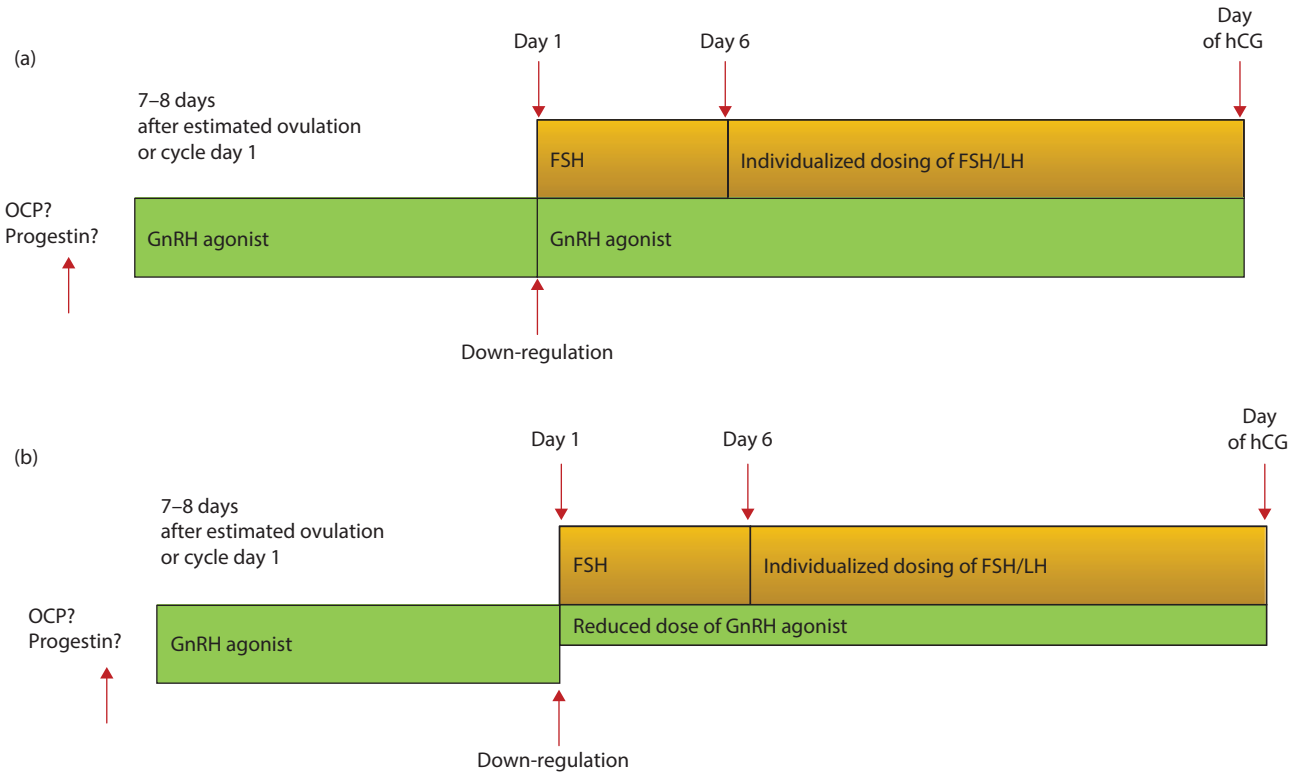


Figure 50.3 (a) The long GnRH agonist protocol. (b) The "mini-dose" long agonist protocol. *Abbreviations*: FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; OCP, oral contraceptive pill.

administration in an IVF/gamete intrafallopian transfer program (94). No spontaneous premature LH surges were recorded in any of the groups, and all parameters of ovarian response to stimulation were found to be comparable for both groups. A trend towards a higher pregnancy rate per ET was noted in the discontinuous BA arm. Simons et al. (95) compared the efficacy of two early cessation protocols of triptorelin treatment with the conventional long protocol in IVF. In a multicenter RCT, 178 women were randomized to one of three treatment groups at the start of stimulation. s.c. triptorelin was started at the mid-luteal phase of the previous cycle and continued until the first day of gonadotropin treatment, or up to and including the fourth day of gonadotropin treatment or the day of hCG injection. One premature LH surge was observed in the second group. Both early cessation protocols were at least as effective as the standard long protocol with regard to the number of oocytes, number of embryos, and ongoing pregnancy rate. It was concluded that early cessation of triptorelin on day 1 of gonadotropin treatment is as effective as the traditional long protocol at preventing a premature LH surge and results in similar reproductive outcomes.

In contrast, Fujii et al. (97) reported on an RCT where 900 μg/day of intranasal BA was administered from the mid-luteal phase of the previous cycle until cycle day 7, when normal-responding patients were randomized to receive either gonadotropin stimulation alone or combined BA and gonadotropin therapy. The duration and total dose of gonadotropins administered were significantly increased in the early GnRHa cessation group compared with the conventional long protocol. The numbers of fertilized oocytes and embryos transferred were significantly lower and the cancelation rate and rate of failed oocyte retrieval were significantly higher in the discontinuous long protocol. Although premature LH surges were not recorded in either group, serum progesterone and LH concentrations were significantly increased on the day of hCG administration with the discontinuous long protocol. Clinical pregnancy rates per transfer were similar for both protocols. It was concluded that early discontinuation of the GnRHa is not beneficial and not cost-effective because of its adverse effects on follicular development and increased exogenous gonadotropin requirements, respectively. A reason for this could be because stopping daily agonist administration combined with ovarian stimulation leads to a further reduction in circulating LH concentrations (98), which supports the concept that there is still a small release of LH following daily agonist administration.

Discontinuous protocols were considered to be potentially beneficial for poor-responder patients undergoing IVF-ET (99). Several trials with contradictory results have been reported. Faber et al. (100) conducted a single-group uncontrolled study in which poor-responder patients were treated with LA 0.5 mg/day starting at the mid-luteal phase of the previous cycle. With the onset of menses, LA was discontinued and high-dose gonadotropin therapy was initiated. The cancelation rate was 12.5% (28/224 cycles), and only one case of premature LH surge was observed. Despite the uncontrolled nature of the study, a clinical pregnancy rate per transfer of 32% and an ongoing pregnancy rate per transfer of 23%, which seemed highly favorable for the specific subgroup of poor-responder patients, were achieved.

Subsequently, Wang et al. (101) conducted a prospective non-randomized study to determine the efficacy of a "stop" protocol in previously poor responders to a standard long protocol. Fifty patients were scheduled for 52 cycles of the modified "stop" agonist protocol. All patients received GnRHa from the mid-luteal phase of the previous cycle to the onset of menstruation, followed by high-dose gonadotropin stimulation. Six of the 52 cycles (11.8%) were canceled because of POR. One premature ovulation was noted, and in the other 45 cycles, an average of 6.3 mature oocytes were retrieved. A favorable embryo implantation rate (11.5%) and clinical pregnancy rate (20.5%) were noted.

In a prospective study with historical controls involving 36 poor responders, the use of intranasal nafarelin (600 μg/day) commenced in the mid-luteal phase and discontinued on day 5 of ovarian stimulation was evaluated (102). The cancelation rate was 8.3%, and there was a trend towards increased peak E2 levels and an increase in the number of oocytes retrieved. The ongoing pregnancy rate per ET was 15%. A significant improvement in both the number and the quality of cleaving embryos was observed, and it was suggested therefore that discontinuation of the GnRHa leads to improved oocyte quality.

In another prospective study with historical controls (103), 39 "stop" nafarelin cycles in 30 previously poor-responder patients were compared to 60 past cycles in the same individuals. A significantly higher number of oocytes were retrieved and a higher number of embryos were available for transfer. No cases of premature LH surge were recorded. Pregnancy rates per ET and per cycle were 10.4% and 7.7%, respectively.

In contrast, Dirnfeld et al. (104) reported on an RCT involving 78 cycles in which a "stop agonist" regimen was compared with a standard long luteal protocol. Intranasal BA (1 mg/day) or s.c. triptorelin (100 μg/day) were initiated on day 21 of the previous cycle and ceased once pituitary suppression was confirmed. Ovarian stimulation was induced with the use of 225–375 IU/day hMG or purified FSH, commencing on the day of down-regulation. A significantly higher cancelation rate was noted with the stop regimen compared with the controls (22.5% vs. 5.0%, respectively). The stop and long regimens resulted in similar stimulation characteristics and clinical pregnancy rates (11% vs. 10.3%, respectively). Only in patients with a basal FSH level that was not persistently high did the stop regimen result in a significantly higher number of retrieved oocytes compared with the standard long protocol (7.6 vs. 4.0, respectively). It was concluded that, for most poor responders, the stop regimen offers no further advantage over the standard long protocol.

Garcia-Velasco et al. (105) designed an RCT in order to evaluate whether early cessation of the GnRHa (LA) is more beneficial than just increasing the doses of gonadotropins in poor-responder patients. Seventy poor-responder patients with normal basal FSH concentrations and a previous canceled IVF cycle were randomly allocated to either a standard long protocol or a stop protocol. A significantly higher number of mature oocytes were obtained with the stop protocol compared with the standard long protocol (8.7 vs. 6.2). The stop protocol significantly reduced the gonadotropin requirements. Both protocols resulted in a similar cancelation rate (2.7% vs. 5.8%), pregnancy rate (14.3% vs. 18.7%), and implantation rate (12.1% vs. 8.8%). It was concluded that the stop protocol combined with high doses of gonadotropins permitted the retrieval of a significantly higher number of oocytes, but did not influence the reproductive outcome.

Short GnRHa regimens

The short protocol consists of early follicular-phase initiation of GnRHa, with minimal delay before commencing gonadotropin ovarian stimulation. It takes advantage of the initial agonistic stimulatory effect of GnRHa on endogenous FSH and LH secretion, also known as the flare-up effect. In theory, it eliminates excessive ovarian suppression associated with prolonged agonist use. The duration of the endogenous gonadotropin flare has not been completely characterized, but pituitary desensitization is generally achieved within five days of initiating treatment (106), and therefore patients are protected from premature LH surges by the end of the stimulation phase. The short protocol has been proposed by many authors as a better stimulation protocol for poor responders (107–109).

In an early prospective study with historical controls and using an ultrashort protocol, Howles et al. (109) treated seven patients who had previously responded poorly to stimulation with clomiphene citrate (CC) and hMG with 0.5 mg/day BA during only the first three days of the cycle (ultrashort protocol). All seven patients had oocytes recovered and embryos replaced, and three out of these seven conceived (42.9%). Similarly, Katayama et al. (110) reported improved cycle outcomes in seven prior poor-responder patients with the short regimen. Garcia et al. (108) conducted a non-randomized prospective trial comparing long luteal and short flare-up agonist initiation in 189 cycles. They noted a significant decrease in exogenous gonadotropin requirements, higher pregnancy rates, and decreased miscarriage rates in patients receiving the flare-up regimen. In a retrospective comparison, Toth et al. (111) also reported that pregnancy and implantation rates were significantly higher and cancelation rates lower in patients with basal serum FSH levels $\geq$15 mIU/mL undergoing a flare-up regimen versus a long luteal agonist regimen. In a prospective uncontrolled study, Padilla et al. (107) administered a flare-up protocol with high-dose gonadotropins to 53 patients who were thought to be at risk for poor response after a "leuprolide acetate screening test." The cancelation rate was higher in poor flare-up LA test responders (11.3%) compared with good flare-up LA responders (1.1%) and luteal-phase long protocol cycles (1.8%). Despite a low number of oocytes retrieved, the ongoing pregnancy rate was 29% per retrieval and was considered favorable for this group of potentially poor-responder patients.

Despite these encouraging findings, other authors failed to confirm any substantial benefit of using a classic flare-up protocol. In a prospective study with historical controls (112), 80 poor responders were treated using a classic flare-up regimen with LA 0.5 mg/day from cycle day 2 and high-dose hMG from cycle day 3. While the number of retrieved oocytes was increased (10 ± 6.6), the cancelation rate was high (23.4%), and the ongoing pregnancy rates of 6.5% per retrieval and 7.6% per transfer were disappointing. Brzyski et al. (113) reported that not only did concomitant initiation of GnRHa with purified urinary FSH result in poorer cycle outcome, but also an increased number of atretic oocytes were retrieved. A significant increase in LH and progesterone levels during the follicular phase was noted. Other groups using this approach also reported failure to improve ovarian response or cycle outcome in generally similar patient populations (114–116).

Despite the rationale for use of the short agonist protocol, the RCT comparing the long agonist versus the short agonist versus the antagonist protocols showed that the short agonist protocol was less effective than the long agonist protocol for poor responders (89). In an RCT, San Roman et al. (117) have shown that a combination of early follicular-phase LA administration and hMG stimulation was associated with a significant increase in serum LH levels beginning with the first follicular-phase agonist dose, and with significant increases in serum progesterone and T levels during the follicular phase compared with mid-luteal GnRHa administration. The live birth rate/retrieval for the long protocol was 25% compared with 3.8% in the flare-up group. This may be the result of the initial flare-up effect of GnRHa on LH secretion causing raised LH levels. Evidence of an adverse effect of high endogenous LH levels during the follicular phase has led to the establishment of the ceiling theory (118). According to this theory, beyond a certain ceiling level, LH suppresses GC proliferation and initiates atresia of less mature follicles.

Further support for this view comes from a study of Gelety et al. (119), who performed a prospective randomized crossover study of five regularly cycling women in order to determine the short-term pituitary and ovarian effects of GnRHa administered during differing phases of the menstrual cycle in the absence of gonadotropin stimulation. Each patient was administered LA 1 mg/day s.c. for five days beginning on cycle day 3, eight days post-LH surge, and 13 days post-LH surge with an intervening "washout" month. Significant increases in serum LH, E2, estrone, androgens, and progesterone levels were noted in the early follicular-phase group compared with the mid-luteal group. Early follicular initiation of the agonist resulted in a more pronounced suppression of FSH. It

was suggested that relative FSH suppression and marked LH elevations could have potential detrimental effects on oocytes of the developing cohort that are often observed with flare-up regimens.

Can the adverse effects of the gonadotropin flare be prevented without losing the potential benefits of the short protocol? Two possible solutions have been suggested: the first is pretreatment with an oral contraceptive pill (OCP) or a progestin. Cédrin-Durnerin et al. (120) noted that pretreatment with a 12–20-day course of the progestin norethisterone before initiation of a flare-up regimen effectively lowered LH and progesterone levels during the early stages of gonadotropin stimulation. Many clinicians thus regard pretreatment with an OCP or a progestin as integral in flare-up regimens, although this issue also became a matter of controversy (121). The second solution is dose reduction of the GnRHa causing the flare, which forms the basis for "micro-dose flare" regimens (Figure 50.4).

Micro-dose flare GnRHa regimens

In theory, micro-dose flare regimens decrease the enhanced LH and progesterone secretion associated with standard flare-up regimens, as described above. Bstandig et al. (122) studied the hormonal profiles during the flare-up period using 25 and 100 μg of triptorelin in the short protocol. No significant difference in the magnitude of FSH and E2 release was observed between the two groups, but the maximal plasma LH level was significantly reduced after injection of 25 μg of triptorelin. It was suggested that in the flare protocol, a lower dose of GnRHa induces a hormonal flare-up that is more conducive to optimal follicular recruitment. Deaton et al. (123) have demonstrated that an extremely low dose of LA (25 or 50 μg) is needed to cause a pituitary flare of gonadotropins. Following a flare from 25 μg of LA on cycle day 2, the pituitary is able to recover and respond with a repeat flare on cycle day 5. These observations support the rationale behind the so-called micro-dose flare protocols.

Navot et al. (124) studied the effect of very low doses of GnRHa in cynomolgus monkeys and humans and established that 10 mg of historelin in four divided doses (micro-doses) could induce ovarian hyperstimulation in humans. Scott et al. (125) reported that an increase in gonadotropin levels could be induced in baboons with LA doses as low as 0.017 mg/kg. Although the minimal and optimal effective dose of GnRHa that can be successfully used to induce a gonadotropin flare in humans has not been thoroughly evaluated, several investigators have reported an improved outcome with doses as low as 20–40 μg of LA twice daily in poor responders.

In a prospective study with historical controls, Scott and Navot (126) treated 34 poor-responder patients with an OCP followed by 20 μg LA twice daily beginning on cycle day 3

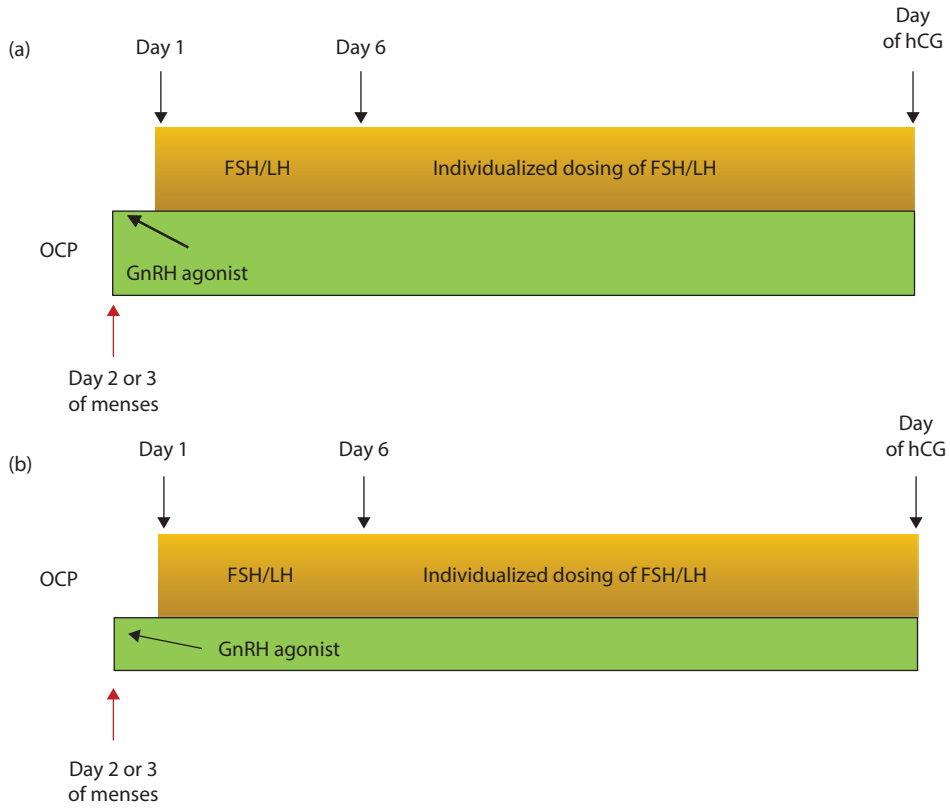


Figure 50.4 (a) The short GnRH agonist protocol. (b) The "micro-dose" flare GnRH agonist protocol. *Abbreviations*: FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; OCP, oral contraceptive pill.

and supplemented with exogenous gonadotropins beginning on cycle day 5. Ovarian responsiveness was enhanced with the micro-dose GnRHa stimulation cycle when compared with previous stimulation cycles. Specifically, the patients had a more rapid rise in E2 levels, much higher peak E2 levels, the development of more mature follicles, and the recovery of larger numbers of mature oocytes. None of the patients had a premature LH surge.

Impressive results using the micro-dose flare protocol were also reported in a prospective study with historical controls by Schoolcraft et al. (127). Thirty-two patients, whose prior long luteal agonist cycles had been canceled because of poor response, were now pretreated with an OCP followed by follicular-phase administration of 40 μg LA twice daily beginning on cycle day 3 and high-dose FSH supplemented with human growth hormone (hGH) beginning on cycle day 5. Compared with the prior long luteal GnRHa cycle, there was a higher E2 response, more oocytes retrieved (10.9 per patient), fewer cycle cancelations (12.5%), and no premature LH surge or luteinization. For patients who were not canceled, a favorable ongoing pregnancy rate of 50% was achieved.

In a prospective non-randomized trial with historical controls, Surrey et al. (128) treated 34 patients with a prior poor response to a standard mid-luteal long protocol with an OCP followed by LA 40 μg twice daily and high-dose gonadotropins. Cycle cancelation rates were dramatically reduced, and the mean maximal serum E2 levels obtained were significantly higher. The ongoing pregnancy rates per ET were 33% in patients aged ≤39 years and 18.2% in patients aged >39 years. Significant increases in circulating FSH levels occurred after five days of gonadotropin stimulation. No abnormal rises in LH, progesterone, or T during the follicular phase were noted. This could result from either the lower GnRHa dose, the OCP pretreatment, or a combination of the two.

Detti et al. reported on a retrospective cohort study that assessed the efficacy of three different GnRHa stimulation regimens to improve ovarian response in poor responders (99). Women diagnosed as poor responders underwent three different stimulation regimens during IVF cycles:

1. Stop protocol: LA 500 μg/day administered from the mid-luteal phase to the start of menses, then gonadotropins from day 2 of the cycle.
2. Micro-dose flare: LA 20 μg administered twice daily with gonadotropins from day 2 to the day of hCG administration.
3. Regular dose flare: gonadotropins beginning with LA on day 2 at 1 mg/day for three days, followed by 250 LA μg/day until the day of hCG administration.

Since only 61 cycles were included in the analysis, none of the comparisons reached statistical significance; however, the micro-dose flare group demonstrated a trend toward a higher delivery rate.

It is noteworthy that, in a general IVF population (excluding poor responders), retrospective analysis failed to find the micro-dose flare protocol to be superior over the long mid-luteal agonist regimen (129). Significantly higher cancelation rates (22.5% vs. 8.2%), lower clinical pregnancy rates (47.3% vs. 60%, non-significant), and a decreased number of oocytes retrieved per cycle (13.3 vs. 16.5, non-significant) were noted with the micro-dose flare-up regimen.

Overall, all studies evaluating the micro-dose flare protocol were retrospective in nature. Obviously, large prospective RCTs are needed to validate the true efficacy of the micro-dose flare-up GnRHa regimens in poor-responder patients.

GnRH-ANTS IN THE TREATMENT OF POOR RESPONDERS

GnRH-ants competitively block the GnRH receptor in the pituitary gland, producing an immediate dose-related suppression of gonadotropin release. Within six hours of GnRH-ant administration, LH levels are significantly reduced. On the principle of maximizing potential endogenous pituitary stimulation, a GnRH-ant can be administered later in the follicular phase to suppress the LH surge (130,131), thus avoiding suppression during the phase of early follicular recruitment (Figure 50.5). In the general IVF population, the GnRH-ants offer comparable therapeutic efficacy to agonists and have a number of potential advantages over agonists for use in ovarian stimulation protocols, such as avoiding the initial "flare-up" of LH, shortening the overall treatment period, reducing the risk of OHSS, and reducing menopausal side effects (130–132).

The GnRH-ants are administered in the late follicular phase, either according to the fixed or according to the flexible protocol (see Chapter 43). Thus, at the beginning of COS, the pituitary is fully susceptible to GnRH pulses. This may allow us to obtain a more natural follicular recruitment without any inhibitory effect possibly induced by the GnRHa. It has therefore been suggested as a suitable protocol for poor responders. GnRH-ants also permit the revival of stimulation protocols of the pre-agonist era using, for example, CC (133). The combination of CC treatment in the early follicular phase and subsequent overlapping gonadotropin stimulation has been a standard therapy in the past (133,134). Owing to the synergistic

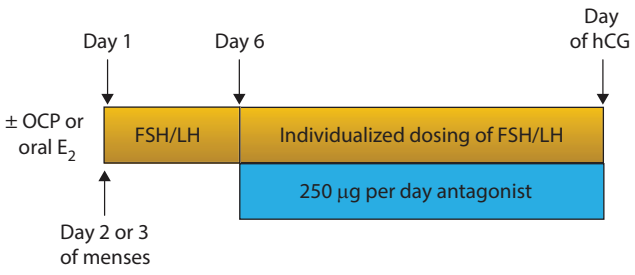


Figure 50.5 Gonadotropin-releasing hormone antagonist protocol. *Abbreviations*: E2, estradiol; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; OCP, oral contraceptive pill.

effect of these compounds, the amount of gonadotropins required is lower and so are the costs (135,136). In addition, the gonadotropins counteract the detrimental effects of CC on the endometrium (135). As a result of the high rate of premature LH surges, and therefore the high cancelation rate, this stimulation regimen was abandoned when GnRHas were introduced in IVF.

Craft et al. (137) were the first to suggest the use of GnRH-ants for COS in poor responders. In a small retrospective series, 18 previously poor responders were stimulated with a combination of gonadotropins and CC, and started on a GnRH-ant according to the flexible protocol. Compared to their poor response in a previous GnRHa cycle, modest improvements in cycle cancelation rates (29% vs. 57%), oocyte yield (6.4 vs. 4.7), and gonadotropin requirements (4506 vs. 5468 IU) were noted with the GnRH-ant. Two live births resulted (11.8%). Several studies were subsequently undertaken in order to examine the efficacy of GnRH-ants in COS regimens designed for poor responders. The majority of these studies were of a small scale and retrospective. Retrospective studies will be presented first, followed by more recently reported RCTs.

Retrospective studies

Nikolettos et al. (138) compared 21 poor responders who underwent IVF–ICSI and were treated with a GnRH-ant protocol with 21 matched poor responders treated according to the long GnRHa protocol. Fifteen patients of the GnRH-ant group were treated with the combination of CC plus gonadotropins, while six patients were treated with gonadotropins alone. The use of the GnRH-ant protocol resulted in a significantly shorter treatment duration and lower gonadotropin consumption as compared with the use of the long GnRHa protocol. Three pregnancies (14.3%) were achieved with the antagonist and two (9.5%) with the long agonist protocol (non-significant).

Several retrospective studies have compared the GnRH-ant protocol with GnRHa flare-up and micro-dose flare regimens. In a retrospective cohort study, Posada et al. (139) compared the clinical outcome of COS in unselected patients undergoing IVF with a GnRH-ant (133 cycles) versus a four-day ultrashort GnRHa regimen (236 cycles). The GnRH-ant protocol was shown to reduce treatment duration and amount of gonadotropin used. In younger women, the antagonist protocol was associated with significantly better pregnancy and implantation rates, but no difference was observed in pregnancy rates in patients aged >38 years.

Mohamed et al. (140) retrospectively compared the agonist flare-up and antagonist protocols in the management of poor responders to the standard long protocol. A total of 134 patients undergoing IVF–ICSI treatment who responded poorly to the standard long protocol in their first treatment cycle were studied. In the second cycle, 77 patients received a short GnRHa flare-up regimen and 57 patients received an antagonist protocol, based solely on physician preference. There were no cycle cancelations in the flare-up protocol and there was a 7% cancelation rate in the antagonist protocol due to lack of response. A significantly higher number of patients had ET in the flare-up protocol. Similar numbers of oocytes (5.4 vs. 5.2) and similar implantation and pregnancy rates per cycle (12.8% and 17.5% vs. 12.8% and 24.7%) were reported in the antagonist and flare-up groups, respectively. It was concluded that both the flare-up and the antagonist protocols significantly improved the ovarian response of previously poor responders. However, a significantly higher cycle cancelation rate and fewer patients having ET in the antagonist group suggested a higher efficacy for the flare-up regimen.

Conflicting results were reported by Fasouliotis et al. (141), who also conducted a retrospective analysis between the flare-up and antagonist regimens in poor responders. Of 56 poor responders treated with the flare-up protocol, 53 who failed to conceive were subsequently treated in the next cycle with a GnRH-ant regimen. While ovarian response did not differ between the two protocols, the number of embryos transferred was significantly higher in the GnRH-ant group (2.5 ± 1.6 vs. 2.0 ± 1.4, respectively). The clinical pregnancy and implantation rates per transfer in the GnRH-ant group tended to be higher than in the flare-up group, but did not reach significance (26.1% and 10.7% compared with 12.2% and 5.9%, respectively). However, the ongoing pregnancy rate per transfer was significantly higher in the GnRH-ant than in the GnRHa flare-up group (23.9% vs. 7.3%, respectively).

Copperman (142) conducted a retrospective analysis with historic controls comparing cycle outcomes in poor responders who had stimulation protocols that included an antagonist with those with the micro-dose flare protocol. Patients were placed in the antagonist or micro-dose flare treatment groups usually after failing in an LA down-regulation cycle, and often according to physician preference. The results of this retrospective analysis indicated that, for poor responders, the inclusion of a GnRH-ant in the treatment regimen significantly increased clinical pregnancy rates and significantly lowered cancelation rates compared with patients treated with the micro-dose flare protocol.

The use of OCP pretreatment in antagonist cycles for poor-responder patients is also of clinical relevance, as their ovarian reserve may be especially sensitive to suppression of endogenous gonadotropins by the pill. Copperman (142) reported a retrospective study of 1343 patients, where poor responders were given a starting dose of 450 IU of gonadotropin. In the OCP pretreatment group, patients were administered OCP for 18–24 days, beginning on cycle day 3. Patients were first administered a combination of r-hFSH and hMG on cycle day 3, and were administered GnRH-ant when their lead follicle reached 14 mm. An additional 75 IU of hMG was administered beginning on the first day of antagonist treatment. Patients whose antagonist stimulation cycle included OCP pretreatment had a significantly higher pregnancy rate and a significantly lower cancelation rate. In addition, a higher proportion of patients obtained more than eight oocytes

following OCP pretreatment. In contrast, Shapiro et al. (143) reported significantly increased cancelation rates (23%) in a group of poor-responder patients pretreated with an OCP compared with patients not receiving OCP pretreatment (9%). The two studies, however, differed both in inclusion criteria and in the use of LH in the stimulation protocol.

Prospective studies

Akman et al. (144) compared a GnRH-ant protocol to a protocol using gonadotropins alone in poor responders. In total, 20 women were randomized to each group. Women assigned to the antagonist arm received 0.25 mg of cetrorelix according to the flexible protocol, and all women were initially stimulated with 600 IU of urinary-derived gonadotropin. There was no statistically significant difference between the groups for cancelation rates, gonadotropin requirements, number of mature oocytes retrieved, E2 concentrations on the day of hCG administration, fertilization rates, and number of embryos transferred. The clinical pregnancy and implantation rates in the antagonist group appeared higher, but were not significantly different (20.00% and 13.33% compared with 6.25% and 3.44%, respectively) because of the small numbers involved.

There are several RCTs that compare the agonist flare-up with the antagonist protocols. Akman et al. (145) compared clinical outcomes of 48 poor-responder patients who were treated with either a micro-dose flare (LA 40 μg s.c. per day) protocol or the antagonist (cetrorelix 0.25 mg daily) protocol. All patients received 300 IU of highly purified FSH and 300 IU of hMG for four days, followed by individual adjustments in the dose of highly purified FSH. Patients in the micro-dose flare group also received OCP pretreatment. There was no difference in the median total treatment doses of gonadotropins between the two groups. Serum E2 levels on the day of hCG administration and the number of oocytes retrieved were significantly lower in the antagonist group. No differences were observed between the two groups for fertilization rates, number of embryos transferred, and, most importantly, implantation rates and ongoing pregnancy rates per transfer. It was concluded that the efficacy of these stimulation protocols in poor-responder patients was comparable, but larger studies were needed.

De Placido et al. (146) randomized 133 women "at risk for poor ovarian response" to undergo COS by either a modified GnRH-ant protocol or a short flare-up regimen. Patients in the antagonist arm were treated by the flexible regimen with 300 IU of r-hFSH given from cycle day 2. When the lead follicle reached a diameter of 14 mm, cetrurelix 0.125 mg was given daily for two days followed by cetrurelix 0.25 mg daily until the day of hCG administration. Beginning on the same day of GnRH-ant administration, a daily dose of 150 IU of r-hLH (Luveris) was also added until the day of hCG administration. Patients in the flare-up arm received a daily dose of triptorelin (0.1 mg s.c.), beginning on the same day of the first r-hFSH administration. In addition, in this group, a dose of 150 IU/day of r-hLH was added when at least one follicle reached 14 mm. The mean number of metaphase II oocytes (primary endpoint) was significantly higher in the antagonist group (5.73 ± 3.57 vs. 4.64 ± 2.23, respectively; $p < 0.05$). Cancelation rates, gonadotropin requirements, implantation rates, and clinical and ongoing pregnancy rates were all comparable for the two groups.

Demirol and Gurgan (147) conducted an RCT comparing the short micro-flare and the flexible GnRH-ant protocols in 90 poor-responder patients. In the micro-flare group, 45 patients received an OCP and, on the third day of menstruation, 40 μg s.c. twice daily of LA followed by 450 IU/day of hMG. In the antagonist group, 45 patients received 450 IU/day hMG starting on day 3 and 0.25 mg cetrorelix administered daily when two or more follicles reached 13–14 mm in diameter. The total gonadotropin dose used was significantly higher in the antagonist group, while the number of oocytes retrieved was significantly greater in the micro-flare group (4.3 ± 2.13 vs. 3.1 ± 1.09; $p = 0.001$). The implantation rate was significantly higher in the micro-flare group than in the antagonist group (22% vs. 11%; $p = 0.017$). It was concluded that the short micro-flare protocol seems to have a better outcome in poor-responder patients, with a significantly higher mean number of mature oocytes retrieved and a higher implantation rate.

Kahraman et al. (148) conducted another RCT comparing the micro-flare and the antagonist protocols in patients who previously had a low response to the long GnRHa protocol. Twenty-one patients received LA (50 μg twice daily) starting on the second day of post-OCP bleeding. The other 21 patients received 0.25 mg of cetrorelix daily when the leading follicle reached 14 mm in diameter. Stimulation in both groups consisted of 300–450 IU daily doses of r-hFSH. The mean serum E2 concentration on the day of hCG administration was significantly higher in the micro-flare group than in the antagonist group (1904 vs. 1362 pg/mL; $p = 0.042$), but all other outcome variables studied were found to be comparable for the two groups. It was concluded that the micro-flare agonist and multiple-dose GnRH-ant protocol have similar efficacy in terms of improving treatment outcomes of poor-responder patients. Very similar findings were recently reported by Devesa et al. (149), who compared the micro-flare agonist and multiple-dose antagonist protocols in 221 poor-prognosis patients based on previous cycles or clinical criteria. Except for significantly higher serum E2 levels on hCG administration day in the micro-flare group, all other outcome variables were found to be comparable for the two groups.

Schmidt et al. (150) randomized 48 previously poor-responder patients to either a GnRH-ant protocol (ganirelix 0.25 mg daily in a flexible manner) or a micro-dose flare regimen (LA, 40 μg twice daily, after OCP pretreatment). Ovarian stimulation consisted of 300 IU of r-hFSH every morning and 150 IU of hMG every evening. Cancelation rates due to an inadequate response were equally high,

being close to 50% in both groups. While only 13 women in the antagonist group and 11 women who received a micro-dose flare completed their cycles, no significant differences in oocyte yield (8.9 vs. 9), fertilization rate (69.1% vs. 63.5%), or clinical pregnancy rate (38.5% vs. 36.4%) were detected. It was concluded that the antagonist protocol appears to be as effective as the micro-dose flare protocol for COS in poor responders, but could be a superior choice in terms of cost and convenience for the patient.

Malmusi et al. (151) compared the efficacy of the flare-up GnRHa protocol to the flexible GnRH-ant protocol in poor responders. Fifty-five poor-responder patients undergoing IVF–ICSI were randomized to receive either triptorelin (100 μg daily) from the first day of menstruation followed by exogenous gonadotropins from the second day of menstruation (30 cycles), or exogenous gonadotropins from the first day of menstrual cycle and later ganirelix (0.25 mg daily) once the leading follicle reached 14 mm in diameter (25 cycles). Gonadotropin requirements were significantly reduced with the flare-up protocol. The number of mature oocytes retrieved, fertilization rate, and top-quality embryos transferred were significantly increased in the flare-up compared to the GnRH-ant group. The implantation and pregnancy rates were similar in both groups.

Very few RCTs comparing the long GnRHa and the GnRH-ant for COS in poor responders have been published (Table 50.1) (152–155). Studies vary and suffer from considerable heterogeneities in terms of almost all possible aspects, such as inclusion criteria, agonist and antagonist administration regimens, and outcome variables reported. For example, in two studies (152,154), a depot preparation of a GnRHa was used, which is not a recommended administration route for low responders. In contrast, in the study by Tazegul et al. (153), a mini-dose agonist protocol was used, which is certainly a more appropriate administration route for low responders. Since these studies are not readily comparable, only general conclusions can be made. It appears that the GnRH-ant protocol is as effective as the long agonist protocol in poor responders. Gonadotropin consumption and stimulation duration both appear to be reduced with the antagonist protocol, a considerable practical advantage for patients. Clinical pregnancy and live birth rates appear to be similar.

Alternative approaches and treatment protocols using GnRH-ants

One of the problems often seen in poor-responding patients is a shortened follicular phase, which limits the ability to recruit a sizable cohort of follicles. Frankfurter et al. (156) described a novel use of a GnRH-ant before ovarian stimulation in an attempt to lengthen the follicular phase, aiming to lengthen the recruitment phase of the cycle to allow for the rescue of more follicles once gonadotropin stimulation was initiated. Twelve patients who previously exhibited a poor response to a standard (long, short, or antagonist) protocol were included. According to the new regimen, patients received two doses of 3 mg of cetrorelix, the first on cycle days 5–8 and the second four days later. With cetrorelix commencement, medroxyprogesterone acetate (MPA; 10 mg daily) was given and was continued until ovarian suppression was confirmed. Then, a combination of r-hFSH (225 IU s.c. twice daily) and r-hCG (2.5 mg s.c. four times a day) was initiated, and MPA was discontinued to allow for vaginal bleeding. When a lead follicle size of 13 mm was observed, daily cetrorelix (0.25 mg s.c.) was started and continued until hCG triggering. By using a GnRH-ant in the follicular phase before ovarian stimulation, significant improvements in oocyte, zygote, and embryo yields were achieved. A trend toward improved implantation (21%), clinical pregnancy (41.7%), and ongoing pregnancy (25%) rates in the follicular GnRH-ant cycle was also noted. More prospective studies are needed in order to examine the efficacy of this novel therapeutic approach.

Orvieto et al. (157) described the combination of the micro-flare GnRHa protocol and a GnRH-ant protocol in poor responders. This protocol combines the benefits of the stimulatory effect of the micro-flare on endogenous FSH release with the immediate LH suppression induced by the GnRH-ant, and was therefore suggested as a valuable new tool for treating poor responders (157,158). The stimulation characteristics of 21 consecutive ultrashort GnRHa/GnRH-ant cycles in 21 patients were compared with their previous failed cycles (157). Triptorelin (100 μg s.c.) was started on the first day of menses and continued for three consecutive days, followed by high-dose gonadotropins, which were initiated two days later. Once the lead follicle had reached a size of 14 mm and/or E2 levels exceeded 400 pg/mL, cetrorelix (0.25 mg/day) was introduced and continued up to and including the day of hCG administration. The number of follicles >14 mm on the day of hCG administration, the number of oocytes retrieved, and the number of embryos transferred were all significantly higher in the study protocol as compared with the historic control cycles. A reasonable clinical pregnancy rate (14.3%) was achieved.

Another innovative protocol using GnRHa/GnRH-ant conversion with estrogen priming (AACEP) in poor responders has been reported by Fisch et al. (159) and is described later in this chapter (in the section entitled "Luteal-phase manipulations").

NCs AND MODIFIED NCs

The yield of lengthy, high-dose, and cost-stimulation regimens used in poor responders to increase the number of oocytes retrieved is often disappointing. It was therefore suggested to perform NC-IVF in such cases, an approach that is less invasive and less costly for the patient.

Terminology

The International Society for Mild Approaches in Assisted Reproduction (ISMAAR) has recommended revised definitions and terminology for NC-IVF and different protocols used in ovarian stimulation for IVF (160). This was the result of the broad inconsistencies existing in the

Table 50.1 Cycle characteristics of randomized controlled trials comparing the long gonadotropin-releasing hormone agonist and gonadotropin-releasing hormone antagonist protocols in poor-responder patients undergoing *in vitro* fertilization

Study	Inclusion criteria	Number of patients on agonist	Number of patients on antagonist	Long protocol	Antagonist protocol	Gonadotropin type and dose	Cancelation rate (%)	Stimulation duration (days)	Gonadotropin consumption ampoules or FSH units	No. of oocytes retrieved	Implantation rate (%)	Clinical pregnancy rate started cycle (%)	Ongoing/ live birth rate (%)
D'Amato et al. (152)	<3 oocytes retrieved in >2 long agonist cycles or canceled cycles	60	85	Depot leuprolide, dose not given	Cetrurelix, multi-dose, flexible	Agonist: r-FSH, individualized dose Antagonist: clomiphene 100 mg days 2–6 plus r-FSH 300 IU	34 (agonist) 4.8 (antagonist)	—	50.05 ± 5.11 (agonist) 83.48 ± 7.05 (antagonist)	3.36 ± 1.3 (agonist) 5.56 ± 1.13 (antagonist)	7.6 (agonist) 13.5 (antagonist)	15.3 (agonist) 22.2 (antagonist)	NA
Cheung et al. (155)	<3 mature follicles on previous long protocol or basal FSH >10 IU/L	31	32	Luteal, nasal buserelin 60 μg daily following OCP	Cetrurelix, multi-dose, fixed (S6)	R-FSH 300 IU	34.4 (agonist) 38.7 (antagonist)	11.5 ± 2.4 (agonist) 10.5 ± 2.7 (antagonist)	3445 ± 730 (agonist) 3150 ± 813 (antagonist)	5.62 ± 4.17 (agonist) 5.89 ± 3.02 (antagonist)	13.3 (agonist) 13.6 (antagonist)	9.4 (agonist) 16.1 (antagonist)	NA
Marci et al. (154)	<3 oocytes retrieved and E2 maximum <600 pg/mL on a previous standard long protocol	30	30	Luteal, depot leuprolide 3.75 mg	Cetrurelix, multi-dose, flexible	R-FSH 375 IU	13.3 (agonist) 3.3 (antagonist)	14.6 ± 1.2 (agonist) 9.8 ± 0.8 (antagonist)	72.6 ± 6.8 (agonist) 49.3 ± 4.3 (antagonist)	4.3 ± 2.2 (agonist) 5.6 ± 1.6 (antagonist)	NA	6.6 (agonist) 16.6 (antagonist)	0 ag 13.3 antag
Tazegul et al. (153)	FSH <13 mIU/mL, E2 maximum <500 pg/mL on hCG day, <3 mature follicles, <4 oocytes retrieved	45	44	Luteal leuprolide 1 mg, decreased to 0.5 mg upon down-regulation	Cetrurelix/ ganirelix, multi-dose, flexible	R-FSH 300 IU and 300 IU hMG	6.8 (agonist) 9.0 (antagonist)	12.03 ± 2.86 (agonist) 10.6 ± 1.63 (antagonist)	3872.7 ± 1257.1 (agonist) 2467.7 ± 342.4 (antagonist)	5.47 ± 2.45 (agonist) 5.44 ± 1.29 (antagonist)	NA	24.4 (agonist) 22.7 (antagonist)	22.2 ag 18.1 antag

Abbreviations: FSH, follicle-stimulating hormone; r-FSH, recombinant follicle-stimulating hormone; E2, estradiol; OCP, oral contraceptive pill; S6, Stimulation day 6; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin.

terminology used for definitions and protocols for ovarian stimulation in IVF cycles, as will be seen later in this text. The term "natural cycle IVF" should be used when IVF is carried out with oocytes collected from a woman's ovary or ovaries in a spontaneous menstrual cycle without administration of any medication at any time during the cycle. The aim of this cycle is to collect a naturally selected single oocyte at the lowest possible cost. The term "modified natural cycle" (MNC) should be applied when exogenous hormones or any drugs are used when IVF is being performed during a spontaneous cycle with the aim of collecting a naturally selected single oocyte but with a reduction in the chance of cycle cancelation. This could include the following scenarios: (i) the use of hCG to induce final oocyte maturation (luteal support may/may not be administered); and (ii) the administration of GnRH-ant to block the spontaneous LH surge with or without FSH or hMG as add-back therapy (an hCG injection and luteal support are administered).

The above-mentioned terminology has not yet been well incorporated into clinical practice. In all of the following studies presented on NC-IVF, hCG was used for ovulation triggering, and the term MNC is used when a combination of GnRH-ant and gonadotropins is given. In a prospective study with historical controls, Bassil et al. (161) analyzed 11 patients who underwent 16 NCs (with hCG administration) for IVF. These were compared with 25 previous failed cycles with poor response in the same patients. The cancelation rate in NCs was 18.8% compared with 48% in stimulated cycles. Three ongoing pregnancies were obtained in NCs (18.8% per started cycle) compared with none in stimulated cycles. In another prospective study with historical controls, Feldman et al. (162) compared 44 unstimulated IVF cycles in 22 poor-responder patients with those of 55 stimulated cycles of the same patients during the 12 months prior to the study. Eighteen (82%) patients had at least one oocyte retrieved, while nine (41%) had at least one cycle with ET. Two (9%) patients each gave birth to a healthy term baby. These results were comparable with those of the stimulated cycles. In a small retrospective study (163), 30 patients who had previously been canceled because of POR underwent 35 NCs, achieving an ongoing pregnancy rate of 16.6% per oocyte retrieval and an implantation rate of 33%. All patients, however, were <40 years old and had a mean day-3 FSH of 11.1 IU/L.

Similar results were found in an observational study with no controls, in which patients aged 44–47 years were included (164). These patients were recruited based on age only, without prior demonstration of poor response. Out of 48 treatment cycles conducted in 20 women, oocyte retrieval was successful in 22 cycles (46%). Fertilization and cleavage rates of 48% and 100%, respectively, were obtained. One biochemical and one ongoing pregnancy were achieved. Thus, the ongoing pregnancy rate was 5% per patient and 2.08% per cycle.

Check et al. (165) reported on 259 retrieval cycles and 72 transfers in poor responders using minimal or no gonadotropin stimulation and without GnRHas or GnRH-ants. These patients were divided into four age groups (<35, 36–39, 40–42, and >43 years) and their mean serum day-3 FSH levels were 19.7, 20.6, 18.8, and 21.9 mIU/mL, respectively. In total, 12 deliveries were achieved after 259 IVF cycles (4.6%). Eliminating the oldest age group, the delivery rate for 47 ETs in women aged ≤42 years was 25.5%. Approximately 50% of retrievals resulted in an embryo (about half were transferred fresh and half frozen). The median number of embryos transferred was one. The implantation rate was 21.6% for the three groups, 33.3% for patients aged <35 years, and 28.6% for women aged 36–39 years. It was concluded that pregnancies and live births can be achieved in poor-prognosis/poor-responder patients with elevated basal FSH levels, and age was found to be a more adverse infertility factor than elevated serum FSH.

The only RCT on this topic (166) compared the efficacy of NC-IVF with the micro-dose GnRHa flare protocol in poor responders. A total of 129 patients who were poor responders in a previous IVF cycle were included: 59 women underwent 114 attempts of NC-IVF and 70 women underwent 101 attempts of IVF with COS by micro-dose agonist flare. In the NC patients, the oocyte retrieval procedure was performed in 114 cycles, and oocytes were found in 88 of these (77.2%). The poor responders treated with NC-IVF and those treated with micro-dose GnRHa flare showed similar pregnancy rates per cycle and per transfer (6.1% and 14.9% vs. 6.9% and 10.1%, respectively). The women treated with NC-IVF showed a statistically significant higher implantation rate (14.9%) compared with controls (5.5%). When subdivided into three groups according to age (≤35 years, 36–39 years, and >40 years), younger patients had a better pregnancy rate than the other two groups. It was concluded that in poor responders, NC-IVF is at least as effective as COS, especially in younger patients, with a higher implantation rate.

Papaleo et al. (167) reported on a series of poor-prognosis patients, all of them with AMA, elevated serum FSH, and reduced AFC, who underwent NC-IVF. A total of 26 NCs in 18 patients were analyzed. Pregnancy was achieved in three patients, of which two patients were ongoing (11.5% per cycle, 20.0% per ET). It was suggested that since the overall pregnancy rates achieved were comparable with those of conventional IVF-ET in poor responders, considering the lower costs and risks and the patient-friendly nature of such protocols, NC-IVF can provide an acceptable alternative option for persistent poor responders.

There have been recent studies addressing the efficacy of NC-IVF in the Bologna criteria poor responders. In a retrospective cohort study by Polyzos et al. (168), 164 consecutive patients undergoing 469 NC-IVF cycles were included, with 136 patients (390 cycles) fulfilling the Bologna criteria definition of POR and 28 women (79 cycles) considered as normal responders. The live birth rates per cycle were 2.6% versus 8.9% among Bologna criteria poor responders and normal responders and the live birth rates per treated patient were 7.8% versus 25%. The conclusion from this study was that Bologna criteria poor responders did not experience substantial benefits with NC-IVF.

Modified NC

The efficacy of NC-IVF is hampered by high cancelation rates because of premature LH rises and premature ovulations (169). The possibility of enhancing the efficacy of unstimulated IVF cycles by the concomitant addition of a GnRH-ant and exogenous gonadotropins in the late follicular phase was introduced by Paulson et al. as early as 1994 (170). This protocol, later known as the MNC, is expected to reduce the rate of premature ovulation and to improve control of gonadotropin delivery to the developing follicle.

In a preliminary report on 44 cycles in 33 young, normal-responder patients (171), the cancelation rate was 9%, and in 25% of retrievals, no oocyte was obtained. ET was performed in 50% of the started cycles, leading to a clinical pregnancy rate of 32.0% per transfer and 17.5% per retrieval, of which five (22.7% per transfer) were ongoing. It was suggested that the MNC could represent a first-choice IVF treatment with none of the complications and risks of current COS protocols, a considerably lower cost, and an acceptable success rate.

Considerable experience with the MNC protocol in the general IVF population has been accumulated by the Dutch group in Groningen (172–174). In a preliminary report, the cumulative ongoing pregnancy rate after three cycles with this protocol was 34% and the live birth rate per patient was 32% (173). Summarizing a much larger experience, the same group (174) later reported on a total of 336 patients who completed 844 cycles (2.5 per patient). The overall ongoing pregnancy rate per started cycle was 8.3% and the cumulative ongoing pregnancy rate after up to three cycles was 20.8% per patient. In a recent report of further follow-up of up to nine cycles (172), a total of 256 patients completed 1048 cycles (4.1 per patient). The ET rate was 36.5% per started cycle. The ongoing pregnancy rate was 7.9% per started cycle and 20.7% per ET. Including treatment-independent pregnancies, the observed clinical pregnancy rate after up to nine cycles was 44.4% (95% confidence interval [CI] 38.3%–50.5%) per patient. Pregnancy rates per started cycle did not decline in higher cycle numbers (overall 9.9%), but drop-out rates were high (overall 47.8%).

Several studies have been reported on the use of the MNC protocol in poor responders. Kolibianakis et al. (175) evaluated the use of the MNC for IVF in poor responders with an extremely poor prognosis as a last resort prior to oocyte donation. Thirty-two patients with regular menstrual cycles, basal FSH levels >12 IU/L, and one or more failed IVF cycles with five or fewer oocytes retrieved were included. Recombinant hFSH 100 IU and ganirelix 0.25 mg/day were started concomitantly when a follicle with a mean diameter of 14 mm was identified. hCG was administered as soon as the mean follicular diameter was ≥16 mm. Twenty-five out of 78 cycles performed (32.1%) did not result in oocyte retrieval. In nine out of 53 cycles (16.9%) in which oocyte retrieval was performed, no oocytes were retrieved. ET was performed in 19 out of 44 cycles in which oocytes were retrieved (43.2%), but no ongoing pregnancy was achieved in 78 MNC cycles. It was concluded that the MNC does not offer a realistic chance of live birth in poor-prognosis/poor-responder patients when offered as a last resort prior to oocyte donation.

Studies with somewhat more encouraging outcomes were also reported. Elizur et al. (176) retrospectively evaluated 540 cycles in 433 poor responders who were divided by treatment protocol into MNC, GnRH-ant, and long agonist groups: there were 52 MNC cycles, 200 GnRH-ant cycles, and 288 long GnRHa cycles. In the MNC protocol, a GnRH-ant 0.25 mg/day and two to three ampules of hMG were administered daily once the lead follicle reached a diameter of 13 mm. The mean number of oocytes retrieved in the MNC group was significantly lower than in the stimulated antagonist and long agonist groups (1.4 ± 0.5 vs. 2.3 ± 1.1 and 2.5 ± 1.1, respectively; $p < 0.05$). The respective implantation and pregnancy rates were comparable (10% and 14.3%, 6.75% and 10.2%, and 7.4% and 10.6%). The number of canceled cycles was significantly higher in the MNC group. Cancelations due to premature luteinization or failure to respond to stimulation were significantly more common in patients aged >40 years. As pregnancy rates were comparable for all groups, it was concluded that the MNC is a reasonable alternative to COS in poor responders.

The only RCT on this issue was performed to investigate the value of MNC-IVF compared with the conventional GnRH-ant cycle in low responders (177). The study population consisted of 90 patients with low response in previous cycles who had undergone 90 IVF cycles. Forty-five patients were randomly allocated into the MNC-IVF protocol and 45 into the GnRH-ant protocol. In the MNC arm, s.c. injections of 0.25 mg cetrorelix and 150 IU r-hFSH were started concomitantly when the lead follicle reached 13–14 mm in diameter and were continued daily until the day of hCG administration. In the antagonist group, patients received a conventional, multiple-dose, flexible GnRH-ant protocol with 225 IU of r-hFSH administered daily from cycle day 3. In the MNC group, 8 out of 45 cycles initiated (17.8%) had to be canceled before ET because no oocytes were available. Four out of 45 cycles initiated (8.9%) did not result in oocyte retrieval owing to no follicular development or premature ovulation and no oocytes were found in 4 out of 41 cycles (9.8%) in which oocyte retrieval was performed. In the antagonist group, 3 out of 45 cycles initiated (6.7%) were canceled before ET. Despite the difference in cancelation rate between the two groups, it was not statistically significant. The numbers of oocytes, mature oocytes, fertilized oocytes, grade 1 or 2 embryos, and embryos transferred were all significantly lower in the MNC group. Gonadotropin requirements and number of days of r-hFSH required for COS were significantly fewer in the MNC group than in the antagonist group. Finally, clinical pregnancy rates per cycle initiated and per ET of the MNC group were similar to those of the antagonist group (13.3% and 17.8%; 16.2% and 19%, respectively). Live birth rates per ET and implantation rates were also comparable between the two groups (13.5%

and 16.7%; 12.5% and 9.8%, respectively). It was concluded that the MNC provides comparable pregnancy rates to GnRH-ant-based COS with lower doses and shorter durations of FSH administration, and thus could be a patient-friendly and cost-effective alternative in low responders.

In summary, the options of NC- or MNC-IVF are safe, patient-friendly treatments with low costs of medication, especially in those who are refractory to COS and decline the option of oocyte donation. Despite the advantages of this approach, its low efficiency has restricted its widespread use. Patients should be fully informed of the advantages and disadvantages of NC- or MNC-IVF protocols. From the above studies, it is evident that the likelihood of retrieving an oocyte is between 45% and 80%, the likelihood of reaching ET is around 50%, and the likelihood of pregnancy and live birth is between 0% and 20% (generally around 5%), depending largely on age and ovarian reserve. Younger patients with diminished ovarian reserve (DOR) have a much better prognosis (178). The use of indomethacin during the late follicular phase has been suggested in order to decrease the spontaneous ovulation rate and hence provide a higher oocyte retrieval success rate in MNC-IVF (178,179).

The exact role of NC and MNC protocols in patients with DOR has yet to be determined, as are several key issues that have not yet been subjected to testing, such as:

1. Is the MNC protocol superior to the simple NC protocol? No study so far has evaluated these two regimens.
2. What is the best timing for hCG administration and what is the ideal time interval between hCG administration and egg retrieval? Different authors used different criteria for triggering ovulation. While many authors regard follicle size ≥16 mm as the threshold (166,175,180,181), other prefer to administer hCG at 17–18 mm (177), or even ≥18 mm (163,176). Segawa et al. (182) prefer the use of GnRHa for ovulation triggering than hCG. While no consensus exists, the best estimate is that early ovulation triggering (i.e., ≥16 mm) is beneficial (183).
3. Are oocyte and embryo quality improved in NCs? While there is a common belief that "natural" is better, this assumption has never been directly tested.
4. How many attempts should be made? Schimberni et al. have reported fairly constant implantation and pregnancy rates through five NC cycles (180). Castelo Branco et al. (181) have reported a cumulative pregnancy rate of 35.2% after three MNC cycles. The best estimate is that three to five cycles should be offered.
5. What is the role of follicle flushing? While in the general IVF population the use of follicle flushing was abandoned, there are studies suggesting that flushing may improve oocyte yield in poor responders (184–186). Others (187), however, have failed to show any beneficial effect.
6. Should cleavage- or blastocyst-stage transfers be performed?
7. Which dose of gonadotropins should be administered in the MNC protocol? Different authors have used doses ranging from 100 IU r-hFSH (196) or 150 IU (181) and up to 225 IU (176). The optimal dose needed to support a single follicle in conjunction with GnRH-ant administration has not been determined.
8. Should LH be included in the gonadotropin regimen? In patients with POR, the addition of LH to the stimulation regimen might be beneficial (188), as will be discussed later.

More research is needed before these questions can be effectively answered.

MANIPULATING ENDOCRINOLOGY

The role of FSH

Inherent biological mechanisms such as follicle sensitivity to FSH and pharmacodynamics of drug metabolism or receptor interaction (189) may affect the individual ovarian response to stimulation. Recent genetic and pharmacogenomic research has revealed other factors that may facilitate improved cycle management.

FSH secreted from the pituitary is a heterodimer glycoprotein hormone with two covalently linked subunits, α and β. The molecule is glycosylated by post-translational modification, and the presence and composition of the carbohydrate glycan moieties determine its *in vivo* biological activity (Figure 50.6) (190,191). *In vivo*, the native FSH consists of a family of at least 20 different isohormones that differ in their pattern of glycosylation. For

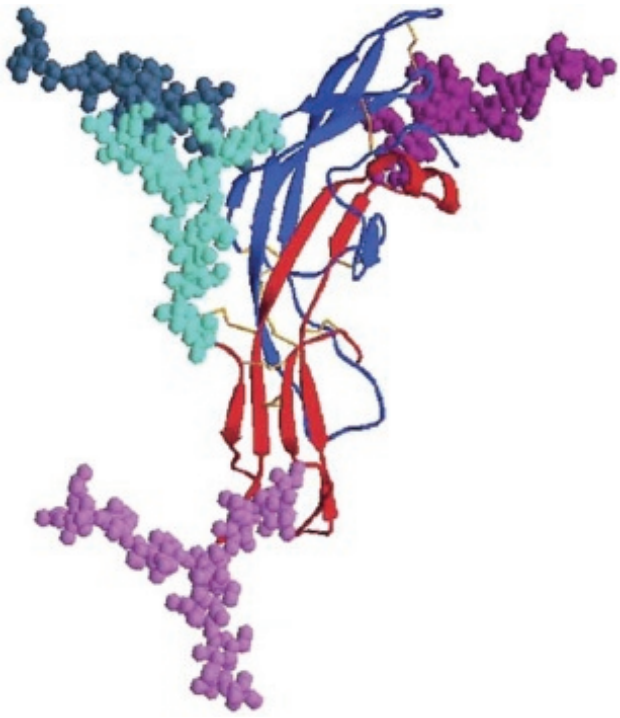


Figure 50.6 Follicle-stimulating hormone is a complex glycoprotein with two non-covalently associated α- and β-protein subunits. Two oligosaccharides are linked to each protein subunit. (Molecular model created by Merck Serono Reproductive Biology Unit, U.S.A.; reproduced with permission.)

follitropin-α, isoelectric focusing has identified seven major bands of FSH isoforms between pI 4.2 and 5.05, five minor bands between pI 5.25 and 6.30, and one minor band at pI 4.20. These have been demonstrated to be consistent between different manufactured batches (192). The ovarian response to stimulation by FSH relies on an interaction of the hormone with membrane receptors (FSHR) on GCs, and a normal response is dependent on the correct molecular structure of the hormone, the receptor, and factors associated with their interaction. Any defect in the genes encoding FSH or its receptor may result in ovarian resistance, and therefore genotype may play a fundamental role in determining the physiological response to FSH stimulation.

The FSHR is a member of the family of G-protein receptors linked to adenyl cyclase signaling, with extensive extracellular ligand-binding domains. The gene encoding the FSHR is located on the short arm of chromosome 2 and is made up of 2085 nucleotides that translate into a polypeptide with 695 amino acids. This molecule has four potential N-linked glycosylation sites located at amino acids 191, 199, 293, and 318. Mutations in the receptor gene can result in amino acid changes that affect function, and mutations that result in complete FSH resistance (193) as well as partial loss of FSHR function have been identified (194). Screening different populations for mutations of the FSHR gene have shown that single nucleotide polymorphisms can be identified, and two discrete polymorphisms have been studied: (1) position 307 (Ala or Thr) in the extracellular domain; and (2) position 680 (Asn or Ser) in the intracellular domain. Both polymorphic sites give rise to two discrete allelic variants of the FSHR (i.e., Thre307/Asn680 and Ala307/Ser680). There is an association between these polymorphisms and ovarian response in patients undergoing ART (195,196), and their frequency may vary among different ethnic groups. Women with the Ser/Ser polymorphism at position 680 have an increased total menstrual cycle length and time from luteolysis to ovulation compared with Asn/Asn controls (197). This Ser/Ser genotype occurs less frequently in Asian women than in Caucasians (Table 50.2).

In a Korean IVF patient population, Jun et al. (196) grouped 263 young patients according to their FSHR genotype and found that basal FSH levels differed between the groups. The Ser/Ser (p.N680S) homozygous group required higher total doses of gonadotropins to achieve multiple follicular development compared with the other two groups (Asn/Asn and Asn/Ser at position 680). Additionally, significantly fewer oocytes were recovered in patients with the Ser/Ser FSHR genotype.

Perez Mayorga et al. (195) also suggest that the FSHR genotype plays a fundamental role in determining the physiological response to FSH stimulation, and that subtle differences in FSHR might fine-tune the action of FSH in the ovary. In a study conducted in 161 ovulatory young (<40 years) women who underwent IVF treatment, a wide variation in the number of ampules of FSH required to achieve an adequate response was observed. They confirmed that this observation could be correlated with the patient's FSHR genotype (i.e., type of polymorphism).

Behre et al. (198) also carried out an RCT to further investigate this observation and found that the Ser/Ser (p.N680S) homozygous group results in lower E2 levels following FSH stimulation. This lower FSHR sensitivity could be overcome by higher FSH doses in the trial patients.

Achrekar et al. have shown that the AA genotype at the –29 position in the 5′-untranslated region of the FSHR gene may be associated with the POR to COS (199). Women with the AA genotype required a large total dose of exogenous FSH and only low numbers of pre-ovulatory follicles were produced and oocytes retrieved. In addition, E2 levels on the day of hCG administration were significantly lower in women with the AA versus GA genotypes.

Table 50.2 The frequency of the follicle-stimulating hormone receptor polymorphism at p.N680S in published reports

Study	Ethnic origin	Patient number (diagnosis)	SNP680		
			Asn/Asn (%)	Asn/Ser (%)	Ser/Ser (%)
Perez Mayorga et al. (195)	Caucasian	161 (male/tubal)	29	45	26
Sudo et al. (324)	Japanese	522 (mixed)	41	46.9	12.1
Laven et al. (325)	Caucasian	148 (anovulatory)	16	44	40
Laven et al. (325)	Caucasian	30 (ovulatory)	23	61	16
De Castro et al. (326)	Caucasian	102 (male/tubal/both)	31.4	50	18.6
Daelemans et al. (327)	Caucasian	99 (non-IVF control)	38	45	17
Daelemans et al. (327)	Caucasian	130 (mixed?)	24	51	25
Daelemans et al. (327)	Caucasian	37 (mixed–OHSS)	16	54	32
Choi et al. (328)	Korean	172 (mixed, non-PCOS)	41.9	47.7	10.5
Schweickhardt 2004—unpublished thesis	Not stated (U.S.A.)	663 (mixed)	30.6	48.7	20.7

Note: The Ser/Ser (p.N680S) homozygous group is generally lower in Asian populations than in Caucasian populations.
Abbreviations: IVF, *in vitro* fertilization; OHSS, ovarian hyperstimulation syndrome; PCOS, polycystic ovary syndrome.

In recent years, there has been a paradigm shift in the use of gonadotropins. The outdated "one-size-fits-all" approach to fertility treatment has been superseded by individualized COS (200–202). Individualized COS is designed to maximize the efficacy and safety for each patient, and is discussed more fully in Chapter 46.

Accordingly, an analysis was undertaken to assess whether specific factors could optimally predict a response to stimulation in ART, and then to develop a corresponding treatment algorithm that could be used to calculate the optimal starting dose of r-hFSH (follitropin-α) for selected patients (51). Backwards stepwise regression modeling indicated that in ART patients aged <35 years (n = 1378) who were treated with r-hFSH monotherapy, predictive factors for ovarian response included basal FSH, BMI, age, and number of follicles <11 mm at baseline screening. The concordance probability index was 59.5% for this model. Using these four predictive factors, a follitropin-α starting dose calculator was developed that can be used to select the FSH starting dose required for an optimal response. A prospective cohort study has been completed using this r-hFSH starting dose calculator and demonstrated a similar number of oocytes and pregnancy rates across the doses used (203).

Taken together, these studies suggest that, in the future, it might be possible to tailor FSH therapy to the patient's genetic background, and thereby adjust the doses and the timing of stimulation. This would be of particular benefit in the treatment of older women, who cannot afford any delay in their race against the biological clock.

The role of LH

Ovulation induction studies in hypogonadotropic women using r-hFSH have demonstrated that FSH can induce follicular growth to the preovulatory stage, but E2 and androstenedione concentrations remain extremely low (204,205). This suggests that follicular maturation depends on the action of LH to stimulate androstenedione biosynthesis as a substrate for aromatase activity. Below a minimal level of LH, follicular development will plateau—this has been observed in patients with profound pituitary down-regulation after GnRHa depot (206,207). In women with hypogonadotropic hypogonadism, E2 concentrations may be inadequate for cytoplasmic maturation of the follicle, endometrial proliferation, and corpus luteum function (204,205).

Adequate folliculogenesis and steroidogenesis required for successful fertilization and implantation therefore depend upon a certain threshold level of LH. Although the amount of LH necessary for normal follicle and oocyte development is not known, it is likely to be very low, since a maximal steroidogenic response can be elicited when <1% of follicular LH receptors are occupied (208). On this basis, resting levels of LH (1–10 IU/L) should be sufficient to provide maximal stimulation of thecal cells (209). There is also evidence that excessive levels of LH can have an adverse effect on follicular development (210) associated with impaired fertilization and pregnancy rates, as well as higher miscarriage rates, through the so-called "ceiling" effect (Figure 50.7). LH levels must be below this ceiling in order for the LH-dependent phase of development to proceed normally. It seems that there is a clinical therapeutic window (211,212): "low-dose" treatment with LH generally enhances steroidogenesis, but "high-dose" treatment can enhance progesterone synthesis, suppress aromatase activity, and inhibit cell growth.

Huirne et al. (213) administered different GnRH-ant doses to five groups of patients and measured the subsequent change in LH levels between the groups. The aim of this study was to deliberately induce different LH levels and to assess the effect of an LH range on IVF outcome in order to estimate what the optimal level might be. No

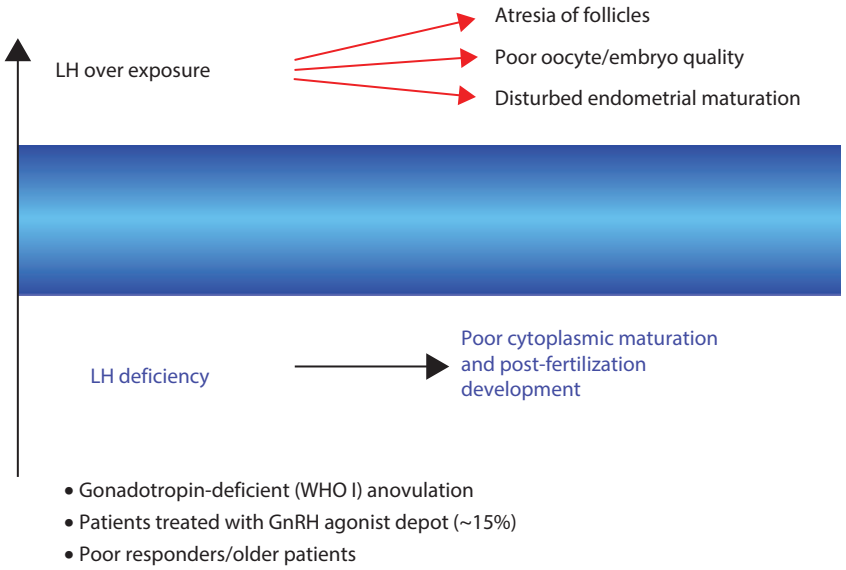


Figure 50.7 The LH therapeutic window concept. *Abbreviations*: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; WHO, World Health Organization.

pregnancies were observed in relation to either very high or very low LH, suggesting an optimal window. However, their data led them to conclude that not the absolute level, but instead excessive change in LH—either increases or decreases—was the more significant parameter. They suggest that the correct sequence of stages in oocyte maturation, together with synchrony between nuclear and cytoplasmic maturation, is dependent upon an appropriate endocrine milieu. Excessive fluctuations in LH levels might disrupt this balance, as well as affect maturation of the endometrium (i.e., stable and appropriate LH levels are needed during IVF cycles). It is possible that specific patient groups, such as those with PCOS or DOR, may be prone to larger changes in LH levels and sensitive to high fluctuations. In addition, serum LH levels assayed by immunoassay do not necessarily reflect circulating LH bioactivity, particularly in these specific patient groups.

A common variant of the LH gene is recognized ($Trp^{8}Arg$ and $Ile^{15}Thr$ of the β-subunit) that encodes a protein with altered *in vitro* and *in vivo* activity (214). It has been suggested that this variant may be less effective at supporting FSH-stimulated multi-follicular growth, resulting in suboptimal ovarian response to standard COS regimens and higher drug consumption (215). An increased prevalence of this gene has been reported in Japanese patients with infertility (216) and premature ovarian failure (217), and it has been postulated that women with this gene variant could benefit from exogenous LH supplementation during COS.

The initial availability of a pure r-hLH (Luveris®, Merck Darmstadt, Germany) preparation has provided a new tool that allows the endocrinology of ovarian stimulation to be examined more accurately. This has been followed by the more recent commercial availability of a combination r-hFSH/r-hLH (2:1 ratio) preparation (Pergoveris®, Merck Darmstadt), which is indicated for use in women with severe gonadotropin deficiency. The use of r-hLH in COS protocols has been reviewed (218). A clear relationship between the dose of r-hLH and serum E2 has been found in hypogonadotropic patients (219). The optimal LH levels required to provide the best results in IVF are still a matter of debate, and a number of studies have tried to assess the role of LH supplementation in GnRHa and GnRH-ant cycles.

LH supplementation may have an effect via intraovarian mechanisms that affect steroid biosynthesis, and therefore oocyte maturation. Foong et al. (220) conducted a study that included patients who showed an inadequate response to r-hFSH-only stimulation, and reported that although peak E2 levels were similar to those found in normal responders, intrafollicular E2 levels were significantly lower, and progesterone was significantly higher in poor responders to FSH. E2 plays an important role in human oocyte cytoplasmic maturation *in vitro* (221), as manifested by improved fertilization and cleavage rates. hGH has also been shown to stimulate E2 production by follicular cells (222,223). High intrafollicular E2 concentrations in the pre-ovulatory follicle predict an increased chance of pregnancy (224). On the other hand, androstenedione can irreversibly block the effect of E2 (225), and it is clear that maintaining an appropriate steroid balance within the follicle is very important. In the ovine, E2 is associated with an up-regulation of oocyte DNA repair enzymes (226). In the rhesus monkey, adding an aromatase inhibitor during the late stages of follicular development, just prior to the period of ovulation, resulted in a reduced capacity of the oocyte to mature and a reduced rate of fertilization *in vitro* (227). Overall, it seems that LH may have a beneficial effect through a mechanism that improves oocyte cytoplasmic maturation (increasing mitochondrial function and/or up-regulating DNA repair enzymes), either through E2 or some other intraovarian factor. However, an additional effect on the endometrium itself cannot be excluded.

A number of further studies have examined the effect of LH supplementation in poor responders (228) or patients who respond inadequately to FSH stimulation (206,207,229). Following stratification of the data, a subset of patients aged ≥35 years have been identified who seem to benefit from LH supplementation in terms of an increased number of mature oocytes retrieved and improved implantation and pregnancy rates. This benefit was maintained even when LH supplementation was initiated from stimulation days 6 or 8. This seems logical in terms of physiology, as the GCs, through FSH stimulation, acquire LH receptors only after the follicle reaches a diameter of at least 11 mm (230). In hyporesponsive women, the need for higher FSH doses might be an individual biological index of LH deficiency, with an effect on oocyte competence.

A requirement for LH supplementation in order to achieve good ovarian response and follicular maturation in patients of AMA could be based on a number of theoretical explanations. With age and the onset of the menopause, endogenous LH as well as FSH levels increase and T levels decrease (231,232). The number of functional LH receptors also decreases with age (233). Kim et al. (234) found that the best predictor of ovarian reserve (reproductive age) in normally cycling women was the combination of the FSH and LH levels on menstrual cycle day 1. There is also evidence that endogenous LH may be less biologically active or potent than it should be, or the immunologic LH may not be comparable to the biologically active LH (235,236). Overall, this could result in increasing ovarian resistance to LH-mediated events.

It has been suggested that follicular recruitment in women aged >38 years can be improved by supplementing r-hFSH stimulation with LH-containing preparations (237,238). Since hMG contains hCG and a number of unknown contaminating proteins in addition to FSH and LH, Gomez-Palomares et al. (239) conducted a prospective randomized cohort study comparing the effects of hMG with r-hLH supplementation in a group of women aged 38–40 years in order to determine whether LH is the hMG component that favors early follicular recruitment. The patients were randomly assigned to one of two groups: 58 patients received r-hFSH 225 plus hMG (one ampule), and 36 were treated with r-hFSH 225 plus r-hLH 75 until day 6.

Follicular recruitment was evaluated on day 6, and stimulation was continued with r-hFSH alone, without further hMG or r-hLH. Both groups recruited a similar number of follicles after five days of stimulation, but the r-hLH group showed a significant increase in the number of metaphase II oocytes retrieved and a higher clinical pregnancy rate (47% vs. 26%; non-significant).

In a group of patients representing about 10%–15% of young women, ovarian response to COS using r-hFSH in GnRHa protocols is suboptimal (rather than poor), despite the presence of normal circulating FSH and/or LH levels (240). Such patients have normal follicular development up to cycle days 5–7, but this response plateaus on days 8–10. As described previously, suboptimal ovarian response to FSH may be due to an LH-β variant polymorphism (241), or to polymorphic variants of the FSH receptor (189,242). Early evidence suggests that LH supplementation may improve outcomes in patients with suboptimal response to FSH stimulation (206,228,229). Lisi et al showed a significant improvement in fertilization and clinical pregnancy rates with the addition of r-hLH to r-hFSH in women who required high doses of r-hFSH in previous cycles (228). Ferraretti et al. demonstrated that supplementation from the mid-to-late stimulation phase with r-hLH but not hMG was associated with significantly improved implantation and clinical pregnancy rates in patients who responded inadequately to FSH-only stimulation (229). This is an interesting category of ART patients and it seems that such a response may be more common in a GnRHa depot regimen (206,207). De Placido and colleagues also described the beneficial use of r-hLH supplementation administered following the occurrence of a plateau in E2 secretion and a lack of continued follicle growth at around day 7 of FSH stimulation (206,207).

Several studies have also supported the need for additional LH in poor responders when short and long protocols of GnRHas are used (243–245). From such studies, it has been theorized that ovarian stimulation in patients with diminished ovarian reserve may be enhanced by the LH-induced production of E2 precursors such as androstenedione.

Because of the sudden and often dramatic inhibition of LH secretion associated with the use of GnRH-ant, there has been interest in the potential need for exogenous LH supplementation. A recent meta-analysis of data on 1764 women (aged 18–39 years) from six RCTs showed that the amount of endogenous LH during GnRH-ant protocols was sufficient to support r-hFSH in COS prior to IVF or ICSI (246). No association between endogenous LH level and pregnancy rate in normogonadotropic women was found (246).

There is, however, a paucity of data on the potential use of LH supplementation in poor responders or patients with AMA undergoing COS using a GnRH-ant protocol. In a retrospective cohort study (247), 240 GnRH-ant cycles in poor responders were evaluated. Of 153 that reached the stage of oocyte retrieval, 75 patients received r-hFSH for ovarian stimulation, and 66 received hMG in combination with r-hFSH. In patients aged <40 years, there were no significant differences between treatment groups in the amount and duration of treatment, number of oocytes retrieved, and number of embryos. In patients aged ≥40 years, significantly fewer oocytes were retrieved in patients who received exogenous LH in their stimulation, resulting in significantly fewer fertilized embryos. Implantation and clinical pregnancy rates did not differ by treatment group. It was concluded that outcomes in poor responders undergoing IVF with GnRH-ants are comparable whether COS is performed with or without supplementary LH.

Similar results from an RCT using a GnRHa flare-up protocol were reported by Barrenetxea et al. (248). Patients (n = 84) who had a basal FSH level of >10 mIU/mL, were aged >40 years, and undergoing their first IVF cycle were randomly allocated into two study groups: group A, in which ovarian stimulation included GnRHa flare-up and r-hFSH and r-hLH; and group B, in which patients received no LH. The overall pregnancy rate was 22.6%. The pregnancy wastage rate was 30.0% in group A and 22.2% in group B. There were no differences in the ongoing pregnancy rate per retrieval and implantation rate per ET. The duration of stimulation, E2 level on hCG administration day, number of developed follicles, number of retrieved oocytes, number of normally fertilized zygotes, cumulative embryo score, and number of transferred embryos were all comparable for the two groups. It was concluded that the addition of r-hLH at a given time of follicular development produces no further benefit in poor-responder patients stimulated with the short protocol, and a reduced ovarian response cannot be overcome by changes in the COS protocol.

A Cochrane systematic review reported that a statistical difference was not found in clinical or ongoing pregnancy rates in all ART patients who received r-hFSH alone or r-hFSH plus r-hLH (188). However, a sub-analysis of data on poor responders from three trials using GnRHa protocols showed a significant increase in the ongoing pregnancy rate in favor of co-administration of r-hLH (odds ratio [OR] 1.85, 95% CI 1.10–3.11). The authors recommended further work to elucidate a potentially beneficial effect of r-hLH in poor responders (Figure 50.8). A multicenter RCT has recently been completed examining the efficacy of r-hLH supplementation in Bologna criteria poor responders (249). Preliminary results of this study involving POR women randomized to receive either a fixed-dose combination of r-hFSH 300 IU plus r-hLH 150 IU or r-hFSH 300 IU fixed-dose monotherapy with the long GnRHa protocol suggest no benefit from r-hLH supplementation with regards to the number of oocytes retrieved.

The role of androgens

The human follicle has internal (granulosa) and external (theca) cell layers, and folliculogenesis is regulated by endocrine and paracrine factors that interact within a microenvironment; steroidogenesis is coordinated by the two different cell types (Figure 50.9). Pituitary LH acts on theca cells through surface receptors to promote androgen synthesis in the early follicular phase, and FSH acts through membrane-associated GC receptors to promote their proliferation and differentiation. GCs then express an

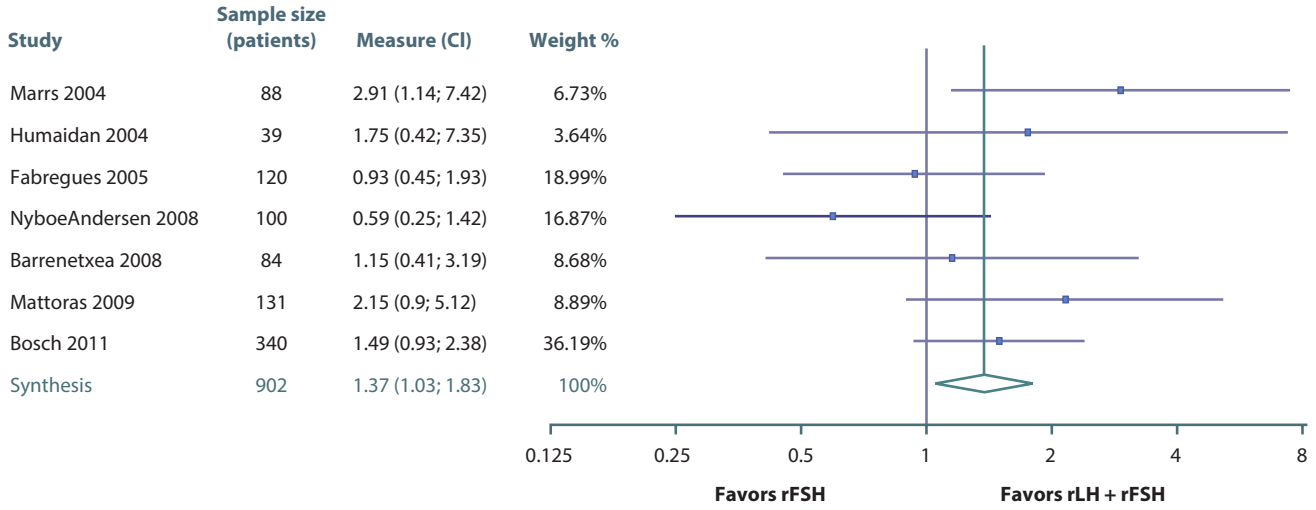


Figure 50.8 Comparison of ongoing pregnancy rates in poor responders undergoing controlled ovarian stimulation using rFSH plus rLH and rFSH alone in gonadotropin-releasing hormone agonist cycles (188). *Abbreviations*: CI, confidence interval; rFSH, recombinant follicle-stimulating hormone; rLH, recombinant luteinizing hormone.

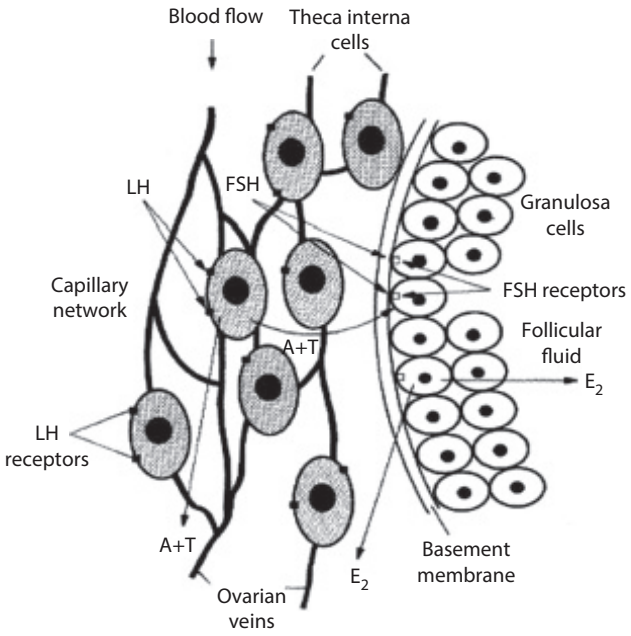


Figure 50.9 Schematic diagram of the "two cells–two gonadotropins theory," which interact to produce E2. LH acts on the theca cells, which produce androgens. Through FSH stimulation, granulosa cell aromatase converts androgens into E2. *Abbreviations*: A+T, Androstenedione + Testosterone; E2: estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

aromatase enzyme system that catalyzes the conversion of androgens to estrogen (250). In order for normal folliculogenesis to continue, adequate levels of bioavailable estrogen are needed, and paracrine signaling activated by FSH and LH (steroids, cytokines, and other growth factors) sustains growth and estrogen secretion as the follicle develops. Theca cell enzyme activity is increased to enhance androgen production, thus contributing further to estrogen synthesis within the follicle. FSH also stimulates GC LH receptor expression in the late follicular phase, so that they become receptive to LH stimulation. Larger follicles with GC LH receptors continue to grow, and LH can then also stimulate the GC aromatase system. FSH and LH together stimulate GCs to produce inhibin, which has a synergistic effect on theca cells to further promote androgen synthesis.

Androgens are synthesized in thecal cells through cell-specific expression of P450C17α (CYP17), which is under LH control, and GCs express androgen receptors (ARs) throughout antral development. During the late stages of follicle development prior to ovulation, transcription of the granulosa AR gene and AR protein levels decline, so that GC responsiveness to gonadotropins is diminished. This mechanism could delay terminal differentiation until the LH surge signals the onset of ovulation, when the cells begin to switch their steroid synthesis to progesterone for the luteal phase.

The rate-limiting step in androgen synthesis—conversion of cholesterol to pregnenolone—occurs within theca cells, and close cooperation between the two types of somatic cells ensures that sufficient E2 is produced during oocyte maturation (220). In the primate ovary, androgens stimulate early stages of follicular growth (251), and primate experiments indicate that androgens may influence the responsiveness of ovaries to gonadotropins. In rhesus monkeys, treatment with dehydrotestosterone or T augments follicular FSHR expression in GCs, promotes initiation of primordial follicle growth, and increases the number of growing preantral and small antral follicles. These studies strongly suggest that androgen treatment may amplify the effects of FSH on the ovary. Hillier and Tetsuka (252) confirmed that T enhances FSH-induced GC gene expression, and that androgens also exert a paracrine effect in the early follicular phase (253).

Hugues and Cédrin Durnerin (254) reviewed the role of androgens in fertility treatment and suggested that

androgen status should be more carefully assessed prior to treatment. Thus, androgens might have two separate roles:

1. During early follicle growth before the follicle becomes sensitive to gonadotropins (reducing apoptosis?)
2. Enhancing FSH action during the early gonadotropin-sensitive phase of follicular growth

In premenopausal cycling women, circulating T is derived from direct secretion by the ovary and adrenal gland, and from conversion of precursors such as androstenedione (Figure 50.10). T circulates in three forms: free, bound to albumin, and bound to sex hormone-binding globulin (SHBG). The free and albumin-bound fractions are believed to be bioavailable, and the fraction bound to SHBG is thought to be unavailable for action in the periphery. With increasing age, androgen levels in women decline significantly (255,256).

Frattarelli and Peterson (257) evaluated androgen levels in 43 normo-ovulatory women before IVF treatment, and observed that patients who had a low level of T after down-regulation (<20 ng/dL) required a higher FSH dose, a longer duration of stimulation, and were less likely to achieve a pregnancy than patients with higher baseline T levels.

Barbieri et al. (258) investigated the association of BMI, age, and cigarette smoking with serum T levels in women undergoing IVF. They observed that T levels decreased significantly with advancing age, and suggest that this may be because LH stimulation of ovarian androgen secretion begins to decline during the decade of the 30s. This effect occurs before a decline in ovarian estrogen secretion, possibly due to the fact that a compensatory increase in FSH with ovarian aging at 35–45 years of age maintains ovarian estrogen secretion but does not maintain ovarian androgen secretion. They also found a positive correlation between serum T and the number of oocytes retrieved; advancing age and years of cigarette smoking were associated with a decreased number of oocytes.

GnRHa administration during ART treatment cycles reduces the level of circulating LH, and therefore the amount or bioactivity of aromatizable androgen substrate available for FSH-induced E2 synthesis is reduced. It is therefore suggested that in women with diminished ovarian reserve who undergo ART treatment, boosting intraovarian androgens might increase the number of follicles available to enter the recruitment stage, as well as the process of follicle recruitment itself. Three different strategies have been proposed:

1. Stimulating theca cells with r-hLH prior to r-hFSH stimulation in the long agonist protocol
2. T or dehydroepiandrosterone (DHEA) supplementation prior to gonadotropin stimulation
3. Blocking intraovarian androgen conversion with the use of an aromatase enzyme inhibitor

Androgens might have two separate roles in enhancing follicular recruitment and function: firstly, during early follicle growth, before the follicles reach gonadotropin sensitivity. Clinical applications of androgens targeted at these stages would require a protracted course. One example is supplementation with DHEA. The second role might be enhancing FSH action during the early gonadotropin-sensitive phase of follicular growth. This would require a relatively short treatment approach, and would lead to improved function rather than increased follicular numbers, as the number of antral follicles present has been determined by other preceding factors. Examples for short-course treatment include supplementation with T and blocking androgen conversion to estrogen with aromatase inhibitors.

T supplementation

Massin et al. (259) treated poor responders with transdermal T application for 15 days prior to FSH stimulation. T gel application resulted in a significant increase in plasma T levels but did not significantly improve the AFC.

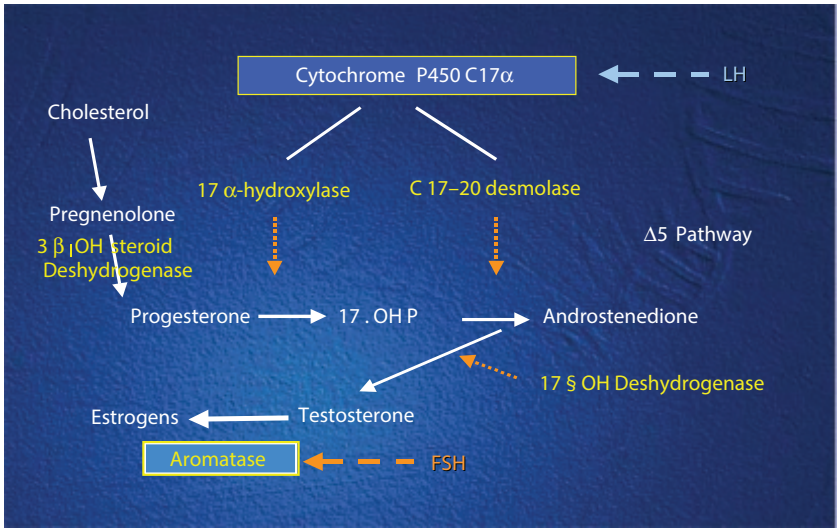


Figure 50.10 Steroid biosynthesis in the human ovary; the Δ5 pathway predominates in the follicle for estrogen synthesis. *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone.

Furthermore, after gel application, the main parameters of ovarian response (numbers of preovulatory follicles and total and mature oocytes and embryos) did not significantly differ between T and placebo-treated patients. It was concluded that no significant beneficial effects of androgen administration on ovarian response to FSH can be demonstrated.

Balasch et al. (260) investigated the usefulness of T pretreatment in poor responders. In a prospective, therapeutic, self-controlled clinical trial, 25 consecutive infertile patients who had a background of first and second IVF treatment cycle cancelations due to poor follicular response, in spite of vigorous gonadotropin ovarian stimulation and having normal basal FSH levels, were included. In their third IVF attempt, all patients received transdermal T treatment (20 μg/kg per day) during the five days preceding gonadotropin treatment. Twenty patients (80%) showed an increase of over five-fold in the number of recruited follicles, produced 5.8 oocytes, received two or three embryos, and achieved a clinical pregnancy rate of 30% per oocyte retrieval. There were 20% canceled cycles. It was concluded that pretreatment with transdermal T may be a useful approach for women known to be poor responders but having normal basal FSH concentrations.

Further evidence of a potentially beneficial effect of T was recently provided by Kim et al. in an RCT of 110 poor responders to COS (261). Poor responders were defined as patients who failed to produce three follicles with a mean diameter of ≥16 mm, leading to the retrieval of three or fewer oocytes, despite the use of a high gonadotropin dose (>2500 IU) in a previous failed IVF/ICSI cycle. Transdermal T gel (12.5 mg) was applied daily for 21 days in the cycle preceding COS. T pretreatment significantly increased the numbers of oocytes retrieved, mature oocytes, fertilized oocytes, and good-quality embryos versus the control group. The embryo implantation rate and clinical pregnancy rate per started cycle were also significantly higher in the women pretreated with T gel. Given the inconsistent evidence on the potential benefit of T pretreatment, further RCTs testing the efficacy in Bologna criteria poor responders are required.

Dehydroepiandrosterone

Casson et al. (262) postulated that DHEA administration to poor responders might augment the effect of gonadotropin stimulation via a paracrine effect mediated by insulin-like growth factor I (IGF-I). In a preliminary small series of five patients (aged <41 years) with documented poor response to high doses of gonadotropins, DHEA was administered orally (80 mg/day) for two months and continued during ovarian stimulation prior to intrauterine insemination (IUI). After two months of treatment, all patients showed increased serum androgen and E2 levels during the stimulation cycle, and all had an improved response to stimulation by approximately two-fold, even after controlling for gonadotropin dose. One of the patients delivered twins after IUI.

This preliminary report was extended by several publications reported primarily by Gleicher, Barad, and their colleagues (263–269). The initiative for the use of DHEA was an unusual case report (265) of a 42.7-year-old woman with presumed severe DOR who requested embryo banking. This patient underwent serial COS cycles with concomitant use of DHEA dietary supplementation as well as acupuncture. In her first treatment cycle, peak E2 was 1211 pmol/mL. After seven months of DHEA supplementation, her peak E2 in cycle 8 was >18,000 pmol/mL. Because of fear of hyperstimulation, her gonadotropin dose was reduced by 25%. The patient had undergone nine treatment cycles while continuously and dramatically improving her ovarian response and banking of 66 embryos overall. Subsequently, the effect of DHEA supplementation on fertility outcomes among women with DOR was evaluated in a case–control study (264). Twenty-five women with significant DOR had one IVF cycle before and after DHEA treatment, with otherwise identical hormonal stimulation. Women received 75 mg of DHEA daily for an average of 17.6 ± 2.1 weeks. Paired analysis of IVF cycle outcomes before and after DHEA supplementation demonstrated significant increases in fertilized oocytes, normal day-3 embryos, embryos transferred, and average embryo scores per oocyte after DHEA treatment. This study supported the previously reported beneficial effects of DHEA supplementation on ovarian function in women with diminished ovarian reserve.

Another case–control study from the same group (263) included 190 women with DOR. The study group included 89 patients who used supplementation with 75 mg/day of oral, micronized DHEA for up to four months prior to entry into IVF. The definition on DOR was based on age-specific FSH concentrations. The control group comprised 101 couples who received infertility treatment but did not use DHEA. Cumulative clinical pregnancy rates were significantly higher in the study group (28.4% vs. 11.9%; $p < 0.05$).

Data from the same group also suggest improvement in ovarian function and oocyte and embryo quality following DHEA supplementation. This is reflected by: (1) an increase in AMH levels in patients with DOR in parallel with the duration of DHEA use (269); (2) significantly lower miscarriage rates in women who had used DHEA compared with those rates reported in the national U.S. IVF database (267); and (3) a lower rate of embryonic aneuploidy as detected by preimplantation genetic screening (268). A recent review by Gleicher and Barad summarizes the experience with DHEA supplementation suggesting that DHEA improves ovarian function, increases pregnancy chances, and, by reducing aneuploidy, lowers miscarriage rates (266). The authors further comment that DHEA may represent a first agent beneficially affecting aging ovarian environments.

In another self-controlled study from Turkey, Sonmezer et al. (270) compared the IVF performance of 19 women with POR before and after DHEA supplementation. The definition of POR was a history of cycle cancelation due to low E2 levels (<130 pg/mL) on the sixth day of cycle or on the hCG administration day (<450 pg/mL) or fewer

than four retrieved oocytes. After 90–180 days of DHEA supplementation, these patients had an increased number of follicles (3 ± 0.7 vs. 1.9 ± 1.3; $p < 0.05$) and metaphase II oocytes (4 ± 1.8 vs. 2.1 ± 1.8; $p < 0.05$), an increased number of day-3 high-quality embryos (1.9 ± 0.8 vs. 0.7 ± 0.6; $p < 0.05$), and higher pregnancy rates (47.4% vs. 10.5%; $p < 0.01$).

Wiser et al. (271) reported the results of the first RCT on the use of DHEA in patients with POR. A total of 33 women received either DHEA 75 mg/day before and during IVF ($n = 17$) or no supplementation ($n = 16$). The long GnRHa protocol was used for COS, and up to two cycles per patient (total 51 cycles) were carried out. The DHEA group demonstrated a non-significant improvement in E2 levels on the day of hCG administration ($p = 0.09$) and improved embryo quality during treatment ($p = 0.04$) between first and second cycles. Although there was no significant difference in live birth rates between the study and control groups following the first cycle, the DHEA group had a significantly higher live birth rate compared with controls following two consecutive IVF cycles in both groups (23.1% vs. 4.0%; $p = 0.05$). Two subsequent RCTs on the use of DHEA did not demonstrate a benefit among women with poor ovarian reserve (272,273). It should be mentioned that the current level of evidence on the effectiveness of DHEA is rather low (274) and does not support its routine use in poor responders.

Blocking intraovarian androgen conversion

Aromatase inhibitors were initially approved to suppress estrogen levels in postmenopausal women with breast cancer. They inhibit the enzyme by competitive binding to the heme of the cytochrome P450 subunit, blocking androgen conversion into estrogens so that there is a temporary accumulation of intraovarian androgens. Mitwally and Casper (275) were first to show that aromatase inhibition improves ovarian response to FSH in poor-responder patients undergoing COS and IUI. Garcia-Velasco et al. (276) assessed whether aromatase inhibitors improve ovarian response and IVF outcomes in patients with POR using an OCP/GnRH-ant protocol. Patients with at least one previously canceled IVF attempt with four or fewer 16-mm follicles received a high-dose gonadotropin regimen supplemented with 2.5 mg letrozole for the first five days of COS ($n = 71$), or the high-dose gonadotropin regimen alone ($n = 76$). In this study, letrozole-treated patients showed significantly higher levels of follicular fluid T and androstenedione and had more oocytes retrieved (6.1 vs. 4.3) and a higher implantation rate (25% vs. 9.4%), despite similar doses of gonadotropins.

In a recent RCT, Ozmen et al. (277) randomized 70 poor-responder patients into two groups. In the study group, letrozole (5 mg/day) was administered along with a fixed dosage (450 IU/day) of r-hFSH, whereas controls were treated with the same r-hFSH dosage alone. A flexible regimen of GnRH-ant was administered in both groups. The mean total dose of r-hFSH and serum concentrations of E2 on the day of hCG administration were significantly lower in the letrozole group compared with controls. The rate of cycle cancelation due to POR was lower in the study group (8.6%) than in controls (28.6%; $p < 0.05$). The costs of achieving a clinical pregnancy were significantly reduced in the letrozole group, and the clinical pregnancy rates per ET were comparable (25.8% and 20% in the letrozole group and controls, respectively). It was concluded that adjunctive letrozole administration is beneficial since it reduces both cycle cancelation rate and cycle cost without an adverse effect on outcome.

Lee et al. (278) compared the sequential use of letrozole and hMG with hMG only in poor responders undergoing IVF. Patients ($n = 53$) with fewer than four oocytes retrieved in previous IVF cycles or fewer than five antral follicles were randomized to either letrozole for five days followed by hMG or hMG alone. The letrozole group required a lower dosage of hMG ($p < 0.001$) and had a shorter duration of hMG treatment ($p < 0.001$), but fewer oocytes were retrieved ($p = 0.001$) when compared with controls. The live birth rate was comparable, with a lower miscarriage rate in the letrozole group ($p = 0.038$). Serum E2 concentrations were lower in the letrozole group from day 4 until the hCG administration day (all $p < 0.001$). Follicular fluid concentrations of T, androstenedione, FSH, and AMH were all significantly increased in the letrozole group.

Studies with less favorable outcomes with letrozole use were also reported. In a prospective controlled trial, Schoolcraft et al. (279) compared the efficacy of a micro-dose GnRHa flare with a GnRH-ant/letrozole protocol in poor responders. A total of 534 infertile women classified as past or potential poor responders based on clinic-specific criteria were prospectively assigned to a micro-dose flare or antagonist/letrozole protocol in a 2:1 ratio, respectively. There were no significant differences in mean age, number of oocytes, fertilization rates, number of embryos transferred, or embryo score. Peak E2 levels were significantly lower in the antagonist/letrozole group. Ongoing pregnancy rates were significantly higher in the micro-dose flare group (52% vs. 37%). Trends toward increased implantation and lower cancelation rates were also noted, but did not reach statistical significance. In another study involving 94 patients, the administration of letrozole with FSH/hMG in a GnRH-ant protocol resulted in significantly lower implantation and fertilization rates and significantly lower metaphase II oocytes and top-quality embryos compared with a micro-dose GnRHa flare protocol with FSH or hMG (280).

A meta-analysis of controlled trials of androgen adjuvants, such as T and DHEA, and androgen-modulating agents, such as letrozole, showed no significant difference in the number of oocytes retrieved or pregnancy/live birth rates with androgen supplementation or modulation compared with controls (281).

The role of hGH

The use of hGH in the management of female and male subfertility was reported in the early 1990s (282,283), and

there has been a great deal of controversy about its use in patient management since that time. There are substantial data that demonstrate the critical importance of the IGF–IGFBP family (the growth factors IGF-I, IGF-II, and their binding proteins) to follicular development. In particular, IGF-I is GH dependent and is involved in potentiating the effect of FSH (284,285).

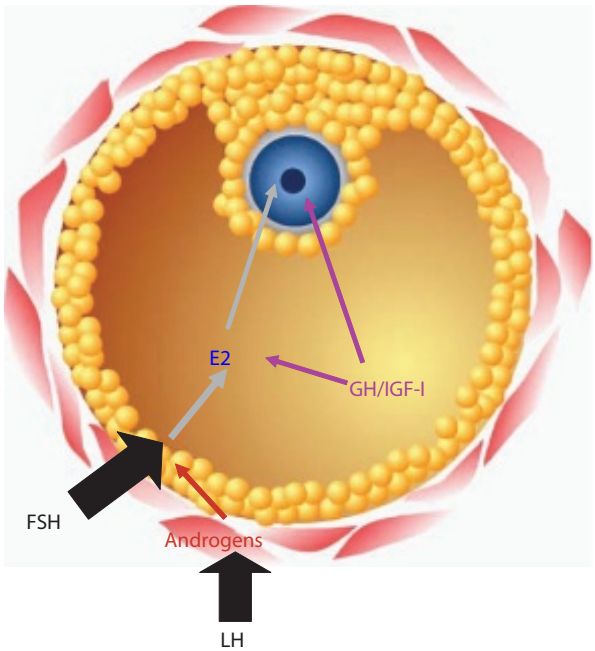
Abir et al. (286) have demonstrated the expression of hGH receptor in human ovaries from fetuses as well as women/girls and of GH in human fetal ovaries. The hGH receptor mRNA is also expressed in human oocytes and throughout preimplantation embryonic development (Figure 50.11). Mendoza et al. (287) reported that the low hGH concentrations in follicular fluid were associated with cleavage failure and poor embryo morphology, whereas the addition of hGH to culture medium improves *in vitro* maturation of immature human oocytes.

Early trials were promising, showing improvements in follicular responsiveness and pregnancy rates (288–290). Similarly, in a cohort of poor responders, Kim et al. (291) found that co-treatment with pyridostigmine, an hGH-releasing agent, enhanced the ovarian response to stimulation and resulted in a non-statistically significantly higher clinical pregnancy rate. Subsequent studies using hGH or GH-releasing factor in IVF poor responders demonstrated no improvement in stimulation characteristics, clinical pregnancy, or live birth rates, and the interest and use of adjuvant hGH has subsided (292–297).

Several studies again raised the interest in the use of hGH for COS in poor responders. In a randomized, placebo-controlled study, Tesarik et al. reported the use of hGH in women aged >40 years undergoing ART (298). They used a high-dose FSH stimulation regimen (600 IU FSH) supplemented with 8 IU/day hGH from FSH stimulation day 7 until day +1 of hCG administration. Although no improvement in the number of oocytes retrieved was observed, significantly higher plasma and intrafollicular E2 levels were found in the hGH group, and the clinical pregnancy and live birth rates were also significantly higher in the hGH-treated group. The authors concluded that hGH may improve the potential for oocyte development.

In an RCT, Kucuk et al. (299) assessed the efficacy of hGH co-stimulation in a long luteal GnRHa regimen in poor responders. The study involved 61 patients who previously responded poorly to high-dose gonadotropin treatment. The study group (n = 31) received prolonged hGH co-treatment, daily subcutaneous injection of 4 mg (equivalent to 12 IUI) from day 21 preceding the cycle along with GnRHa, until the day of hCG administration. A control group (n = 30) received the same treatment protocol except for the hGH co-treatment. Although the gonadotropin requirements were significantly reduced, the average cost of the cycle was more than double with hGH co-treatment. Significantly more mature oocytes, zygotes, and embryos available for transfer were achieved following hGH administration. This, however, did not translate into improved cycle outcome, as the implantation rate was significantly higher in the control group as compared to the hGH-treated group (31.5% vs. 11.7%, respectively; $p < 0.05$). Pregnancy rates were comparable for both groups.

In a sequential crossover study (300), hGH supplementation was assessed in poor-prognosis patients, categorized on the basis of past failure to conceive (mean 3.05 cycles)



- In vitro GH stimulates E_2 production 240,241
- Oocytes from follicles with high antral fluid GH levels have better developmental potential than oocytes from low GH follicles 304
- GH receptor mRNA in human oocyte and preimplantation embryos (Menezo et al. 304a)
- Nuclear and cytoplasmic maturation enhanced by GH (bovine, Izadyar et al. 305)
- Role for GH in stimulation of DNA repair (as in liver, Thompson et al. 307)
- Improvement in normal fertilization and embryo development

Figure 50.11 E2 and GH/IGF-I may enhance oocyte quality by enhancing and coordinating cytoplasmic and nuclear maturation. *Abbreviations*: E2: estradiol; FSH, follicle-stimulating hormone; GH, growth hormone; IGF-I, insulin-like growth factor I; LH, luteinizing hormone.

due to low response to high-dose stimulation (fewer than three metaphase II oocytes) or poor-quality embryos. Pregnancy rates in both fresh and frozen transfer cycles and the total productivity rates (fresh and frozen pregnancies per egg collection) were compared. In all, 159 patients had 488 treatment cycles: 221 with hGH and 241 without hGH. These cycles were also compared with 1572 uncategorized cycles from the same period. hGH co-treatment significantly improved the clinical pregnancy rate per fresh transfer ($p < 0.001$), as well as per frozen–thawed embryos derived from hGH cycles ($p < 0.05$), creating a highly significant productivity rate ($p < 0.001$). The effect was significant across all age groups, especially in younger patients, and was independent of stimulation modality or number of transfers. hGH cycles resulted in significantly more babies delivered per transfer than non-hGH cycles (20% vs. 7%; $p < 0.001$), although this was less than in the uncategorized cycles (53%). The data uniquely show that the effect of hGH is directed at oocyte and subsequent embryo quality.

Meta-analyses regarding hGH have been recently updated. A meta-analysis of 22 RCTs that evaluated 15 interventions to increase pregnancy rates in poor responders to IVF found that the addition of hGH to ovarian stimulation was one of only two interventions that increased the probability of pregnancy (301). Interestingly, the other beneficial intervention was ET on day 2 rather than day 3 (301). Similarly, an updated meta-analysis (302) focusing on use of hGH co-treatment in poor responders has shown that hGH addition increases the probability of clinical pregnancy and live birth. However, it was mentioned that the total number of patients analyzed was small, and thus further RCTs are warranted to prove or disprove this finding.

In summary, considering the extra cost and limited data available, there is currently no well-established clinical role for adjuvant hGH in the treatment of poor responders. Further studies should be directed at defining the dose of hGH and determining if select populations may benefit from hGH co-treatment.

OCP pretreatment

It has been suggested that the use of an OCP in the previous cycle may increase pregnancy rates in IVF (303). Because OCPs have a putative role in the enhancement of estrogen receptor sensitization due to their estrogen content, in addition to exerting pituitary suppression, they have been used in combination with GnRHa. Biljan et al. (304) reported that pituitary suppression with OCP and a GnRHa was superior to GnRHa alone regarding the time required to achieve pituitary suppression, as well as pregnancy and implantation rates.

Because of these promising effects, OCPs have also been used in poor responders. However, there are only very few retrospective studies evaluating the actual contribution of OCPs in this group of patients. Lindheim et al. (305) found higher pregnancy rates with OCP alone compared with GnRHa-treated cycles (both long and short protocols). They concluded that the good outcome associated with OCP pretreatment might reflect the production or alteration of local ovarian growth factors and/or changes at the endometrial level. In contrast with the above observations, Kovacs et al. (306) also retrospectively compared the use of OCPs with GnRHa for hypothalamic–pituitary suppression in poor-responder IVF patients. Hypothalamic–pituitary suppression was performed with either an OCP or a GnRHa followed by stimulation with gonadotropins. Cycle outcomes, including cancelation rates, gonadotropin requirements, number of oocytes retrieved, number of embryos transferred, and embryo quality, were similar. Patients in the OCP group required fewer days of stimulation to reach oocyte retrieval. Pregnancy rates were similar in the two groups. Overall, there was no improvement in IVF cycle outcome in poor responders who received OCPs to achieve pituitary suppression instead of a GnRHa.

In summary, although there is a general feeling that OCP pretreatment might be of assistance in the ovarian response of poor responders, especially in flare-up regimens, only a minimal amount of published data exist to support this approach.

Luteal-phase manipulations

During the early follicular phase of the menstrual cycle, antral follicle sizes are often markedly heterogeneous. These follicle size discrepancies may, at least in part, result from the early exposure of FSH-sensitive follicles to gradient FSH concentrations during the preceding luteal phase. This phenomenon, which often occurs in women with poor ovarian reserve, and in particular those with short cycles, may potentially affect the results of ovarian stimulation. Pre-existing follicle size discrepancies may encumber coordinated follicular growth during ovarian stimulation, thereby reducing the number of follicles that reach maturation at once. Interventions aimed at coordinating follicular growth by manipulation at the mid-luteal phase of the preceding cycle are largely based on the innovative work of Fanchin et al. (307).

To investigate this issue, three clinical studies were conducted to test the hypothesis that luteal FSH suppression could coordinate subsequent follicular growth. First, luteal FSH concentrations were artificially lowered by administering physiological E2 doses and follicular characteristics were measured on the subsequent day 3 in healthy volunteers (308). In this study, luteal E2 administration was found to reduce the size and to improve the homogeneity of early antral follicles on day 3.

Secondly, it was verified whether luteal E2 administration could promote the coordination of follicular growth during ovarian stimulation and improve its results (309). Ninety IVF patients were randomly pretreated with 17β-estradiol (4 mg/day) from cycle day 20 until next cycle day 2 ($n = 47$) or controls ($n = 43$). On cycle day 3, all women started r-hFSH treatment followed by a GnRH-ant in the flexible protocol. The authors focused on the dynamics of follicular development, including magnitude of size discrepancy of growing follicles on day 8 of r-hFSH

treatment and number of follicles >16 mm in diameter on the day of hCG administration. On day 8, follicles were significantly smaller (9.9 ± 2.5 vs. 10.9 ± 3.4 mm) and their size discrepancies were attenuated in the treatment group compared with controls. This was associated with more >16-mm follicles and more mature oocytes and embryos in the E2-treated group. It was concluded that luteal E2 administration reduces the pace of growth, improves size homogeneity of antral follicles on day 8 of r-hFSH treatment, and increases the number of follicles reaching maturation at once. A recently published meta-analysis on the role of luteal E2 priming in poor responders found a significantly lower cycle cancelation rate among women with luteal E2 priming. This meta-analysis comprised pooled results of eight studies, of which only one study was an RCT (310).

Thirdly, the effects of premenstrual GnRH-ant administration on follicular characteristics were assessed during the early follicular phase (311). Twenty-five women underwent measurements of early antral follicles by ultrasound and serum FSH and ovarian hormones on cycle day 2 (control/day 2). On day 25, they received a single dose of 3 mg cetrorelix acetate. On the subsequent day 2 (premenstrual GnRH-ant/day 2), participants were re-evaluated as on control/day 2. The main outcome measure was the magnitude of follicular size discrepancies. Follicular diameters (4.1 ± 0.9 vs. 5.5 ± 1.0 mm) and follicle-to-follicle size differences decreased on premenstrual GnRH-ant/day 2 compared with control/day 2. Consistently, FSH (4.5 ± 1.9 vs. 6.7 ± 2.4 mIU/mL), E2 (23 ± 13 vs. 46 ± 26 pg/mL), and inhibin-B (52 ± 30 vs. 76 ± 33 pg/mL) were lower on GnRH-ant/day 2 than on control/day 2. It was concluded that premenstrual GnRH-ant administration reduces diameters and size disparities of early antral follicles, probably through the prevention of luteal FSH elevation and early follicular development.

Taken together, the results of the above studies suggest that luteal FSH suppression by either E2 or GnRH-ant administration could improve the size homogeneity of early antral follicles during the early follicular phase, an effect that persists during ovarian stimulation. Coordination of follicular development could have the potential to optimize ovarian response to COS protocols, and constitutes an attractive approach for improving their outcome, which needs to be evaluated in well-designed RCTs.

An opposite approach of enhancing follicular recruitment by initiating FSH therapy during the late luteal as opposed to the early follicular phase has been attempted in prior poor responders but without success. In an RCT, Rombauts et al. (312) failed to demonstrate any benefit of this regimen, with the exception that follicular maturation was achieved sooner after the onset of menses.

Several studies evaluated the effects of combining pretreatment with E2 and/or GnRH-ant during the luteal phase of the preceding cycle on the outcome of COS in poor responders. Dragisic et al. (313) reported lower cancelation rates and improved IVF outcomes via a combination of estrogen patch therapy and GnRH-ant started in the mid-luteal phase of the preceding menstrual cycle. Frattarelli et al. (314) reported a retrospective paired cohort analysis where they compared embryo and oocyte data between a standard protocol and a luteal-phase E2 protocol. The results of 60 poor-responder patients who underwent IVF with a luteal-phase oral E2 protocol were compared to 60 cycles in the same patients without E2 pretreatment. The luteal-phase E2 protocol showed significant increases in the number of embryos with more than seven cells, number of oocytes retrieved, number of mature oocytes, and number of embryos generated than did the standard protocol. There was no difference between the two protocols with respect to basal AFC, days of stimulation, number of follicles >14 mm on day of hCG administration, or endometrial thickness. A trend toward improved pregnancy outcomes was found with the luteal-phase E2 protocol.

Several studies compared the luteal-phase E2 protocol with a subsequent GnRH-ant protocol with the short micro-flare agonist protocol. DiLuigi et al. (315) performed an RCT to compare IVF outcomes in 54 poor-responder patients undergoing a micro-dose LA flare protocol or a GnRH-ant protocol incorporating both a luteal-phase E2 patch and GnRH-ant in the preceding menstrual cycle. Cancelation rates (32.1% vs. 23.1%), number of oocytes retrieved (5.4 ± 4.7 vs. 5.2 ± 4), clinical pregnancy rates (28.6% vs. 34.6%), and ongoing pregnancy rates (25% vs. 23.1%) were similar for the micro-flare and luteal E2/GnRH-ant protocols, respectively. Similarly, Weitzman et al. (316) retrospectively compared IVF outcomes in poor-responder patients undergoing COS after luteal-phase E2 patch and subsequent GnRH-ant protocol (n = 45) versus micro-dose GnRHa flare protocol (n = 76). The cancelation rate (28.9% vs. 30.3%), mean number of oocytes (9.1 ± 4.1 vs. 8.9 ± 4.3), fertilization rate (70.0 ± 24.2% vs. 69.9 ± 21.5%), number of embryos transferred (2.5 ± 1.1 vs. 2.7 ± 1.3), implantation rate (15.0% vs. 12.5%), clinical pregnancy rate (43.3% vs. 45.1%), and ongoing pregnancy rate per transfer (33.3% vs. 26.0%) were all comparable for both groups. Focusing on young poor responders (aged <35 years), Shastri et al. (317) retrospectively compared COS with a luteal E2 and subsequent GnRH-ant protocol versus an OCP micro-dose LA flare protocol. Patients in the luteal E2/GnRH-ant group had increased gonadotropin requirements (71.9 ± 22.2 vs. 57.6 ± 25.7 ampoules) and lower E2 levels (1178.6 ± 668 vs. 1627 ± 889 pg/mL), yet achieved similar numbers of oocytes retrieved and fertilized, and a greater number of embryos transferred (2.3 ± 0.9 vs. 2.0 ± 1.1), with a better mean grade (2.14 ± 0.06 vs. 2.70 ± 1.80) compared with the micro-flare group. The luteal E2/GnRH-ant group exhibited a trend toward improved implantation rates (30.5% vs. 21.1%) and ongoing pregnancy rates per started cycle (37% vs. 25%). From the above studies (315–317), it can be concluded that both protocols remain viable options for poor responders undergoing IVF, and that adequately powered, randomized clinical comparison appears justified.

When luteal E2 and antagonist (n = 256) was compared with luteal E2 only (n = 57) before a GnRH-ant protocol in low responders (318), the addition of GnRH-ant to luteal E2 for luteal suppression did not improve IVF outcome.

Elassar et al. (319) recently compared IVF outcomes after COS using letrozole/antagonist (LA) versus luteal-phase E2/GnRH-ant in poor responders. In a retrospective study, 99 women with two or more prior failed cycles with poor response were included. In the luteal intervention group (n = 52), both transdermal E2 and GnRH-ant were administered in the preceding luteal phase, with gonadotropins started on the second day of menstruation. In the LA group (n = 47), letrozole 5 mg/day was initiated on the second day of spontaneous menstruation for five days then gonadotropins were added on day 5; for both groups, a flexible antagonist protocol was used. The total dose of gonadotropins administered and E2 levels on the day of hCG administration were significantly lower with the LA protocol. Cancelation rate (55.3% vs. 36.5%), number of oocytes retrieved (6.1 ± 3.0 vs. 7.9 ± 4.8), number of transferred embryos (2.2 ± 1.0 vs. 2.4 ± 1.4), and ongoing pregnancy rate per transfer (40% vs. 21.2%) and per initiated cycle (19.1% vs. 13.5%) were similar in the LA and luteal intervention groups, respectively. It was concluded that both aromatase inhibitor regimens and luteal intervention regimens can be feasible alternatives in recurrent POR.

Using a slightly different approach, Fisch et al. (159) described their experience with a protocol using AACEP in poor responders with prior IVF failures. The AACEP protocol focuses on promoting estrogenic dominance in the stimulated ovary and opposing the potential ill effects of the LH flare and overproduction of androgens, which are commonly seen in GnRHa flare and in antagonist protocols. Patients received an OCP and a GnRHa overlapping the last five to seven days of the pill until the onset of menses. From cycle day 2, low-dose GnRH-ant (0.125 mg/day) and estradiol valerate (2 mg) were given intramuscularly every three days for two doses, followed by estrogen suppositories until a dominant follicle was detected. Ovarian stimulation consisted of high-dose FSH/hMG. Although women aged <38 years and those on 600 IU/day produced more mature eggs and fertilized embryos than women aged 38–42 years, there were no differences in peak serum E2, endometrial thickness, or embryos transferred. Outcomes were similar for all patients, regardless of age or FSH dosage. Ongoing pregnancy rates were 27% for all patients, 25% for patients aged <38 years, and 28% for patients aged 38–42 years. It was concluded that the AACEP protocol may improve the prognosis and outcomes for poor responders with prior IVF failures.

In summary, manipulating the luteal phase preceding the IVF treatment cycle may improve the coordination of follicular development and increase the number and quality of embryos achieved in poor-responder patients. Ultimately, this may translate into improved cycle and pregnancy outcomes in these patients. It remains to be seen whether this approach is superior to pretreatment with an OCP, which is commonly practiced in various protocols designed for poor responders. Properly designed RCTs are needed to test this innovative therapeutic approach.

RECENT STRATEGIES

Double stimulation

The double stimulation strategy proposed for poor responders involves the combination of two stimulations in one menstrual cycle targeting antral follicles in the follicular and luteal phases. It involves two oocyte pickups in a single menstrual cycle aiming to achieve more oocytes and viable embryos. Kuang et al. (320) explored the efficacy of double stimulations during the follicular and luteal phases in women with POR defined by the Bologna criteria. Thirty-eight women began with mild ovarian stimulation. After the first oocyte retrieval, hMG and letrozole were administrated to stimulate follicle development, and oocyte retrieval was carried out a second time when dominant follicles had matured. The primary outcome measure was the number of oocytes retrieved (stage 1: 1.7 ± 1.0; stage 2: 3.5 ± 3.2). From the double stimulation, 167 oocytes were collected and 26 out of 38 (68.4%) succeeded in producing one to six viable embryos that were cryopreserved for later transfer. Twenty-one women underwent 23 cryopreserved ETs, resulting in 13 clinical pregnancies. The study concluded that double ovarian stimulations in the same menstrual cycle provide more opportunities for retrieving oocytes in poor responders. Stimulation can start in the luteal phase, resulting in retrieval of more oocytes in a short period of time. Although encouraging, the results need to be reproduced across other units and the success of this strategy is dependent on a good laboratory set-up for gamete/embryo cryopreservation.

Oocyte accumulation and embryo banking

Improvements in cryopreservation and vitrification techniques have led to the increased uptake of elective oocyte and embryo cryopreservation with deferred ET with the advantage of avoiding the risk of OHSS without jeopardizing pregnancy outcomes (321,322). In a prospective study, Cobo et al. (323) have demonstrated that the strategy of oocyte accumulation could increase the inseminated cohort in poor responders, thereby creating a similar situation to normal responders. The study included 242 low-responder (LR) patients (594 cycles) whose mature oocytes were accumulated by vitrification and inseminated simultaneously (LR-Accu-Vit) and 482 patients (588 cycles) undergoing IVF-ET with fresh oocytes in each stimulation cycle (LR-fresh). The drop-out rate in the LR-fresh group was >75%. The ET cancelation rate per patient was significantly lower in the LR-Accu-Vit group (9.1%) than the LR-fresh group (34.0%). The live birth rate/patient was higher in the LR-Accu-Vit group (30.2%) than the LR-fresh group (22.4%). The cumulative live birth rate/patient was statistically higher in the LR-Accu-Vit group (36.4%) than the LR-fresh group (23.7%), and a similar outcome was observed among patients aged ≥40 years (LR-Accu-Vit

15.8% vs. LR-fresh 7.1%). The LR-Accu-Vit group had more cycles with embryo cryopreservation (LR-Accu-Vit 28.9% vs. LR-fresh 8.7%). The authors' conclusion was that accumulation of oocytes by vitrification and simultaneous insemination represents a successful alternative for LR patients, yielding comparable success rates to those in normal responders and avoiding adverse effects of a low response.

SUMMARY: PRACTICAL CONSIDERATIONS

There are several key issues that make the development of treatment strategies for poor-responder patients difficult and frustrating:

1. Historically, there was no universally accepted definition of POR until the ESHRE definition (12). While many papers referenced in this text use a large variety of inclusion criteria and are therefore not readily comparable, it is hoped that the few studies referred to in the text and upcoming future studies using the ESHRE consensus definition will provide the necessary evidence.
2. There is a need for large-scale RCTs to test the efficacy of interventions such as T and hGH supplementation.

The following practical considerations represent a combination of the evidence presented above with long-standing clinical experience.

High-dose gonadotropins

Patients with either diminished ovarian reserve (by testing prior to treatment) or POR in previous cycles may benefit from high-dose gonadotropin therapy (300 FSH daily) in order to maximize oocyte yield.

Long GnRHa protocol

The long GnRHa protocol is one of the protocols of choice for poor responders. If the long protocol is to be used, progestagen pretreatment may reduce the incidence of cyst formation. Reducing the dose of the GnRHa once pituitary down-regulation has been achieved (mini-dose agonist) is one suggested strategy with the long GnRHa regimen.

GnRH-ant protocol

The GnRH-ant protocol is also a protocol of choice for poor responders. The GnRH-ant protocol results in lower gonadotrophin consumption and shorter duration of stimulation compared to the long GnRHa protocol.

Short or micro-dose flare GnRHa protocol

The short GnRHa protocol can also be applied in COS regimens for poor responders. Oral contraceptive pretreatment is an important consideration with use of short GnRHa regimens, as it may prevent the adverse effects of elevated LH and androgen secretion caused by the endogenous gonadotropin flare. Reducing the dose of the GnRHa to micro-doses, as is done in micro-flare regimens, is an effective and popular approach in stimulating poor responders.

CONCLUSIONS

Women who have entered the declining years of fecundity and then require assisted reproduction have always been a major challenge in ART treatment. The poor response that is commonly observed in women of AMA is directly related to diminished ovarian reserve. The associated reduction in oocyte quality as manifested by the increase in aneuploid embryos is most likely due to suboptimal cytoplasmic maturation (including reduced capacity of oocyte mitochondria to generate sufficient quantities of energy required for fertilization and cell division). In addition to the obstacles of diminished ovarian reserve, resistance to ovarian stimulation, and higher frequency of potential gynecological disorders, these women are also at higher risk of producing aneuploid oocytes and embryos. Uterine factors, as well as the possibility of aneuploid embryos, result in an increased miscarriage rate. Their situation is further compounded by the psychological stress of knowing that the "biological clock" is ticking, and that time is against them.

Although the use of donor oocytes has proved to be a very successful alternative treatment, this is not an option in many parts of the world, and efforts must be made to maximize each patient's potential to use her own oocytes. If a sufficient number of oocytes and embryos can be obtained, aneuploidy screening by PGS could be considered. However, the role of PGS needs to be evaluated in poor responders following the introduction of newer and more reliable techniques for genetic testing. In the future, accurate noninvasive methods for assessing oocyte and embryo quality may also become available, such as gene expression profiling of the cumulus cells surrounding the oocyte, as well as metabolomics and proteomics. These strategies, utilizing pharmacogenomics and manipulating endocrinology, may provide a means of augmenting follicular recruitment and cytoplasmic integrity, and thus improve the prognosis for these women.

REFERENCES

1. Wallace WH, Kelsey TW. Human ovarian reserve from conception to menopause. *PLoS One* 2010; 5: e8772.
2. Borini A, Bafaro G, Violini F, Bianchi L, Casadio V, Flamigni C. Pregnancies in postmenopausal women over 50 years old in an oocyte donation program. *Fertil Steril* 1995; 63: 258–61.
3. Laufer N, Simon A, Samueloff A, Yaffe H, Milwidsky A, Gielchinsky Y. Successful spontaneous pregnancies in women older than 45 years. *Fertil Steril* 2004; 81: 1328–32.
4. Gielchinsky Y, Mazor M, Simon A, Mor-Yossef S, Laufer N. Natural conception after age 45 in Bedouin women, a uniquely fertile population. *J Assist Reprod Genet* 2006; 23: 305–9.
5. Spandorfer SD, Bendikson K, Dragisic K, Schattman G, Davis OK, Rosenwaks Z. Outcome of *in vitro* fertilization in women 45 years and older who use autologous oocytes. *Fertil Steril* 2007; 87: 74–6.

6. Baker TG. Radiosensitivity of mammalian oocytes with particular reference to the human female. *Am J Obstet Gynecol* 1971; 110: 746–61.
7. Gougeon A. Dynamics of follicular growth in the human: A model from preliminary results. *Hum Reprod* 1986 Feb; 1(2): 81–7.
8. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, Calhaz-Jorge C, De Geyter C, Goossens V. Assisted reproductive technology in Europe, 2010: Results generated from European registers by ESHRE. *Hum Reprod* 2014; 29: 2099–113.
9. Assisted Reproductive Technology National Summary Report Figures, 2013. http://www.cdc.gov/art/reports/2013/national-summary-figures.html
10. Alviggi C, Humaidan P, Howles CM, Tredway D, Hillier SG. Biological versus chronological ovarian age: Implications for assisted reproductive technology. *Reprod Biol Endocrinol* 2009; 7: 101.
11. Surrey ES, Schoolcraft WB. Evaluating strategies for improving ovarian response of the poor responder undergoing assisted reproductive techniques. *Fertil Steril* 2000; 73: 667–76.
12. Polyzos NP, Devroey P. A systematic review of randomized trials for the treatment of poor ovarian responders: Is there any light at the end of the tunnel? *Fertil Steril* 2011; 96: 1058–61.
13. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of "poor response" to ovarian stimulation for *in vitro* fertilization: The Bologna criteria. *Hum Reprod* 2011; 26: 1616–24.
14. Papathanasiou A. Implementing the ESHRE "poor responder" criteria in research studies: Methodological implications. *Hum Reprod* 2014; 29: 1835–8.
15. La Marca A, Grisendi V, Giulini S, Sighinolfi G, Tirelli A, Argento C, Re C, Tagliasaachi D, Marsella T, Sunkara SK. Live birth rates in the different combinations of the Bologna criteria poor ovarian responders: A validation study. *J Assist Reprod Genet* 2015; 32: 931–7.
16. Busnelli A, Papaleo E, Del Prato D, La Vecchia I, Lachini E, Paffoni A, Candiani M, Somigliana E. A retrospective evaluation of prognosis and cost-effectiveness of IVF in poor responders according to the Bologna criteria. *Hum Reprod* 2015; 30: 315–22.
17. Chuang CC, Chen CD, Chao KH, Chen SU, Ho HN, Yang YS. Age is a better predictor of pregnancy potential than basal follicle-stimulating hormone levels in women undergoing *in vitro* fertilization. *Fertil Steril* 2003; 79: 63–8.
18. McClure N, McQuinn B, McDonald J, Kovacs GT, Healy DL, Burger HG. Body weight, body mass index, and age: Predictors of menotropin dose and cycle outcome in polycystic ovarian syndrome? *Fertil Steril* 1992; 58: 622–4.
19. Nahum R, Shifren JL, Chang Y, Leykin L, Isaacson K, Toth TL. Antral follicle assessment as a tool for predicting outcome in IVF—Is it a better predictor than age and FSH? *J Assist Reprod Genet* 2001; 18: 151–5.
20. Pohl M, Hohlagschwandtner M, Obruca A, Poschalko G, Weigert M, Feichtinger W. Number and size of antral follicles as predictive factors *in vitro* fertilization and embryo transfer. *J Assist Reprod Genet* 2000; 17: 315–8.
21. Popovic-Todorovic B, Loft A, Lindhard A, Bangsboll S, Andersson AM, Andersen AN. A prospective study of predictive factors of ovarian response in "standard" IVF/ICSI patients treated with recombinant FSH. A suggestion for a recombinant FSH dosage normogram. *Hum Reprod* 2003; 18: 781–7.
22. Scheffer GJ, Broekmans FJ, Looman CW, Blankenstein M, Fauser BC, teJong FH, teVelde ER. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. *Hum Reprod* 2003; 18: 700–6.
23. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: Practical recommendations for better standardization. *Fertil Steril* 2010; 94: 1044–51.
24. Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. The antral follicle count is a better marker than basal follicle-stimulating hormone for the selection of older patients with acceptable pregnancy prospects after *in vitro* fertilization. *Fertil Steril* 2005; 83: 811–4.
25. Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: A randomized controlled trial. *Hum Reprod* 2005; 20: 611–5.
26. Toner JP, Philput CB, Jones GS, Muasher SJ. Basal follicle-stimulating hormone level is a better predictor of *in vitro* fertilization performance than age. *Fertil Steril* 1991; 55: 784–91.
27. Abdalla H, Thum MY. An elevated basal FSH reflects a quantitative rather than qualitative decline of the ovarian reserve. *Hum Reprod* 2004; 19: 893–8.
28. Bancsi LF, Broekmans FJ, Mol BW, Habbema JD, te Velde ER. Performance of basal follicle-stimulating hormone in the prediction of poor ovarian response and failure to become pregnant after *in vitro* fertilization: A meta-analysis. *Fertil Steril* 2003; 79: 1091–100.
29. Fenichel P, Grimaldi M, Olivero JF, Donzeau M, Gillet JY, Harter M. Predictive value of hormonal profiles before stimulation for *in vitro* fertilization. *Fertil Steril* 1989; 51: 845–9.
30. Gurgan T, Urman B, Yarali H, Duran HE. Follicle-stimulating hormone levels on cycle day 3 to predict ovarian response in women undergoing controlled ovarian hyperstimulation for *in vitro* fertilization using a flare-up protocol. *Fertil Steril* 1997; 68: 483–7.
31. Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z. Follicle-stimulating hormone levels on cycle day 3 are predictive of *in vitro* fertilization outcome. *Fertil Steril* 1989; 51: 651–4.

32. Tinkanen H, Blauer M, Laippala P, Tuohimaa P, Kujansuu E. Prognostic factors in controlled ovarian hyperstimulation. *Fertil Steril* 1999; 72: 932–6.
33. Fawzy M, Lambert A, Harrison RF, Knight PG, Groome N, Hennelly B, Robertson WR. Day 5 inhibin B levels in a treatment cycle are predictive of IVF outcome. *Hum Reprod* 2002; 17: 1535–43.
34. Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in *in vitro* fertilization: A prospective study comparing basal markers of ovarian reserve. *Fertil Steril* 2002; 77: 328–36.
35. Eldar-Geva T, Margalioth EJ, Ben-Chetrit A, Gal M, Robertson DM, Healy DL, Diamant YZ, Spitz IM. Serum inhibin B levels measured early during FSH administration for IVF may be of value in predicting the number of oocytes to be retrieved in normal and low responders. *Hum Reprod* 2002; 17: 2331–7.
36. Ficicioglu C, Kutlu T, Demirbasoglu S, Mulayim B. The role of inhibin B as a basal determinant of ovarian reserve. *Gynecol Endocrinol* 2003; 17: 287–93.
37. Seifer DB, Lambert-Messerlian G, Hogan JW, Gardiner AC, Blazar AS, Berk CA. Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. *Fertil Steril* 1997; 67: 110–4.
38. Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: Prediction of live birth and extremes of response in stimulated cycles—Implications for individualization of therapy. *Hum Reprod* 2007; 22: 2414–21.
39. Lekamge DN, Barry M, Kolo M, Lane M, Gilchrist RB, Tremellen KP. Anti-Mullerian hormone as a predictor of IVF outcome. *Reprod Biomed Online* 2007; 14: 602–10.
40. Kwee J, Schats R, McDonnell J, Themmen A, de Jong F, Lambalk C. Evaluation of anti-Mullerian hormone as a test for the prediction of ovarian reserve. *Fertil Steril* 2008; 90: 737–43.
41. Feyereisen E, Mendez Lozano DH, Taieb J, Hesters L, Frydman R, Fanchin R. Anti-Mullerian hormone: Clinical insights into a promising biomarker of ovarian follicular status. *Reprod Biomed Online* 2006; 12: 695–703.
42. Nelson SM, Messow MC, Wallace AM, Fleming R, McConnachie A. Nomogram for the decline in serum antimullerian hormone: A population study of 9,601 infertility patients. *Fertil Steril* 2011; 95: 736–41.e1–3.
43. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, Broekmans FJ. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. *Hum Reprod* 2010; 25: 221–7.
44. Barad DH, Weghofer A, Gleicher N. Comparing anti-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH) as predictors of ovarian function. *Fertil Steril* 2009; 91: 1553–5.
45. Broer SL, Dolleman M, van Disseldorp J, Broeze KA, Opmeer BC, Bossuyt PM, Eijkemans MJ, Mol BW, Broekmans FJ. IPD-EXPORT Study Group. Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparion in subgroups: An individual patient data meta-analysis. *Fertil Steril* 2013; 100: 420–9.e7.
46. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt PM, Eijkemans MJ, Mol BW, Broekmans FJ. IPD-EXPORT Study Group. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: An individual patient data approach. *Hum Reprod Update* 2013; 19: 26–36.
47. Nelson SM, Yates RW, Lyall H, Jamieson M, Traynor I, Gaudoin M, Mitchell P, Ambrose P, Fleming R. Anti-Mullerian hormone-based approach to controlled ovarian stimulation for assisted conception. *Hum Reprod* 2009; 24: 867–75.
48. Nardo LG, Gelbaya TA, Wilkinson H, Roberts SA, Yates A, Pemberton P, Laing I. Circulating basal anti-Mullerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for *in vitro* fertilization. *Fertil Steril* 2009; 92: 1586–93.
49. Yates AP, Rustamov O, Roberts SA, Lim HY, Pemberton PW, Smith A, Nardo LG. Anti-Mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. *Hum Reprod* 2011; 26: 2353–62.
50. Popovic-Todorovic B, Loft A, Bredkjaeer HE, Bangsboll S, Nielsen IK, Andersen AN. A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a "standard" dose of 150 IU/day in "standard" patients undergoing IVF/ICSI treatment. *Hum Reprod* 2003; 18: 2275–82.
51. Howles CM, Saunders H, Alam V, Engrand P. Predictive factors and a corresponding treatment algorithm for controlled ovarian stimulation in patients treated with recombinant human follicle stimulating hormone (follitropin alfa) during assisted reproduction technology (ART) procedures. An analysis of 1378 patients. *Curr Med Res Opin* 2006; 22: 907–18.
52. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: From theory to practice. *Hum Reprod Update* 2014; 20: 124–40.
53. Ubaldi FM, Rienzi L, Ferrero S, Baroni E, Sapienza F, Cobellis L, Greco E. Management of poor responders in IVF. *Reprod Biomed Online* 2005; 10: 235–46.
54. Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhattacharya S. Interventions for "poor responders" to controlled ovarian hyper stimulation (COH) in *in-vitro* fertilisation (IVF). *Cochrane Database Syst Rev* 2010; 1: CD004379.
55. Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. *Br J Obstet Gynaecol* 1997; 104: 521–7.

56. Karande V, Gleicher N. A rational approach to the management of low responders in *in-vitro* fertilization. *Hum Reprod* 1999; 14: 1744–8.
57. Fasouliotis SJ, Simon A, Laufer N. Evaluation and treatment of low responders in assisted reproductive technology: A challenge to meet. *J Assist Reprod Genet* 2000; 17: 357–73.
58. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: A systematic review. *Hum Reprod Update* 2003; 9: 61–76.
59. Ben-Rafael Z, Strauss JF 3rd, Mastroianni L Jr., Flickinger GL. Differences in ovarian stimulation in human menopausal gonadotropin treated woman may be related to follicle-stimulating hormone accumulation. *Fertil Steril* 1986; 46: 586–92.
60. Ben-Rafael Z, Benadiva CA, Ausmanas M, Barber B, Blasco L, Flickinger GL, Mastroianni L, Jr. Dose of human menopausal gonadotropin influences the outcome of an *in vitro* fertilization program. *Fertil Steril* 1987; 48: 964–8.
61. Hofmann GE, Toner JP, Muasher SJ, Jones GS. High-dose follicle-stimulating hormone (FSH) ovarian stimulation in low-responder patients for *in vitro* fertilization. *J In Vitro Fert Embryo Transf* 1989; 6: 285–9.
62. Berkkanoglu M, Ozgur K. What is the optimum maximal gonadotropin dosage used in microdose flare-up cycles in poor responders? *Fertil Steril* 2010; 94: 662–5.
63. Lefebvre J, Antaki R, Kadoch IJ, Dean NL, Sylvestre C, Bissonnette F, Benoit J, Menard S, Lapensee L. 450 IU versus 600 IU gonadotropin for controlled ovarian stimulation in poor responders: A randomized controlled trial. *Fertil Steril* 2015; 104: 1419–25.
64. van Hooff MH, Alberda AT, Huisman GJ, Zeilmaker GH, Leerentveld RA. Doubling the human menopausal gonadotrophin dose in the course of an *in-vitro* fertilization treatment cycle in low responders: A randomized study. *Hum Reprod* 1993; 8: 369–73.
65. Hughes EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA. The routine use of gonadotropin-releasing hormone agonists prior to *in vitro* fertilization and gamete intrafallopian transfer: A meta-analysis of randomized controlled trials. *Fertil Steril* 1992; 58: 888–96.
66. Daya S. Optimal protocol for gonadotropin-releasing hormone agonist use in ovarian stimulation. In: *In Vitro Fertilization and Assisted Reproduction*. Gomel V, Cheung PCK (eds). Bologna, Italy: Monduzzi Editore, 1997, pp. 405–15.
67. Ben-Rafael Z, Bider D, Dan U, Zolti M, Levran D, Mashiach S. Combined gonadotropin releasing hormone agonist/human menopausal gonadotropin therapy (GnRH-a/hMG) in normal, high, and poor responders to hMG. *J In Vitro Fert Embryo Transf* 1991; 8: 33–6.
68. Chetkowski RJ, Rode RA, Burruel V, Nass TE. The effect of pituitary suppression and the women's age on embryo viability and uterine receptivity. *Fertil Steril* 1991; 56: 1095–103.
69. Lessing JB, Cohen JR, Yovel I, Amit A, David MP, Peyser MR. Atypical response to luteinizing hormone-releasing hormone (LH-RH) agonist (suprefact nasal) in induction of ovulation in *in vitro* fertilization (IVF). *J In vitro Fert Embryo Transf* 1991; 8: 314–6.
70. Ben-Nun I, Jaffe R, Goldberger S, Ghetler Y, Kaneti H, Fejgin M. Complete ovarian unresponsiveness to hMG stimulation after prolonged GnRH analogue administration. *Gynecol Endocrinol* 1990; 4: 151–5.
71. Davis OK, Rosenwaks Z. The ovarian factor in assisted reproductive technology. In: *The Ovary*. Leung PCK, Adashi EY (eds). New York, NY: Raven Press, 1993, pp. 545–60.
72. Meldrum DR, Tsao Z, Monroe SE, Braunstein GD, Sladek J, Lu JK, Vale W, Rivier J, Judd HL, Chang RJ. Stimulation of LH fragments with reduced bioactivity following GnRH agonist administration in women. *J Clin Endocrinol Metab* 1984; 58: 755–7.
73. Cedars MI, Surey E, Hamilton F, Lapolt P, Meldrum DR. Leuprolide acetate lowers circulating bioactive luteinizing hormone and testosterone concentrations during ovarian stimulation for oocyte retrieval. *Fertil Steril* 1990; 53: 627–31.
74. Dahl KD, Pavlou SN, Kovacs WJ, Hsueh AJ. The changing ratio of serum bioactive to immunoreactive follicle-stimulating hormone in normal men following treatment with a potent gonadotropin releasing hormone antagonist. *J Clin Endocrinol Metab* 1986; 63: 792–4.
75. Scott RT, Neal GS, Illions EH, Hayslip CA, Hofmann GE. The duration of leuprolide acetate administration prior to ovulation induction does not impact ovarian responsiveness to exogenous gonadotropins. *Fertil Steril* 1993; 60: 247–53.
76. Kowalik A, Barmat L, Damario M, Liu HC, Davis O, Rosenwaks Z. Ovarian estradiol production *in vivo*. Inhibitory effect of leuprolide acetate. *J Reprod Med* 1998; 43: 413–7.
77. Latouche J, Crumeyrolle-Arias M, Jordan D, Kopp N, Augendre-Ferrante B, Cedard L, Haour F. GnRH receptors in human granulosa cells: Anatomical localization and characterization by autoradiographic study. *Endocrinology* 1989; 125: 1739–41.
78. Sandow J, Stoeckemann K, Jerabek-Sandow G. Pharmacokinetics and endocrine effects of slow release formulations of LHRH analogues. *J Steroid Biochem Mol Biol* 1990; 37: 925–31.
79. Janssens RM, Lambalk CB, Vermeiden JP, Schats R, Bernards JM, Rekers-Mombarg LT, Schoemaker J. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: A prospective, randomized, double-blind, placebo-controlled study. *Hum Reprod* 2000; 15: 2333–40.

80. Feldberg D, Farhi J, Ashkenazi J, Dicker D, Shalev J, Ben-Rafael Z. Minidose gonadotropin-releasing hormone agonist is the treatment of choice in poor responders with high follicle-stimulating hormone levels. *Fertil Steril* 1994; 62: 343–6.
81. Olivennes F, Righini C, Fanchin R, Torrisi C, Hazout A, Glissant M, Fernandez H, Frydman F. A protocol using a low dose of gonadotrophin-releasing hormone agonist might be the best protocol for patients with high follicle-stimulating hormone concentrations on day 3. *Hum Reprod* 1996; 11: 1169–72.
82. Weissman A, Farhi J, Royburt M, Nahum H, Glezerman M, Levran D. Prospective evaluation of two stimulation protocols for low responders who were undergoing *in vitro* fertilization–embryo transfer. *Fertil Steril* 2003; 79: 886–92.
83. Keltz MD, Jones EE, Duleba AJ, Polcz T, Kennedy K, Olive DL. Baseline cyst formation after luteal phase gonadotropin-releasing hormone agonist administration is linked to poor *in vitro* fertilization outcome. *Fertil Steril* 1995; 64: 568–72.
84. Session DR, Saad AH, Salmansohn DD, Kelly AC. Ovarian activity during follicular-phase down regulation in *in vitro* fertilization is associated with advanced maternal age and a high recurrence rate in subsequent cycles. *J Assist Reprod Genet* 1995; 12: 301–4.
85. Margalioth EJ, Kafka I, Friedler S, Yehezkel O, Almagor M, Yaffe H. The incidence of ovarian cysts formation following GnRH analog treatment in IVF cycles is related to serum progesterone on the day of analog treatment initiation. In: *7th Anuual Meeting of the ESHRE*. Paris, France: 1991, p 15.
86. Aston K, Arthur I, Masson GM, Jenkins JM. Progestogen therapy and prevention of functional ovarian cysts during pituitary desensitisation with GnRH agonists. *Br J Obstet Gynaecol* 1995; 102: 835–7.
87. Ditkoff EC, Sauer MV. A combination of norethindrone acetate and leuprolide acetate blocks the gonadotrophin-releasing hormone agonistic response and minimizes cyst formation during ovarian stimulation. *Hum Reprod* 1996; 11: 1035–7.
88. Engmann L, Maconochie N, Bekir J, Tan SL. Progestogen therapy during pituitary desensitization with gonadotropin-releasing hormone agonist prevents functional ovarian cyst formation: A prospective, randomized study. *Am J Obstet Gynecol* 1999; 181: 576–82.
89. Sunkara SK, Coomarasamy A, Faris R, Braude P, Khalaf Y. Long gonadotropin-releasing hormone agonist versus short agonist versus antagonist regimens in poor responders undergoing *in vitro* fertilization: A randomized controlled trial. *Fertil Steril* 2014; 101: 147–53.
90. Smitz J, Devroey P, Camus M, Deschacht J, Van Waesberghe L, Wisanto A, Van Steirteghem AC. Inhibition of gonadotropic and ovarian function by intranasal administration of D-Ser (TBU)6-EA10-LHRH in normo-ovulatory women and patients with polycystic ovary disease. *J Endocrinol Invest* 1988; 11: 647–52.
91. Calogero AE, Macchi M, Montanini V, Mongioi A, Maugeri G, Vicari E, Coniglione F, Sipione C, D'Agata R. Dynamics of plasma gonadotropin and sex steroid release in polycystic ovarian disease after pituitary–ovarian inhibition with an analog of gonadotropin-releasing hormone. *J Clin Endocrinol Metab* 1987; 64: 980–5.
92. Sungurtekin U, Jansen RP. Profound luteinizing hormone suppression after stopping the gonadotropin-releasing hormone-agonist leuprolide acetate. *Fertil Steril* 1995; 63: 663–5.
93. Corson SL, Batzer FR, Gocial B, Eisenberg E, Huppert LC, Nelson JR. Leuprolide acetate-prepared *in vitro* fertilization-gamete intrafallopian transfer cycles: Efficacy versus controls and cost analysis. *Fertil Steril* 1992; 57: 601–5.
94. Pantos K, Meimeth-Damianaki T, Vaxevanoglou T, Kapetanakis E. Prospective study of a modified gonadotropin-releasing hormone agonist long protocol in an *in vitro* fertilization program. *Fertil Steril* 1994; 61: 709–13.
95. Simons AH, Roelofs HJ, Schmoutziguer AP, Roozenburg BJ, van't Hof-van den Brink EP, Schoonderwoerd SA. Early cessation of triptorelin in *in vitro* fertilization: A double-blind, randomized study. *Fertil Steril* 2005; 83: 889–96.
96. Beckers NG, Laven JS, Eijkemans MJ, Fauser BC. Follicular and luteal phase characteristics following early cessation of gonadotrophin-releasing hormone agonist during ovarian stimulation for *in-vitro* fertilization. *Hum Reprod* 2000; 15: 43–9.
97. Fujii S, Sagara M, Kudo H, Kagiya A, Sato S, Saito Y. A prospective randomized comparison between long and discontinuous-long protocols of gonadotropin-releasing hormone agonist for *in vitro* fertilization. *Fertil Steril* 1997; 67: 1166–8.
98. Cedrin-Durnerin I, Bidart JM, Robert P, Wolf JP, Uzan M, Hugues JN. Consequences on gonadotrophin secretion of an early discontinuation of gonadotrophin-releasing hormone agonist administration in short-term protocol for *in-vitro* fertilization. *Hum Reprod* 2000; 15: 1009–14.
99. Detti L, Williams DB, Robins JC, Maxwell RA, Thomas MA. A comparison of three downregulation approaches for poor responders undergoing *in vitro* fertilization. *Fertil Steril* 2005; 84: 1401–5.
100. Faber BM, Mayer J, Cox B, Jones D, Toner JP, Oehninger S, Muasher SJ. Cessation of gonadotropin-releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. *Fertil Steril* 1998; 69: 826–30.
101. Wang PT, Lee RK, Su JT, Hou JW, Lin MH, Hu YM. Cessation of low-dose gonadotropin releasing

hormone agonist therapy followed by high-dose gonadotropin stimulation yields a favorable ovarian response in poor responders. *J Assist Reprod Genet* 2002; 19: 1–6.
102. Schachter M, Friedler S, Raziel A, Strassburger D, Bern O, Ron-El R. Improvement of IVF outcome in poor responders by discontinuation of GnRH analogue during the gonadotropin stimulation phase—A function of improved embryo quality. *J Assist Reprod Genet* 2001; 18: 197–204.
103. Pinkas H, Orvieto R, Avrech OM, Rufas O, Ferber A, Ben-Rafael Z, Fisch B. Gonadotropin stimulation following GnRH-a priming for poor responders in *in vitro* fertilization-embryo transfer programs. *Gynecol Endocrinol* 2000; 14: 11–4.
104. Dirnfeld M, Fruchter O, Yshai D, Lissak A, Ahdut A, Abramovici H. Cessation of gonadotropin-releasing hormone analogue (GnRH-a) upon down-regulation versus conventional long GnRH-a protocol in poor responders undergoing *in vitro* fertilization. *Fertil Steril* 1999; 72: 406–11.
105. Garcia-Velasco JA, Isaza V, Requena A, Martinez-Salazar FJ, Landazabal A, Remohi J, Pellicer A, Simon C. High doses of gonadotrophins combined with stop versus non-stop protocol of GnRH analogue administration in low responder IVF patients: A prospective, randomized, controlled trial. *Hum Reprod* 2000; 15: 2292–6.
106. Bider D, Ben-Rafael Z, Shalev J, Goldenberg M, Mashiach S, Blankstein J. Pituitary and ovarian suppression rate after high dosage of gonadotropin-releasing hormone agonist. *Fertil Steril* 1989; 51: 578–81.
107. Padilla SL, Dugan K, Maruschak V, Shalika S, Smith RD. Use of the flare-up protocol with high dose human follicle stimulating hormone and human menopausal gonadotropins for *in vitro* fertilization in poor responders. *Fertil Steril* 1996; 65: 796–9.
108. Garcia JE, Padilla SL, Bayati J, Baramki TA. Follicular phase gonadotropin-releasing hormone agonist and human gonadotropins: A better alternative for ovulation induction in *in vitro* fertilization. *Fertil Steril* 1990; 53: 302–5.
109. Howles CM, Macnamee MC, Edwards RG. Short term use of an LHRH agonist to treat poor responders entering an *in-vitro* fertilization programme. *Hum Reprod* 1987; 2: 655–6.
110. Katayama KP, Roesler M, Gunnarson C, Stehlik E, Jagusch S. Short-term use of gonadotropin-releasing hormone agonist (leuprolide) for *in vitro* fertilization. *J In Vitro Fert Embryo Transf* 1988; 5: 332–4.
111. Toth TL, Awwad JT, Veeck LL, Jones HW, Jr., Muasher SJ. Suppression and flare regimens of gonadotropin-releasing hormone agonist. Use in women with different basal gonadotropin values in an *in vitro* fertilization program. *J Reprod Med* 1996; 41: 321–6.
112. Karande V, Morris R, Rinehart J, Miller C, Rao R, Gleicher N. Limited success using the "flare" protocol in poor responders in cycles with low basal follicle-stimulating hormone levels during *in vitro* fertilization. *Fertil Steril* 1997; 67: 900–3.
113. Brzyski RG, Muasher SJ, Droesch K, Simonetti S, Jones GS, Rosenwaks Z. Follicular atresia associated with concurrent initiation of gonadotropin-releasing hormone agonist and follicle-stimulating hormone for oocyte recruitment. *Fertil Steril* 1988; 50: 917–21.
114. Gindoff PR, Hall JL, Stillman RJ. Ovarian suppression with leuprolide acetate: Comparison of luteal, follicular, and flare-up administration in controlled ovarian hyperstimulation for oocyte retrieval. *J In Vitro Fert Embryo Transf* 1990; 7: 94–7.
115. Anserini P, Magnasco A, Remorgida V, Gaggero G, Testa D, Capitanio GL. Comparison of a blocking vs. a flare-up protocol in poor responders with a normal and abnormal clomiphene citrate challenge test. *Gynecol Endocrinol* 1997; 11: 321–6.
116. Karacan M, Erkan H, Karabulut O, Sarikamis B, Camlibel T, Benhabib M. Clinical pregnancy rates in an IVF program. Use of the flare-up protocol after failure with long regimens of GnRH-a. *J Reprod Med* 2001; 46: 485–9.
117. San Roman GA, Surrey ES, Judd HL, Kerin JF. A prospective randomized comparison of luteal phase versus concurrent follicular phase initiation of gonadotropin-releasing hormone agonist for *in vitro* fertilization. *Fertil Steril* 1992; 58: 744–9.
118. Hillier SG. Roles of follicle stimulating hormone and luteinizing hormone in controlled ovarian hyperstimulation. *Hum Reprod* 1996; 11(Suppl 3): 113–21.
119. Gelety TJ, Pearlstone AC, Surrey ES. Short-term endocrine response to gonadotropin-releasing hormone agonist initiated in the early follicular, midluteal, or late luteal phase in normally cycling women. *Fertil Steril* 1995; 64: 1074–80.
120. Cédrin-Durnerin I, Bulwa S, Herve F, Martin-Pont B, Uzan M, Hugues JN. The hormonal flare-up following gonadotrophin-releasing hormone agonist administration is influenced by a progestogen pretreatment. *Hum Reprod* 1996; 11: 1859–63.
121. al-Mizyen E, Sabatini L, Lower AM, Wilson CM, al-Shawaf T, Grudzinskas JG. Does pretreatment with progestogen or oral contraceptive pills in low responders followed by the GnRHa flare protocol improve the outcome of IVF-ET? *J Assist Reprod Genet* 2000; 17: 140–6.
122. Bstandig B, Cedrin-Durnerin I, Hugues JN. Effectiveness of low dose of gonadotropin releasing hormone agonist on hormonal flare-up. *J Assist Reprod Genet* 2000; 17: 113–7.
123. Deaton JL, Bauguess P, Huffman CS, Miller KA. Pituitary response to early follicular-phase minidose gonadotropin releasing hormone agonist (GnRHa) therapy: Evidence for a second flare. *J Assist Reprod Genet* 1996; 13: 390–4.

124. Navot D, Rosenwaks Z, Anderson F, Hodgen GD. Gonadotropin-releasing hormone agonist-induced ovarian hyperstimulation: Low-dose side effects in women and monkeys. *Fertil Steril* 1991; 55: 1069–75.
125. Scott RT, Carey KD, Leland M, Navot D. Gonadotropin responsiveness to ultralow-dose leuprolide acetate administration in baboons. *Fertil Steril* 1993; 59: 1124–8.
126. Scott RT, Navot D. Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonist during ovulation induction for in vitro fertilization. *Fertil Steril* 1994; 61(5): 880–5.
127. Schoolcraft W, Schlenker T, Gee M, Stevens J, Wagley L. Improved controlled ovarian hyperstimulation in poor responder *in vitro* fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. *Fertil Steril* 1997; 67: 93–7.
128. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW. Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing *in vitro* fertilization. *Fertil Steril* 1998; 69: 419–24.
129. Leondires MP, Escalpes M, Segars JH, Scott RT, Jr., Miller BT. Microdose follicular phase gonadotropin-releasing hormone agonist (GnRH-a) compared with luteal phase GnRH-a for ovarian stimulation at *in vitro* fertilization. *Fertil Steril* 1999; 72: 1018–23.
130. Albano C, Felberbaum RE, Smitz J, Riethmuller-Winzen H, Engel J, Diedrich K, Devroey P. Ovarian stimulation with HMG: Results of a prospective randomized Phase III European study comparing the luteinizing hormone-releasing hormone (LHRH)-antagonist cetrorelix and the LHRH-agonist buserelin. European Cetrorelix Study Group. *Hum Reprod* 2000; 15: 526–31.
131. Howles CM. The place of gonadotrophin-releasing hormone antagonists in reproductive medicine. *Reprod Biomed Online* 2002; 4(Suppl 3): 64–71.
132. Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, Abou-Setta AM. Gonadotrophin releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011; 5: CD001750.
133. Quigley MM. The use of ovulation-inducing agents in *in-vitro* fertilization. *Clin Obstet Gynecol* 1984; 27: 983–92.
134. Lehmann F, Baban N, Webber B. Ovarian stimulation for *in-vitro* fertilization: Clomiphene and HMG. *Hum Reprod* 1988; 3(Suppl 2): 11–21.
135. Ronen J, Bosschieter J, Wiswedel K, Hendriks S, Levin M. Ovulation induction for *in vitro* fertilisation using clomiphene citrate and low-dose human menopausal gonadotrophin. *Int J Fertil* 1988; 33: 120–2.
136. Tummon IS, Daniel SA, Kaplan BR, Nisker JA, Yuzpe AA. Randomized, prospective comparison of luteal leuprolide acetate and gonadotropins versus clomiphene citrate and gonadotropins in 408 first cycles of *in vitro* fertilization. *Fertil Steril* 1992; 58: 563–8.
137. Craft I, Gorgy A, Hill J, Menon D, Podsiadly B. Will GnRH antagonists provide new hope for patients considered "difficult responders" to GnRH agonist protocols? *Hum Reprod* 1999; 14: 2959–62.
138. Nikolettos N, Al-Hasani S, Felberbaum R, Demirel LC, Kupker W, Montzka P, Xia YX, Schopper B, Sturm R, Diedrich K. Gonadotropin-releasing hormone antagonist protocol: A novel method of ovarian stimulation in poor responders. *Eur J Obstet Gynecol Reprod Biol* 2001; 97: 202–7.
139. Posada MN, Vlahos NP, Jurema MW, Bracero NJ, Wallach EE, Garcia JE. Clinical outcome of using ganirelix acetate versus a 4-day follicular phase leuprolide acetate protocol in unselected women undergoing *in vitro* fertilization. *Fertil Steril* 2003; 80: 103–10.
140. Mohamed KA, Davies WA, Allsopp J, Lashen H. Agonist "flare-up" versus antagonist in the management of poor responders undergoing *in vitro* fertilization treatment. *Fertil Steril* 2005; 83: 331–5.
141. Fasouliotis SJ, Laufer N, Sabbagh-Ehrlich S, Lewin A, Hurwitz A, Simon A. Gonadotropin-releasing hormone (GnRH)-antagonist versus GnRH-agonist in ovarian stimulation of poor responders undergoing IVF. *J Assist Reprod Genet* 2003; 20: 455–60.
142. Copperman AB. Antagonists in poor-responder patients. *Fertil Steril* 2003; 80(Suppl 1): S16–24; discussion S32–4.
143. Shapiro D, Carter M, Mitchell-Leef D, Wininger D. Plateau or drop in estradiol (E2) on the day after initiation of the GnRH antagonist Antagon™ in *in-vitro* fertilization (IVF) treatment cycles does not affect pregnancy outcome. *Fertil Steril* 2002; 78: S22–3.
144. Akman MA, Erden HF, Tosun SB, Bayazit N, Aksoy E, Bahceci M. Addition of GnRH antagonist in cycles of poor responders undergoing IVF. *Hum Reprod* 2000; 15: 2145–7.
145. Akman MA, Erden HF, Tosun SB, Bayazit N, Aksoy E, Bahceci M. Comparison of agonistic flare-up-protocol and antagonistic multiple dose protocol in ovarian stimulation of poor responders: Results of a prospective randomized trial. *Hum Reprod* 2001; 16: 868–70.
146. De Placido G, Mollo A, Clarizia R, Strina I, Conforti S, Alviggi C. Gonadotropin-releasing hormone (GnRH) antagonist plus recombinant luteinizing hormone vs. a standard GnRH agonist short protocol in patients at risk for poor ovarian response. *Fertil Steril* 2006; 85: 247–50.
147. Demirol A, Gurgan T. Comparison of microdose flare-up and antagonist multiple-dose protocols for poor-responder patients: A randomized study. *Fertil Steril* 2009; 92: 481–5.
148. Kahraman K, Berker B, Atabekoglu CS, Sonmezer M, Cetinkaya E, Aytac R, Satiroglu H. Microdose gonadotropin-releasing hormone agonist flare-up protocol

versus multiple dose gonadotropin-releasing hormone antagonist protocol in poor responders undergoing intracytoplasmic sperm injection–embryo transfer cycle. *Fertil Steril* 2009; 91: 2437–44.
149. Devesa M, Martinez F, Coroleu B, Tur R, Gonzalez C, Rodriguez I, Barri PN. Poor prognosis for ovarian response to stimulation: Results of a randomised trial comparing the flare-up GnRH agonist protocol vs. the antagonist protocol. *Gynecol Endocrinol* 2010; 26: 509–15.
150. Schmidt DW, Bremner T, Orris JJ, Maier DB, Benadiva CA, Nulsen JC. A randomized prospective study of microdose leuprolide versus ganirelix in *in vitro* fertilization cycles for poor responders. *Fertil Steril* 2005; 83: 1568–71.
151. Malmusi S, La Marca A, Giulini S, Xella S, Tagliasacchi D, Marsella T, Volpe A. Comparison of a gonadotropin-releasing hormone (GnRH) antagonist and GnRH agonist flare-up regimen in poor responders undergoing ovarian stimulation. *Fertil Steril* 2005; 84: 402–6.
152. D'Amato G, Caroppo E, Pasquadibisceglie A, Carone D, Vitti A, Vizziello GM. A novel protocol of ovulation induction with delayed gonadotropin-releasing hormone antagonist administration combined with high-dose recombinant follicle-stimulating hormone and clomiphene citrate for poor responders and women over 35 years. *Fertil Steril* 2004; 81: 1572–7.
153. Tazegul A, Gorkemli H, Ozdemir S, Aktan TM. Comparison of multiple dose GnRH antagonist and minidose long agonist protocols in poor responders undergoing *in vitro* fertilization: A randomized controlled trial. *Arch Gynecol Obstet* 2008; 278: 467–72.
154. Marci R, Caserta D, Dolo V, Tatone C, Pavan A, Moscarini M. GnRH antagonist in IVF poor-responder patients: Results of a randomized trial. *Reprod Biomed Online* 2005; 11: 189–93.
155. Cheung LP, Lam PM, Lok IH, Chiu TT, Yeung SY, Tjer CC, Haines CJ. GnRH antagonist versus long GnRH agonist protocol in poor responders undergoing IVF: A randomized controlled trial. *Hum Reprod* 2005; 20: 616–21.
156. Frankfurter D, Dayal M, Dubey A, Peak D, Gindoff P. Novel follicular-phase gonadotropin-releasing hormone antagonist stimulation protocol for *in vitro* fertilization in the poor responder. *Fertil Steril* 2007; 88: 1442–5.
157. Orvieto R, Kruchkovich J, Rabinson J, Zohav E, Anteby EY, Meltcer S. Ultrashort gonadotropin-releasing hormone agonist combined with flexible multidose gonadotropin-releasing hormone antagonist for poor responders in *in vitro* fertilization/embryo transfer programs. *Fertil Steril* 2008; 90: 228–30.
158. Berger BM, Ezcurra D, Alper MM. The agonist-antagonist protocol: A novel protocol for treating the poor responder [abstract]. *Fertil Steril* 2004; 82: S126.
159. Fisch JD, Keskintepe L, Sher G. Gonadotropin-releasing hormone agonist/antagonist conversion with estrogen priming in low responders with prior *in vitro* fertilization failure. *Fertil Steril* 2008; 89: 342–7.
160. Nargund G, Fauser BC, Macklon NS, Ombelet W, Nygren K, Frydman R. The ISMAAR proposal on terminology for ovarian stimulation for IVF. *Hum Reprod* 2007; 22: 2801–4.
161. Bassil S, Godin PA, Donnez J. Outcome of *in-vitro* fertilization through natural cycles in poor responders. *Hum Reprod* 1999; 14: 1262–5.
162. Feldman B, Seidman DS, Levron J, Bider D, Shulman A, Shine S, Dor J. *In vitro* fertilization following natural cycles in poor responders. *Gynecol Endocrinol* 2001; 15: 328–34.
163. Lindheim SR, Vidali A, Ditkoff E, Sauer MV, Zinger M. Poor responders to ovarian hyperstimulation may benefit from an attempt at natural-cycle oocyte retrieval. *J Assist Reprod Genet* 1997; 14: 174–6.
164. Bar-Hava I, Ferber A, Ashkenazi J, Dicker D, Ben-Rafael Z, Orvieto R. Natural-cycle *in vitro* fertilization in women aged over 44 years. *Gynecol Endocrinol* 2000; 14: 248–52.
165. Check ML, Check JH, Wilson C, Choe JK, Krotec J. Outcome of *in vitro* fertilization-embryo transfer according to age in poor responders with elevated baseline serum follicle stimulation hormone using minimal or no gonadotropin stimulation. *Clin Exp Obstet Gynecol* 2004; 31: 183–4.
166. Morgia F, Sbracia M, Schimberni M, Giallonardo A, Piscitelli C, Giannini P, Aragona C. A controlled trial of natural cycle versus microdose gonadotropin-releasing hormone analog flare cycles in poor responders undergoing *in vitro* fertilization. *Fertil Steril* 2004; 81: 1542–7.
167. Papaleo E, De Santis L, Fusi F, Doldi N, Brigante C, Marelli G, Persico P, Cino I, Ferrari A. Natural cycle as first approach in aged patients with elevated follicle-stimulating hormone undergoing intracytoplasmic sperm injection: A pilot study. *Gynecol Endocrinol* 2006; 22: 351–4.
168. Polyzos NP, Blockeel C, Verpoest W, De Vos M, Stoop D, Vloeberghs V, Camus M, Devroey P, Tournaye H. Live birth rates following natural cycle IVF in women with poor ovarian response according to the Bologna criteria. *Hum Reprod* 2012; 12: 3481–6.
169. Pelinck MJ, Hoek A, Simons AH, Heineman MJ. Efficacy of natural cycle IVF: A review of the literature. *Hum Reprod Update* 2002; 8: 129–39.
170. Paulson RJ, Sauer MV, Lobo RA. Addition of a gonadotropin releasing hormone (GnRH) antagonist and exogenous gonadotropins to unstimulated *in vitro* fertilization (IVF) cycles: Physiologic observations and preliminary experience. *J Assist Reprod Genet* 1994; 11: 28–32.

171. Rongieres-Bertrand C, Olivennes F, Righini C, Fanchin R, Taieb J, Hamamah S, Bouchard P, Frydman R. Revival of the natural cycles in *in-vitro* fertilization with the use of a new gonadotrophin-releasing hormone antagonist (cetrorelix): A pilot study with minimal stimulation. *Hum Reprod* 1999; 14: 683–8.
172. Pelinck MJ, Vogel NE, Arts EG, Simons AH, Heineman MJ, Hoek A. Cumulative pregnancy rates after a maximum of nine cycles of modified natural cycle IVF and analysis of patient drop-out: A cohort study. *Hum Reprod* 2007; 22: 2463–70.
173. Pelinck MJ, Vogel NE, Hoek A, Arts EG, Simons AH, Heineman MJ. Minimal stimulation IVF with late follicular phase administration of the GnRH antagonist cetrorelix and concomitant substitution with recombinant FSH: A pilot study. *Hum Reprod* 2005; 20: 642–8.
174. Pelinck MJ, Vogel NE, Hoek A, Simons AH, Arts EG, Mochtar MH, Beemsterboer S, Hondelink MN, Heineman MJ. Cumulative pregnancy rates after three cycles of minimal stimulation IVF and results according to subfertility diagnosis: A multicentre cohort study. *Hum Reprod* 2006; 21: 2375–83.
175. Kolibianakis E, Zikopoulos K, Camus M, Tournaye H, Van Steirteghem A, Devroey P. Modified natural cycle for IVF does not offer a realistic chance of parenthood in poor responders with high day 3 FSH levels, as a last resort prior to oocyte donation. *Hum Reprod* 2004; 19: 2545–9.
176. Elizur SE, Aslan D, Shulman A, Weisz B, Bider D, Dor J. Modified natural cycle using GnRH antagonist can be an optional treatment in poor responders undergoing IVF. *J Assist Reprod Genet* 2005; 22: 75–9.
177. Kim CH, Kim SR, Cheon YP, Kim SH, Chae HD, Kang BM. Minimal stimulation using gonadotropin-releasing hormone (GnRH) antagonist and recombinant human follicle-stimulating hormone versus GnRH antagonist multiple-dose protocol in low responders undergoing *in vitro* fertilization/intracytoplasmic sperm injection. *Fertil Steril* 2009; 92: 2082–4.
178. Kadoch IJ, Phillips SJ, Bissonnette F. Modified natural-cycle *in vitro* fertilization should be considered as the first approach in young poor responders. *Fertil Steril* 2011; 96: 1066–8.
179. Kadoch IJ, Al-Khaduri M, Phillips SJ, Lapensee L, Couturier B, Hemmings R, Bissonnette F. Spontaneous ovulation rate before oocyte retrieval in modified natural cycle IVF with and without indomethacin. *Reprod Biomed Online* 2008; 16: 245–9.
180. Schimberni M, Morgia F, Colabianchi J, Giallonardo A, Piscitelli C, Giannini P, Montigiani M, Sbracia M. Natural-cycle *in vitro* fertilization in poor responder patients: A survey of 500 consecutive cycles. *Fertil Steril* 2009; 92: 1297–301.
181. Castelo Branco A, Achour-Frydman N, Kadoch J, Fanchin R, Tachdjian G, Frydman R. *In vitro* fertilization and embryo transfer in seminatural cycles for patients with ovarian aging. *Fertil Steril* 2005; 84: 875–80.
182. Segawa T, Yelian Y, Kato K, Kobayashi T, Takehera Y, Kato O. Natural cycle IVF is an excellent treatment option for women with advanced age. *Fertil Steril* 2009; 92: S54.
183. Ellenbogen A, Gidoni Y, Atamna R, Karchovski E, Rotfarb N, Michaeli M. Last chance before egg donation: Modified natural cycle *in vitro* fertilization in poor responder patients; the role of follicle diameter on the day of hCG administration in order to improve results. *Fertil Steril* 2009; 92: S162.
184. Ellenbogen A, Michaeli M, Ballas S. Egg collection with a double lumen needle in poor responder patients undergoing *in vitro* fertilization treatment. *Fertil Steril* 2003; 80: 127.
185. Méndez Lozano DH, Brum Scheffer J, Frydman N, Fay S, Fanchin R, Frydman R. Optimal reproductive competence of oocytes retrieved through follicular flushing in minimal stimulation IVF. *Reprod Biomed Online* 2008; 16: 119–23.
186. Mendez Lozano DH, Fanchin R, Chevalier N, Feyereisen E, Hesters L, Frydman N, Frydman R. [The follicular flushing duplicate the pregnancy rate on semi natural cycle IVF]. *J Gynecol Obstet Biol Reprod (Paris)* 2007; 36: 36–41.
187. Levens ED, Whitcomb BW, Payson MD, Larsen FW. Ovarian follicular flushing among low-responding patients undergoing assisted reproductive technology. *Fertil Steril* 2009; 91: 1381–4.
188. Mochtar MH, Van der V, Ziech M, van Wely M. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2007; 2: CD005070.
189. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: Implications for human reproduction. *Hum Reprod Update* 2002; 8: 413–21.
190. Flack MR, Froehlich J, Bennet AP, Anasti J, Nisula BC. Site-directed mutagenesis defines the individual roles of the glycosylation sites on follicle-stimulating hormone. *J Biol Chem* 1994; 269: 14015–20.
191. Howles CM. Genetic engineering of human FSH (Gonal-F). *Hum Reprod Update* 1996; 2: 172–91.
192. Bassett RM, Driebergen R. Continued improvements in the quality and consistency of follitropin alfa, recombinant human FSH. *Reprod Biomed Online* 2005; 10: 169–77.
193. Aittomaki K, Herva R, Stenman UH, Juntunen K, Ylostalo P, Hovatta O, de la Chapelle A. Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *J Clin Endocrinol Metab* 1996; 81: 3722–6.

194. Touraine P, Beau I, Gougeon A et al. New natural inactivating mutations of the follicle-stimulating hormone receptor: Correlations between receptor function and phenotype. *Mol Endocrinol* 1999; 13: 1844–54.
195. Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. *J Clin Endocrinol Metab* 2000; 85: 3365–9.
196. Jun JK, Yoon JS, Ku SY, Choi YM, Hwang KR, Park SY, Lee GH, Lee WD, Kim SH, Kim JG, Moon SY. Follicle-stimulating hormone receptor gene polymorphism and ovarian responses to controlled ovarian hyperstimulation for IVF-ET. *J Hum Genet* 2006; 51: 665–70.
197. Greb RR, Grieshaber K, Gromoll J, Sonntag B, Nieschlag E, Kiesel L, Simoni M. A common single nucleotide polymorphism in exon 10 of the human follicle stimulating hormone receptor is a major determinant of length and hormonal dynamics of the menstrual cycle. *J Clin Endocrinol Metab* 2005; 90: 4866–72.
198. Behre HM, Greb RR, Mempel A, Sonntag B, Kiesel L, Kaltwasser P, Seliger E, Ropke F, Gromoll J, Nieschlag E, Simoni M. Significance of a common single nucleotide polymorphism in exon 10 of the follicle-stimulating hormone (FSH) receptor gene for the ovarian response to FSH: A pharmacogenetic approach to controlled ovarian hyperstimulation. *Pharmacogenet Genomics* 2005; 15: 451–6.
199. Achrekar SK, Modi DN, Desai SK, Mangoli VS, Mangoli RV, Mahale SD. Poor ovarian response to gonadotrophin stimulation is associated with FSH receptor polymorphism. *Reprod Biomed Online* 2009; 18: 509–15.
200. Bosch E, Ezcurra D. Individualised controlled ovarian stimulation (iCOS): Maximising success rates for assisted reproductive technology patients. *Reprod Biol Endocrinol* 2011; 9: 82.
201. Nardo LG, Fleming R, Howles CM, Bosch E, Hamamah S, Ubaldi FM, Hugues JN, Balen AH, Nelson SM. Conventional ovarian stimulation no longer exists: Welcome to the age of individualized ovarian stimulation. *Reprod Biomed Online* 2011; 23: 141–8.
202. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: From theory to practice. *Hum Reprod Update* 2014; 1: 124–40.
203. Olivennes F, Borini A, Germond M, Trew G, Wikland M, Zegers-Hochschild F. Optimising ovarian stimulation in ART using a novel FSH dose algorithm and follitropin alfa filled by mass: Results of the CONSORT multinational study. In: *23rd Annual Meeting of the ESHRE*. Lyon, France: 2007, p. O-009.
204. Balasch J, Miro F, Burzaco I, Casamitjana R, Civico S, Ballesca JL, Puerto B, Vanrell JA. The role of luteinizing hormone in human follicle development and oocyte fertility: Evidence from *in-vitro* fertilization in a woman with long-standing hypogonadotrophic hypogonadism and using recombinant human follicle stimulating hormone. *Hum Reprod* 1995; 10: 1678–83.
205. Hull M, Corrigan E, Piazzi A, Loumaye E. Recombinant human luteinising hormone: An effective new gonadotropin preparation. *Lancet* 1994; 344: 334–5.
206. De Placido G, Alviggi C, Mollo A, Strina I, Ranieri A, Alviggi E, Wilding M, Varricchio MT, Borrelli AL, Conforti S. Effects of recombinant LH (rLH) supplementation during controlled ovarian hyperstimulation (COH) in normogonadotrophic women with an initial inadequate response to recombinant FSH (rFSH) after pituitary downregulation. *Clin Endocrinol (Oxf)* 2004; 60: 637–43.
207. De Placido G, Alviggi C, Perino A, Strina I, Lisi F, Fasolino A, De Palo R, Ranieri A, Colacurci N, Mollo A. Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. *Hum Reprod* 2005; 20: 390–6.
208. Howles CM. Role of LH and FSH in ovarian function. *Mol Cell Endocrinol* 2000; 161: 25–30.
209. Chappel SC, Howles C. Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum Reprod* 1991; 6: 1206–12.
210. Hillier SG. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. *Hum Reprod* 1994; 9: 188–91.
211. Balasch J, Fabregues F. Is luteinizing hormone needed for optimal ovulation induction? *Curr Opin Obstet Gynecol* 2002; 14: 265–74.
212. Shoham Z. Treatment of female infertility with recombinant human luteinising hormone: Is there a benefit over other available drugs? *Expert Opin Pharmacother* 2003; 4: 1985–94.
213. Huirne JA, van Loenen AC, Schats R, McDonnell J, Hompes PG, Schoemaker J, Homburg R, Lambalk CB. Dose-finding study of daily GnRH antagonist for the prevention of premature LH surges in IVF/ICSI patients: Optimal changes in LH and progesterone for clinical pregnancy. *Hum Reprod* 2005; 20: 359–67.
214. Haavisto AM, Pettersson K, Bergendahl M, Virkamaki A, Huhtaniemi I. Occurrence and biological properties of a common genetic variant of luteinizing hormone. *J Clin Endocrinol Metab* 1995; 80: 1257–63.
215. Alviggi C, Clarizia R, Pettersson K, Mollo A, Humaidan P, Strina I, Coppola M, Ranieri A, D'Uva M, De Placido G. Suboptimal response to

GnRHa long protocol is associated with a common LH polymorphism. *Reprod Biomed Online* 2009; 18: 9–14.
216. Takahashi K, Kurioka H, Ozaki T, Kanasaki H, Kohsaka M, Miyazaki K, Karino K. Increased prevalence of luteinizing hormone beta-subunit variant in Japanese infertility patients. *Hum Reprod* 1998; 13: 3338–44.
217. Takahashi K, Ozaki T, Okada M, Kurioka H, Kanasaki H, Miyazaki K. Increased prevalence of luteinizing hormone beta-subunit variant in patients with premature ovarian failure. *Fertil Steril* 1999; 71: 96–101.
218. Caglar GS, Asimakopoulos B, Nikolettos N, Diedrich K, Al-Hasani S. Recombinant LH in ovarian stimulation. *Reprod Biomed Online* 2005; 10: 774–85.
219. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: A dose-finding study. The European Recombinant Human LH Study Group. *J Clin Endocrinol Metab* 1998; 83: 1507–14.
220. Foong SC, Abbott DH, Lesnick TG, Session DR, Walker DL, Dumesic DA. Diminished intrafollicular estradiol levels in *in vitro* fertilization cycles from women with reduced ovarian response to recombinant human follicle-stimulating hormone. *Fertil Steril* 2005; 83: 1377–83.
221. Tesarik J, Mendoza C. Nongenomic effects of 17 beta-estradiol on maturing human oocytes: Relationship to oocyte developmental potential. *J Clin Endocrinol Metab* 1995; 80: 1438–43.
222. Mason HD, Martikainen H, Beard RW, Anyaoku V, Franks S. Direct gonadotrophic effect of growth hormone on oestradiol production by human granulosa cells *in vitro*. *J Endocrinol* 1990; 126: R1–4.
223. Lanzone A, Fortini A, Fulghesu AM, Soranna L, Caruso A, Mancuso S. Growth hormone enhances estradiol production follicle-stimulating hormone-induced in the early stage of the follicular maturation. *Fertil Steril* 1996; 66: 948–53.
224. Mendoza C, Ruiz-Requena E, Ortega E, Cremades N, Martinez F, Bernabeu R, Greco E, Tesarik J. Follicular fluid markers of oocyte developmental potential. *Hum Reprod* 2002; 17: 1017–22.
225. Tesarik J, Mendoza C. Direct non-genomic effects of follicular steroids on maturing human oocytes: Oestrogen versus androgen antagonism. *Hum Reprod Update* 1997; 3: 95–100.
226. Murdoch WJ, Van Kirk EA. Estrogenic upregulation of DNA polymerase beta in oocytes of preovulatory ovine follicles. *Mol Reprod Dev* 2001; 58: 417–23.
227. Zelinski-Wooten MB, Hess DL, Baughman WL, Molskness TA, Wolf DP, Stouffer RL. Administration of an aromatase inhibitor during the late follicular phase of gonadotropin-treated cycles in rhesus monkeys: Effects on follicle development, oocyte maturation, and subsequent luteal function. *J Clin Endocrinol Metab* 1993; 76: 988–95.
228. Lisi F, Rinaldi L, Fishel S, Lisi R, Pepe G, Picconeri MG, Campbell A, Rowe P. Use of recombinant FSH and recombinant LH in multiple follicular stimulation for IVF: A preliminary study. *Reprod Biomed Online* 2001; 3: 190–4.
229. Ferraretti AP, Gianaroli L, Magli MC, D'Angelo A, Farfalli V, Montanaro N. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. *Fertil Steril* 2004; 82: 1521–6.
230. Richards JS, Jahnsen T, Hedin L, Lifka J, Ratoosh S, Durica JM, Goldring NB. Ovarian follicular development: From physiology to molecular biology. *Recent Prog Horm Res* 1987; 43: 231–76.
231. Robertson DM, Burger HG. Reproductive hormones: Ageing and the perimenopause. *Acta Obstet Gynecol Scand* 2002; 81: 612–6.
232. Piltonen T, Koivunen R, Ruokonen A, Tapanainen JS. Ovarian age-related responsiveness to human chorionic gonadotropin. *J Clin Endocrinol Metab* 2003; 88: 3327–32.
233. Vihko KK, Kujansuu E, Morsky P, Huhtaniemi I, Punnonen R. Gonadotropins and gonadotropin receptors during the perimenopause. *Eur J Endocrinol* 1996; 134: 357–61.
234. Kim YK, Wasser SK, Fujimoto VY, Klein NA, Moore DE, Soules MR. Utility of follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol and FSH:LH ratio in predicting reproductive age in normal women. *Hum Reprod* 1997; 12: 1152–5.
235. Mitchell R, Hollis S, Rothwell C, Robertson WR. Age related changes in the pituitary–testicular axis in normal men; lower serum testosterone results from decreased bioactive LH drive. *Clin Endocrinol (Oxf)* 1995; 42: 501–7.
236. Huhtaniemi IT, Pettersson KS. Alterations in gonadal steroidogenesis in individuals expressing a common genetic variant of luteinizing hormone. *J Steroid Biochem Mol Biol* 1999; 69: 281–5.
237. De Placido G, Mollo A, Alviggi C, Strina I, Varricchio MT, Ranieri A, Colacurci N, Tolino A, Wilding M. Rescue of IVF cycles by HMG in pituitary down-regulated normogonadotrophic young women characterized by a poor initial response to recombinant FSH. *Hum Reprod* 2001; 16: 1875–9.
238. Loutradis D, Drakakis P, Milingos S, Stefanidis K, Michalas S. Alternative approaches in the management of poor response in controlled ovarian hyperstimulation (COH). *Ann N Y Acad Sci* 2003; 997: 112–9.
239. Gomez-Palomares JL, Acevedo-Martin B, Andres L, Ricciarelli E, Hernandez ER. LH improves early follicular recruitment in women over 38 years old. *Reprod Biomed Online* 2005; 11: 409–14.

240. Alviggi C, Mollo A, Clarizia R, De Placido G. Exploiting LH in ovarian stimulation. *Reprod Biomed Online* 2006; 12: 221–33.
241. Nilsson C, Pettersson K, Millar RP, Coerver KA, Matzuk MM, Huhtaniemi IT. Worldwide frequency of a common genetic variant of luteinizing hormone: An international collaborative research. International Collaborative Research Group. *Fertil Steril* 1997; 67: 998–1004.
242. de Castro F, Moron FJ, Montoro L, Galan JJ, Hernandez DP, Padilla ES, Ramirez-Lorca R, Real LM, Ruiz A. Human controlled ovarian hyperstimulation outcome is a polygenic trait. *Pharmacogenetics* 2004; 14: 285–93.
243. Loutradis D, Elsheikh A, Kallianidis K, Drakakis P, Stefanidis K, Milingos S, Michalas S. Results of controlled ovarian stimulation for ART in poor responders according to the short protocol using different gonadotrophins combinations. *Arch Gynecol Obstet* 2004; 270: 223–6.
244. Laml T, Obruca A, Fischl F, Huber JC. Recombinant luteinizing hormone in ovarian hyperstimulation after stimulation failure in normogonadotropic women. *Gynecol Endocrinol* 1999; 13: 98–103.
245. Ferrari B, Barusi L, Coppola F. Clinical and endocrine effects of ovulation induction with FSH and hCG supplementation in low responders in the midfollicular phase. A pilot study. *J Reprod Med* 2002; 47: 137–43.
246. Griesinger G, Shapiro DB, Kolibianakis EM, Witjes H, Mannaerts BM. No association between endogenous LH and pregnancy in a GnRH antagonist protocol: Part II, recombinant FSH. *Reprod Biomed Online* 2011; 23: 457–65.
247. Chung K, Krey L, Katz J, Noyes N. Evaluating the role of exogenous luteinizing hormone in poor responders undergoing *in vitro* fertilization with gonadotropin-releasing hormone antagonists. *Fertil Steril* 2005; 84: 313–8.
248. Barrenetxea G, Agirregoikoa JA, Jimenez MR, Lopez de Larruzea A, Ganzabal T, Carbonero K. Ovarian response and pregnancy outcome in poor-responder women: A randomized controlled trial on the effect of luteinizing hormone supplementation on *in vitro* fertilization cycles. *Fertil Steril* 2008; 89: 546–53.
249. Humaidan P, Schertz J, Fischer R. Efficacy and Safety of Pergoveris in Assisted Reproductive Technology—ESPART: Rationale and design of a randomised controlled trial in poor ovarian responders undergoing IVF/ICSI treatment. *BMJ Open* 2015; 7: e008297.
250. Adashi EY. The ovarian follicular apparatus. In: *Reproductive Endocrinology, Surgery and Technology*. Adashi EY, Rock J, Rosenwaks Z (eds). Philadelphia, PA: Lippincott-Raven, 1996, pp. 7–40.
251. Weil SJ, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J, Bondy CA. Androgen receptor gene expression in the primate ovary: Cellular localization, regulation, and functional correlations. *J Clin Endocrinol Metab* 1998; 83: 2479–85.
252. Hillier SG, Tetsuka M. Role of androgens in follicle maturation and atresia. *Baillieres Clin Obstet Gynaecol* 1997; 11: 249–60.
253. Hillier SG. Gonadotropic control of ovarian follicular growth and development. *Mol Cell Endocrinol* 2001; 179: 39–46.
254. Hugues JN, Cédrin Durnerin I. Impact of androgens on fertility—Physiological, clinical and therapeutic aspects. *Reprod Biomed Online* 2005; 11: 570–80.
255. Piltonen T, Koivunen R, Perheentupa A, Morin-Papunen L, Ruokonen A, Tapanainen JS. Ovarian age-related responsiveness to human chorionic gonadotropin in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2004; 89: 3769–75.
256. Guay A, Munarriz R, Jacobson J, Talakoub L, Traish A, Quirk F, Goldstein I, Spark R. Serum androgen levels in healthy premenopausal women with and without sexual dysfunction: Part A. Serum androgen levels in women aged 20–49 years with no complaints of sexual dysfunction. *Int J Impot Res* 2004; 16: 112–20.
257. Frattarelli JL, Peterson EH. Effect of androgen levels on *in vitro* fertilization cycles. *Fertil Steril* 2004; 81: 1713–4.
258. Barbieri RL, Sluss PM, Powers RD, McShane PM, Vitonis A, Ginsburg E, Cramer DC. Association of body mass index, age, and cigarette smoking with serum testosterone levels in cycling women undergoing *in vitro* fertilization. *Fertil Steril* 2005; 83: 302–8.
259. Massin N, Cedrin-Durnerin I, Coussieu C, Galey-Fontaine J, Wolf JP, Hugues JN. Effects of transdermal testosterone application on the ovarian response to FSH in poor responders undergoing assisted reproduction technique—A prospective, randomized, double-blind study. *Hum Reprod* 2006; 21: 1204–11.
260. Balasch J, Fabregues F, Penarrubia J, Carmona F, Casamitjana R, Creus M, Manau D, Casals G, Vanrell JA. Pretreatment with transdermal testosterone may improve ovarian response to gonadotrophins in poor-responder IVF patients with normal basal concentrations of FSH. *Hum Reprod* 2006; 21: 1884–93.
261. Kim CH, Howles CM, Lee HA. The effect of transdermal testosterone gel pretreatment on controlled ovarian stimulation and IVF outcome in low responders. *Fertil Steril* 2011; 95: 679–83.
262. Casson PR, Lindsay MS, Pisarska MD, Carson SA, Buster JE. Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: A case series. *Hum Reprod* 2000; 15: 2129–32.
263. Barad D, Brill H, Gleicher N. Update on the use of dehydroepiandrosterone supplementation among women with diminished ovarian function. *J Assist Reprod Genet* 2007; 24: 629–34.
264. Barad D, Gleicher N. Effect of dehydroepiandrosterone on oocyte and embryo yields, embryo grade and cell number in IVF. *Hum Reprod* 2006; 21: 2845–9.

265. Barad DH, Gleicher N. Increased oocyte production after treatment with dehydroepiandrosterone. *Fertil Steril* 2005; 84: 756.
266. Gleicher N, Barad DH. Dehydroepiandrosterone (DHEA) supplementation in diminished ovarian reserve (DOR). *Reprod Biol Endocrinol* 2011; 9: 67.
267. Gleicher N, Ryan E, Weghofer A, Blanco-Mejia S, Barad DH. Miscarriage rates after dehydroepiandrosterone (DHEA) supplementation in women with diminished ovarian reserve: A case control study. *Reprod Biol Endocrinol* 2009; 7: 108.
268. Gleicher N, Weghofer A, Barad DH. Dehydroepiandrosterone (DHEA) reduces embryo aneuploidy: Direct evidence from preimplantation genetic screening (PGS). *Reprod Biol Endocrinol* 2010; 8: 140–144.
269. Gleicher N, Weghofer A, Barad DH. Improvement in diminished ovarian reserve after dehydroepiandrosterone supplementation. *Reprod Biomed Online* 2010; 21: 360–5.
270. Sonmezer M, Ozmen B, Cil AP, Ozkavukcu S, Tasci T, Olmus H, Atabekoglu CS. Dehydroepiandrosterone supplementation improves ovarian response and cycle outcome in poor responders. *Reprod Biomed Online* 2009; 19: 508–13.
271. Wiser A, Gonen O, Ghetler Y, Shavit T, Berkovitz A, Shulman A. Addition of dehydroepiandrosterone (DHEA) for poor-responder patients before and during IVF treatment improves the pregnancy rate: A randomized prospective study. *Hum Reprod* 2010; 25: 2496–500.
272. Kara M, Aydin T, Aran T, Turktekin N, Ozdemir B. Does dehydroepiandrosterone supplementation really affect IVF–ICSI outcome in women with poor ovarian reserve? *Eur J Obstet Gynecol Reprod Biol* 2014; 173: 63–5.
273. Yeung T, Chai J, Li R, Lee V, Ho PC, Ng E. A randomized, controlled, pilot trial on the effect of dehydroepiandrosterone on ovarian response markers, ovarian response, and *in vitro* fertilization outcomes in poor responders. *Fertil Steril* 2014; 102: 108–15.
274. Sunkara SK, Coomarasamy A, Arlt W, Bhattacharya S. Should androgen supplementation be used for poor ovarian response in IVF? *Hum Reprod* 2012; 3: 637–40.
275. Mitwally MF, Casper RF. Aromatase inhibition improves ovarian response to follicle-stimulating hormone in poor responders. *Fertil Steril* 2002; 77: 776–80.
276. Garcia-Velasco JA, Moreno L, Pacheco A, Guillen A, Duque L, Requena A, Pellicer A. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves *in vitro* fertilization outcome in low responder patients: A pilot study. *Fertil Steril* 2005; 84: 82–7.
277. Ozmen B, Sonmezer M, Atabekoglu CS, Olmus H. Use of aromatase inhibitors in poor-responder patients receiving GnRH antagonist protocols. *Reprod Biomed Online* 2009; 19: 478–85.
278. Lee VC, Chan CC, Ng EH, Yeung WS, Ho PC. Sequential use of letrozole and gonadotrophin in women with poor ovarian reserve: A randomized controlled trial. *Reprod Biomed Online* 2011; 23: 380–8.
279. Schoolcraft WB, Surrey ES, Minjarez DA, Stevens JM, Gardner DK. Management of poor responders: Can outcomes be improved with a novel gonadotropin-releasing hormone antagonist/letrozole protocol? *Fertil Steril* 2008; 89: 151–6.
280. Davar R, Oskouian H, Ahmadi S, Firouzabadi RD. GnRH antagonist/letrozole versus microdose GnRH agonist flare protocol in poor responders undergoing *in vitro* fertilization. *Taiwan J Obstet Gynecol* 2010; 49: 297–301.
281. Sunkara SK, Pundir J, Khalaf Y. Effect of androgen supplementation or modulation on ovarian stimulation outcome in poor responders: A meta-analysis. *Reprod Biomed Online* 2011; 22: 545–55.
282. Shoham Z, Homburg R, Owen EJ, Conway GS, Ostergaard H, Jacobs HS. The role of treatment with growth hormone in infertile patients. *Baillieres Clin Obstet Gynaecol* 1991; 6: 267–81.
283. Owen EJ, Shoham Z, Mason BA, Ostergaard H, Jacobs HS. Cotreatment with growth hormone, after pituitary suppression, for ovarian stimulation in *in vitro* fertilization: A randomized, double-blind, placebo-control trial. *Fertil Steril* 1991; 56: 1104–10.
284. Adashi EY, Resnick CE, Hurwitz A, Ricciarelli E, Hernandez ER, Roberts CT, Leroith D, Rosenfeld R. Insulin-like growth factors: The ovarian connection. *Hum Reprod* 1991; 6: 1213–9.
285. Adashi EY, Resnick CE, Hernandez ER, Hurwitz A, Roberts CT, Leroith D, Rosenfeld R. Insulin-like growth factor I as an intraovarian regulator: Basic and clinical implications. *Ann N Y Acad Sci* 1991; 626: 161–8.
286. Abir R, Garor R, Felz C, Nitke S, Krissi H, Fisch B. Growth hormone and its receptor in human ovaries from fetuses and adults. *Fertil Steril* 2008; 90: 1333–9.
287. Mendoza C, Cremades N, Ruiz-Requena E, Martinez F, Ortega E, Bernabeu S, Tesarik J. Relationship between fertilization results after intracytoplasmic sperm injection, and intrafollicular steroid, pituitary hormone and cytokine concentrations. *Hum Reprod* 1999; 14: 628–35.
288. Ibrahim ZH, Matson PL, Buck P, Lieberman BA. The use of biosynthetic human growth hormone to augment ovulation induction with buserelin acetate/human menopausal gonadotropin in women with a poor ovarian response. *Fertil Steril* 1991; 55: 202–4.
289. Wu MY, Chen HF, Ho HN, Chen SU, Chao KH, Huang SC, Lee TY, Yang YS. The value of human growth hormone as an adjuvant for ovarian stimulation in a human *in vitro* fertilization program. *J Obstet Gynaecol Res* 1996; 22: 443–50.

290. Busacca M, Fusi FM, Brigante C, Bonzi V, Gonfiantini C, Vignali M, Ferrari A. Use of growth hormone-releasing factor in ovulation induction in poor responders. *J Reprod Med* 1996; 41: 699–703.
291. Kim CH, Chae HD, Chang YS. Pyridostigmine cotreatment for controlled ovarian hyperstimulation in low responders undergoing *in vitro* fertilization-embryo transfer. *Fertil Steril* 1999; 71: 652–7.
292. Howles CM, Loumaye E, Germond M, Yates R, Brinsden P, Healy D, Bonaventura LM, Strowitzki T. Does growth hormone-releasing factor assist follicular development in poor responder patients undergoing ovarian stimulation for *in-vitro* fertilization? *Hum Reprod* 1999; 14: 1939–43.
293. Suikkari A, MacLachlan V, Koistinen R, Seppala M, Healy D. Double-blind placebo controlled study: Human biosynthetic growth hormone for assisted reproductive technology. *Fertil Steril* 1996; 65: 800–5.
294. Shaker AG, Fleming R, Jamieson ME, Yates RW, Coutts JR. Absence of effect of adjuvant growth hormone therapy on follicular responses to exogenous gonadotropins in women: Normal and poor responders. *Fertil Steril* 1992; 58: 919–23.
295. Hughes SM, Huang ZH, Morris ID, Matson PL, Buck P, Lieberman BA. A double-blind cross-over controlled study to evaluate the effect of human biosynthetic growth hormone on ovarian stimulation in previous poor responders to *in-vitro* fertilization. *Hum Reprod* 1994; 9: 13–8.
296. Dor J, Seidman DS, Amudai E, Bider D, Levran D, Mashiach S. Adjuvant growth hormone therapy in poor responders to *in-vitro* fertilization: A prospective randomized placebo-controlled double-blind study. *Hum Reprod* 1995; 10: 40–3.
297. Younis JS, Simon A, Koren R, Dorembus D, Schenker JG, Laufer N. The effect of growth hormone supplementation on *in vitro* fertilization outcome: A prospective randomized placebo-controlled double-blind study. *Fertil Steril* 1992; 58: 575–80.
298. Tesarik J, Hazout A, Mendoza C. Improvement of delivery and live birth rates after ICSI in women aged >40 years by ovarian co-stimulation with growth hormone. *Hum Reprod* 2005; 20: 2536–41.
299. Kucuk T, Kozinoglu H, Kaba A. Growth hormone co-treatment within a GnRH agonist long protocol in patients with poor ovarian response: A prospective, randomized, clinical trial. *J Assist Reprod Genet* 2008; 25: 123–7.
300. Yovich JL, Stanger JD. Growth hormone supplementation improves implantation and pregnancy productivity rates for poor-prognosis patients undertaking IVF. *Reprod Biomed Online* 2010; 21: 37–49.
301. Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing *in vitro* fertilization: A systematic review and meta-analysis. *Fertil Steril* 2009; 91: 749–66.
302. Kolibianakis EM, Venetis CA, Diedrich K, Tarlatzis BC, Griesinger G. Addition of growth hormone to gonadotrophins in ovarian stimulation of poor responders treated by *in-vitro* fertilization: A systematic review and meta-analysis. *Hum Reprod Update* 2009; 15: 613–22.
303. Gonen Y, Jacobson W, Casper RF. Gonadotropin suppression with oral contraceptives before *in vitro* fertilization. *Fertil Steril* 1990; 53: 282–7.
304. Biljan MM, Mahutte NG, Dean N, Hemmings R, Bissonnette F, Tan SL. Effects of pretreatment with an oral contraceptive on the time required to achieve pituitary suppression with gonadotropin-releasing hormone analogues and on subsequent implantation and pregnancy rates. *Fertil Steril* 1998; 70: 1063–9.
305. Lindheim SR, Barad DH, Witt B, Ditkoff E, Sauer MV. Short-term gonadotropin suppression with oral contraceptives benefits poor responders prior to controlled ovarian hyperstimulation. *J Assist Reprod Genet* 1996; 13: 745–7.
306. Kovacs P, Barg PE, Witt BR. Hypothalamic–pituitary suppression with oral contraceptive pills does not improve outcome in poor responder patients undergoing *in vitro* fertilization-embryo transfer cycles. *J Assist Reprod Genet* 2001; 18: 391–4.
307. Fanchin R, Mendez Lozano DH, Schonauer LM, Cunha-Filho JS, Frydman R. Hormonal manipulations in the luteal phase to coordinate subsequent antral follicle growth during ovarian stimulation. *Reprod Biomed Online* 2005; 10: 721–8.
308. Fanchin R, Salomon L, Castelo-Branco A, Olivennes F, Frydman N, Frydman R. Luteal estradiol pretreatment coordinates follicular growth during controlled ovarian hyperstimulation with GnRH antagonists. *Hum Reprod* 2003; 18: 2698–703.
309. Fanchin R, Cunha-Filho JS, Schonauer LM, Kadoch IJ, Cohen-Bacri P, Frydman R. Coordination of early antral follicles by luteal estradiol administration provides a basis for alternative controlled ovarian hyperstimulation regimens. *Fertil Steril* 2003; 79: 316–21.
310. Reynolds KA, Omurtag KR, Jimenez PT, Rhee JS, Tuuli MG, Jungheim ES. Cycle cancellation and pregnancy after luteal estradiol priming in women defined as poor responders: A systematic review and meta-analysis. *Hum Reprod* 2013; 11: 2981–9.
311. Fanchin R, Castelo Branco A, Kadoch IJ, Hosny G, Bagirova M, Frydman R. Premenstrual administration of gonadotropin-releasing hormone antagonist coordinates early antral follicle sizes and sets up the basis for an innovative concept of controlled ovarian hyperstimulation. *Fertil Steril* 2004; 81: 1554–9.
312. Rombauts L, Suikkari AM, MacLachlan V, Trounson AO, Healy DL. Recruitment of follicles by recombinant human follicle-stimulating hormone commencing in the luteal phase of the ovarian cycle. *Fertil Steril* 1998; 69: 665–9.

313. Dragisic KG, Davis OK, Fasouliotis SJ, Rosenwaks Z. Use of a luteal estradiol patch and a gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation for *in vitro* fertilization in poor responders. *Fertil Steril* 2005; 84: 1023–6.
314. Frattarelli JL, Hill MJ, McWilliams GD, Miller KA, Bergh PA, Scott RT, Jr. A luteal estradiol protocol for expected poor-responders improves embryo number and quality. *Fertil Steril* 2008; 89: 1118–22.
315. DiLuigi AJ, Engmann L, Schmidt DW, Benadiva CA, Nulsen JC. A randomized trial of microdose leuprolide acetate protocol versus luteal phase ganirelix protocol in predicted poor responders. *Fertil Steril* 2011; 95: 2531–3.
316. Weitzman VN, Engmann L, DiLuigi A, Maier D, Nulsen J, Benadiva C. Comparison of luteal estradiol patch and gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation versus microdose gonadotropin-releasing hormone agonist protocol for patients with a history of poor *in vitro* fertilization outcomes. *Fertil Steril* 2009; 92: 226–30.
317. Shastri SM, Barbieri E, Kligman I, Schoyer KD, Davis OK, Rosenwaks Z. Stimulation of the young poor responder: Comparison of the luteal estradiol/gonadotropin-releasing hormone antagonist priming protocol versus oral contraceptive microdose leuprolide. *Fertil Steril* 2011; 95: 592–5.
318. Elassar A, Mann JS, Engmann L, Nulsen J, Benadiva C. Luteal phase estradiol versus luteal phase estradiol and antagonist protocol for controlled ovarian stimulation before *in vitro* fertilization in poor responders. *Fertil Steril* 2011; 95: 324–6.
319. Elassar A, Engmann L, Nulsen J, Benadiva C. Letrozole and gonadotropins versus luteal estradiol and gonadotropin-releasing hormone antagonist protocol in women with a prior low response to ovarian stimulation. *Fertil Steril* 2011; 95: 2330–4.
320. Kuang Y, Chen Q, Hong Q, Lyu Q, Ai A, Fu Y, Shoham Z. Double stimulations during the follicular and luteal phases of poor responders in IVF/ICSI programmes (Shanghai protocol). *Reprod Biomed Online* 2014; 6: 684–91.
321. Cobo A, Kuwayama M, Pérez S, Ruiz A, Pellicer A, Remohí J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. *Fertil Steril* 2008; 6: 1657–64.
322. Rienzi L, Romano S, Albricci L, Maggiulli R, Capalbo A, Baroni E, Colamaria S, Sapienza F, Ubaldi F. Embryo development of fresh "versus" vitrified metaphase II oocytes after ICSI: A prospective randomized sibling-oocyte study. *Hum Reprod* 2010; 1: 66–73.
323. Cobo A, Garrido N, Crespo J, José R, Pellicer A. Accumulation of oocytes: A new strategy for managing low-responder patients. *Reprod Biomed Online* 2012; 4: 424–32.
324. Sudo A, Kudo M, Wada S et al. Genetic and functional analyses of polymorphisms in women in the human FSH receptor gene. *Mol Hum Reprod* 2002; 8: 89–90.
325. Laven JS, Mulders AG, Suryandari DA et al. Follicle-stimulating hormone receptor polymorphisms in women with normogonadotropic anovulatory infertility. *Fertil Steril* 2003; 80: 986–92.
326. De Castro F, Moron FJ, Montoro L et al. Pharmacogenetics of controlled ovarian hyperstimulation. *Pharmacogenomics* 2005; 6: 629–37.
327. Daelemans C, Smits G, de Maertelaer V et al. Prediction of severity of symptoms in iatrogenic ovarian hyperstimulation syndrome by follicle-stimulating hormone receptor Ser680Asn polymorphism. *J Clin Endocrinol Metab* 2004; 89: 6310–15.
328. Choi D, Lee EY, Yoon S et al. Clinical correlation of cycline D2 mRNA expression in human luteinized granulosa cells. *J Assist Reprod Genet* 2000; 17: 574–9.

Recurrent implantation failure

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OVERVIEW

Human reproduction is inefficient, as evidenced by a mean delivery rate of 37.3% per fresh embryo transfer in the U.S.A. in 2013 (1). Despite advances in assisted reproductive technologies over the past four decades, patients remain who fail to achieve live births following multiple *in vitro* fertilization (IVF)/embryo transfer cycles. Although delivery rates have improved, in the majority of failed implantation cycles, no identifiable etiology is found. Thus, isolating a specific cause for repeated implantation failure can be challenging. Patient age, genetic constitution of embryos, culture conditions, and endometrial receptivity have all been implicated in recurrent implantation failure (RIF).

Maternal age remains the single most important variable in predicting successful implantation. Advanced female age is not only associated with an increase in embryo aneuploidy, but also parallels a decline in ovarian reserve and response to gonadotropin stimulation. Although ovarian reserve testing can help anticipate response to gonadotropin stimulation and may correlate with success rates, it falls short of predicting cycle outcome.

Embryos have a high attrition rate in the laboratory and following implantation, owing mostly to genetic abnormalities. *In vitro*, this rate could be amplified by culture conditions as well as perturbations associated with handling and exposure to ambient air. The introduction of closed incubator systems utilizing continuous time-lapse monitoring of embryos in stable, non-disturbed culture conditions may mitigate the impact of an artificial laboratory environment on embryos. Despite these advances in laboratory conditions, it is reasonable to assume that some degree of embryonic loss may be due to the artificial environment. Once embryos have been selected based on developmental competence, embryonic loss continues after transfer, often necessitating multiple embryos to be replaced in order to achieve a singleton pregnancy.

RIF is generally defined by the absence of implantation after three or more transfers of high-quality embryos, or after transfer of >10 high-quality embryos in multiple cycles. Although this definition may be somewhat arbitrary, it can serve as a guide to demarcating this difficult group of challenging patients. This chapter summarizes specific causes of RIF as well as treatment strategies designed to improve the efficiency of embryo implantation.

PARENTAL GENETICS

Translocations represent a variety of rearrangements between non-homologous chromosomes. They can be reciprocal, whereby two non-homologous chromosomes exchange segments, or Robertsonian, when two acrocentric chromosomes break at their centromere to fuse as a single, large chromosome with the loss of the two short arms. Parental translocations often affect the pattern of segregation during meiosis, resulting in a variety of aneuploidies depending upon which chromosomes are involved as well as the size of the rearrangement.

Although only a small portion of couples with RIF will have abnormal karyotypes, the incidence of parental chromosomal abnormalities is reported to be 2.5% (2). By comparison, the incidence of translocation carriers in couples with recurrent pregnancy loss was found to be 4.7%. In a study examining extreme cases of RIF (either six or more failed IVF attempts or 15 or more transferred embryos), 10 of 65 couples (15.4%) were noted to have either chromosomal translocations, mosaicism, inversions, or deletions (3).

In light of these observations, parental karyotypes should be performed in couples with RIF. Couples with known translocations should be carefully counseled with the aim of providing advice regarding preimplantation genetic diagnosis (PGD).

BLASTOCYST CULTURE

Identification of embryos with a higher implantation potential is key to improving the efficiency of IVF. It is widely acknowledged that cleavage-stage embryo development is controlled by maternal RNA transcripts until the four- to eight-cell stage (4). Activation of the embryonic genome begins on day 3 of development and continues to the blastocyst stage, a stage that confers a higher implantation potential than cleavage-stage embryos. Early attempts to culture embryos to the blastocyst stage used monophasic cultures with unsatisfactory blastulation rates. Sequential culture systems evolved in the 1990s with an increased understanding of physiologic conditions *in vivo*. The first culture stage (pronuclear stage to compaction) consists of non-essential amino acids, ethylenediaminetetraacetic acid (EDTA), and pyruvate and a reduced glucose concentration. The second culture stage (compaction to blastocyst) adds essential amino acids, removes EDTA, reduces the pyruvate concentration, and increases the glucose concentration to meet the increased energy demands of the embryo during rapid cell division. Sequential culture media thus facilitates the selection of embryos most suitable for transfer. Recently, global culture media have been developed that are also suitable for efficient blastocyst development.

While a high proportion of embryos fail to form blastocysts due to genetic aneuploidy, a subset of embryos arrest at the cleavage stage due to suboptimal culture conditions. Earlier studies suggesting a higher implantation rate with blastocyst transfers may have used select

patient populations with favorable prognoses for implantation. The ideal candidates for blastocyst transfer are high ovarian responders to gonadotropins who create excess embryos, allowing one to select the best available blastocysts to enhance implantation rates. Conversely, marginal or poor responders with limited numbers of embryos are not good candidates for prolonged culture conditions as they may arrest at cleavage stages prior to transfer. Most patients with RIF fall into the latter category where prolonged culture of a small number of embryos appears to offer no significant advantages. For those who are high responders, prolonged culture conditions may improve the implantation rate and clinical success.

EMBRYO GENETICS

A direct correlation between female age and oocyte aneuploidy exists, with the steepest rise in aneuploidy occurring in the late 30s and early 40s. This is based on cytogenetic analysis of products of conception from first trimester miscarriages, as well as aneuploidy assessment of biopsied embryos (5). In original studies using fluorescent *in situ* hybridization (FISH) to diagnose numeric abnormalities of X, Y, 18, 13, and 21, Munne (6) noted aneuploidy rates for these five chromosomes to be 37% for women aged 40–47 years. With the advent of comparative genomic hybridization (CGH) for all 24 chromosomes, aneuploidy rates have been found to range from 58% at 40 years of age to 100% at 47 years of age (7). This underscores the importance of female age in predicting the implantation potential of embryos.

Over the past 20 years, methods have evolved to detect aneuploidy in embryos from women undergoing IVF. The purpose of these techniques is to increase implantation and live birth rates, as well as to reduce the risk for spontaneous abortion and associated chromosomal abnormalities discovered at birth. Biopsy and FISH analysis of cleavage-stage embryos has largely been abandoned, owing to a high rate of mosaicism at day 3 and self-correction capabilities that led to misdiagnosis of aneuploidy. In 2007, Mastenbroek et al. reported on a multicenter randomized controlled trial that highlighted the limitations of day-3 biopsy with FISH (8). Women aged 35–41 years were randomized to three cycles of IVF with and without preimplantation genetic screening (PGS). In total, there were 434 PGS cycles and 402 control cycles. The ongoing pregnancy rate was lower in the PGS group (25%) when compared to the control group (37%), as was the live birth rate (24% vs. 35%). Subsequent meta-analyses of randomized controlled trials confirmed that there was a detrimental impact of single day-3 blastomere biopsy for women with advanced maternal age.

As embryo culture techniques and the efficiency of reaching the blastocyst stage *in vitro* have improved, PGS by trophectoderm biopsy has witnessed a resurgence. CGH is a molecular technique that allows for an entire genome to be scanned for variations in DNA copy number. Total genomic DNA is isolated and compared to a reference cell, differentially labeled, and hybridized, allowing for comparison of all 24 chromosomes in a single interphase cell (9). This technique necessitates cryopreservation of blastocysts and transfer in a subsequent cycle. Despite these new techniques for detecting aneuploidy, it is unclear whether RIF is correlated with a higher proportion of aneuploid embryos, or indeed whether these patients benefit from PGS.

Specific trials have looked at the outcomes of PGD for aneuploidy screening in RIF patients. An early study by Pehlivan et al. compared 49 RIF patients with nine fertile controls. Day-3 blastomeres were tested by FISH for chromosomes 13, 16, 18, 21, 22, X, and Y. A higher rate of aneuploidy (67.4% vs. 36.3%) was observed in patients with RIF compared to age-matched controls. The pregnancy rates between the RIF group and controls were comparable (34.0% vs. 33.1%) (10). In a similar study, Gianaroli et al. found that the percentage of chromosomally abnormal embryos increased proportionally with the number of prior IVF failures (11). A recent study utilizing 24-chromosome array CGH analysis of blastocysts failed to demonstrate a difference in 43 RIF patients compared to 45 infertile, good-prognosis patients (aneuploidy 53.8% vs. 48.2%, respectively) (12). Thus, further studies are needed to assess whether embryonic aneuploidy is responsible for RIF in the absence of parental chromosomal abnormalities.

Prospective data examining whether PGS benefits patients with RIF are mixed, and largely rely on data from older FISH technologies. Gianaroli et al. reported a significant improvement in implantation (28.0% vs. 11.9%) in a small prospective study when day-3 biopsy and FISH analysis were employed (11). The same group confirmed their findings in a randomized trial that again included patients with three or more prior IVF failures (13). Conversely, other studies have suggested a negative impact of PGS for RIF patients, with significantly lower live birth rates after biopsy (21%) as compared to no biopsy (39%) (14). Indeed, the negative impact of PGS in individuals with RIF was confirmed by a recent meta-analysis (15).

The role for PGS in patients with RIF remains to be determined, especially given the recent evolution in diagnostic techniques. The potential inaccuracy of FISH-based technology and the developmental susceptibility of day-3 embryos to injury incurred by biopsy might have explained the prior failure to demonstrate benefit for IVF patients (16–18). Trophectoderm biopsy has several advantages over blastomere biopsy, including greater developmental resiliency, less mosaicism, and the ability to analyze multiple cells. Moreover, the greater accuracy of 24-chromosome analysis has been validated (19). Blastocyst-based PGS has been suggested to improve the efficiency of IVF in older individuals (>40 years of age) undergoing IVF (20). Another retrospective study claimed a benefit in implantations and live births when PGS was used in women aged 40–43 years with multiple prior IVF failures, with a live birth rate for PGS frozen embryo transfer (45.5%) being significantly greater than for fresh transfer without PGS (15.8%) or frozen transfer of non-PGS embryos (19.0%) (21).

The benefit of blastocyst biopsy with 24-chromosome PGS is to reduce the incidence of viable trisomies and spontaneous pregnancy loss, both of which affect older patients (>38 years of age) disproportionately. The benefit of PGS for young patients, and specifically young patients with RIF, is less established, and is an area deserving of careful study. PGS should be considered for patients experiencing RIF, as it may provide some important information regarding the incidence of aneuploidy in these susceptible individuals. Biopsy and analysis does not, however, intrinsically increase the implantation potential of any given euploid embryo, and indeed adds cost, invasiveness, and the potential for discarding a normal embryo; thus, PGS should not be viewed as a reflex intervention for such patients.

SPERM GENETICS

Sperm concentration, motility, and morphological assessment are relatively poor predictors of conception with assisted reproductive technology. Recently, tests of sperm DNA integrity have been increasingly used for evaluating spermatozoa in conjunction with semen analyses. Several studies have provided evidence that sperm DNA damage is correlated with poor reproductive outcome, including increased pregnancy loss and chromosomal aneuploidy (22). Damage of sperm DNA has also been associated with poor development of embryos, and both animal and human studies have implied that failure to achieve conception may be associated with markedly elevated sperm DNA fragmentation (23–27). While elevated DNA fragmentation has been associated with an increase in miscarriage risk in spontaneous pregnancies, its role in patients with RIF remains uncertain (28,29). Standardization of techniques and thresholds for measuring sperm DNA fragmentation is lacking between assays and laboratories, making it difficult to validate the published literature.

There are several assays for measuring sperm DNA and chromatin damage. The most common are the sperm chromatin structure assay, single cell gel electrophoresis (COMET), terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL), and the sperm chromatin dispersion assay. Some assays have undergone more rigorous testing than others, and no assay is able to differentiate clinically important DNA damage from insignificant damage.

Sperm DNA damage is lower in the seminiferous tubules as compared to epididymal or ejaculated spermatozoa (30–32). In RIF couples in whom high DNA fragmentation has been documented, the use of testicular-retrieved spermatozoa has been suggested (30,33). Greco et al. studied 18 couples with at least two unsuccessful IVF/intracytoplasmic sperm injection (ICSI) attempts where male partners had ejaculated spermatozoa with >15% DNA damage by TUNEL assay (30). The incidence of DNA fragmentation in their testicular sperm (4.8%) was markedly lower as compared to ejaculated specimens from the same individuals (23.6%), with eight subsequent clinical pregnancies (44.4% clinical pregnancy rate) when testicular sperm was used for ICSI.

A number of techniques have been proposed to select spermatozoa with lower levels of DNA damage from ejaculated samples, including annexin-V columns, sperm hyaluronic acid binding, confocal light absorption scattering spectroscopy, and high-magnification ICSI (intracytoplasmic morphologically selected sperm injection [IMSI]) (34–37). IMSI has been proposed as a useful intervention for patients with RIF (37–40). Spermatozoa noted to have large vacuoles under high-power magnification have increased DNA defects (41–44). The use of IMSI to select spermatozoa that are free of vacuoles has been suggested to improve embryo quality at early cleavage stages and to potentially increase implantation and pregnancy (37,39). Subsequent studies, however, have failed to demonstrate different ongoing pregnancy, miscarriage, or live birth rates with IMSI when compared to conventional ICSI (45). A study specifically examining the use of IMSI (8400× magnification to select morphologically normal sperm) in 200 patients with RIF failed to demonstrate any significant benefits in terms of fertilization, implantation, or pregnancy rates when IMSI was employed as compared to conventional ICSI (40). Given the mixed nature of existing data, further studies are required to assess whether sperm DNA fragmentation testing is warranted in patients experiencing RIF. Moreover, studies are needed to further assess the optimal methods of testing, and whether testicular sperm extraction or IMSI offer any significant benefits over use of ICSI with ejaculated specimens.

Along with sperm DNA fragmentation, sperm aneuploidy may also play a role in RIF. Men with abnormal semen parameters have been noted to have increased aneuploidy rates in randomly collected semen samples. Burrello et al. sampled 48 consecutive male patients to evaluate aneuploidy rates in a swim up preparation used for ICSI. Sperm was evaluated with five-probe FISH and divided into two groups: those with sperm aneuploidy rates in the normal range versus a group with aneuploidy rates above the upper limit of normal (as determined by World Health Organization criteria). Men with lower sperm aneuploidy demonstrated higher implantation (34% vs. 13%) and pregnancy (75% vs. 34%) rates, as well as lower miscarriage rates (38% vs. 11%) (46). However, given that the individual tested sperm is unable to be used for fertilization, the clinical utility of sperm aneuploidy testing remains to be established.

UTERINE PATHOLOGY

An evaluation of the uterine cavity is warranted in patients who have experienced repeated IVF failures after transfer of high-quality embryos. Fibroids, polyps, intrauterine adhesions, chronic endometritis, or Mullerian anomalies have all been implicated in RIF. The incidence of previously unrecognized intrauterine pathology in individuals with RIF may be elevated; in fact, in some studies, it was as high as 25%–50% (47).

There are a number of theories regarding the mechanisms by which fibroids adversely affect implantation: mechanical obstruction of tubal ostia, chronic intracavitary inflammation, and increased uterine contractility are

the most commonly cited (48–50). It is generally accepted that subserous myomas do not adversely affect pregnancy or live birth rates, and thus removal is rarely warranted. It is equally agreed upon that submucous myomas decrease pregnancy rates and increase the incidence of miscarriage. For the majority of patients with submucous myomas, surgical resection restores pregnancy rates to match those of infertile women without myomas. While the benefits of submucous myoma resection are clear, the benefit of myomectomy for intramural myomas located outside of the endometrial cavity is more controversial (51,52). The largest published meta-analysis suggests a 21% relative reduction in live birth rates in women with non-cavity distorting intramural myomas as compared to women without myomas, but there is no clear evidence that removal of these fibroids leads to higher pregnancy rates (53).

Endometrial polyps can also diminish implantation rates (54). Multiple retrospective studies have reported improved spontaneous conception rates when endometrial polyps are resected (55–57). A randomized controlled trial of 215 infertile women with polyps that compared a group undergoing polypectomy with those undergoing a diagnostic hysteroscopy without intervention revealed that pregnancy was 2.1-times more likely (95% confidence interval [CI] 1.5–2.9) after polyp resection (58). Previous uterine instrumentation, especially those complicated with pelvic infection, should prompt investigation for intrauterine adhesions. In one study of patients with RIF undergoing hysteroscopy, the incidence of intrauterine adhesions was 8.5%. Available evidence suggests that subsequent surgical correction improves fertility outcomes (59–63).

Chronic endometritis should be excluded in patients with RIF without apparent cause. Several researchers have implicated endometrial inflammation as a potential etiology of RIF (64–66). The incidence of chronic endometritis based on histological evidence of plasma cells has been estimated to be as high as 30.3% in patients with RIF. Indeed, lower implantation rates have been noted in patients with evidence of endometritis (67). A recent study compared IVF outcomes in RIF patients after successful treatment of endometritis versus RIF patients in whom evidence of endometritis persisted despite three rounds of antibiotic treatment (67). Biopsies were performed in the follicular phase using a 3 mm curette attached to a 20 mL syringe, with samples divided into equal aliquots for culture and histologic analysis. Patients cultured positive for Gram-negative bacteria were treated with ciprofloxacin for 10 days, whereas those with Gram-positive bacteria were treated with an eight-day course of amoxicillin and clavulanate. Patients with histologic evidence of endometritis in the absence of positive cultures were treated with a single dose of intramuscular ceftriaxone followed by a 14-day course of oral doxycycline and metronidazole. Live birth rates were 60.8% in the patients in whom endometritis was successfully treated (based on negative repeat culture and histology) versus 13.3% for those in whom evidence of endometritis persisted after treatment.

Adenomyosis occurs when endometrial glandular cells invade the uterine myometrium. This condition should be considered to be a potential cause of RIF (68). While ultrasound findings can be suggestive of adenomyosis, magnetic resonance imaging (MRI) with contrast is the best diagnostic imaging modality. Though adenomyosis has been implicated as having a significant negative affect on female fertility, the condition is one of the least treatable of all uterine pathologies (69,70). Limited reports in patients with RIF have suggested successful treatments with ultra-long pituitary gonadotropin-releasing hormone agonist down-regulation prior to IVF, although the data are limited and further corroboration is needed (68).

Given the extent to which uterine pathology can be implicated in RIF, diagnostic investigation of the myometrium and endometrial cavity is warranted in all patients with RIF. Hysterosalpingogram, saline infusion sonography, three-dimensional ultrasonography, MRI, and hysteroscopy are all available for evaluation of uterine architecture and the endometrial cavity. Two prospective, randomized controlled trials have suggested a benefit of performing routine hysteroscopy in all patients with RIF, with reported detection rates of abnormal findings ranging between 25% and 50% (71,72). Some have argued that saline hysterography (SHG) offers similar detection rates with less invasiveness and cost; in one study, SHG detected all but one uterine abnormality, missing only a small endometrial polyp (73). Similarly, hysterosalpingography provides data on the endometrial cavity as well providing information on the status of the fallopian tubes (i.e., hydrosalpinges); however, on occasion, hysterosalpingography may miss small intrauterine lesions (74). A prospective study comparing vaginal sonogram, SHG, and diagnostic hysteroscopy concluded that hysteroscopy offered a more thorough detection of intracavitary lesions than SHG and transvaginal ultrasound (75).

Molecular or transcriptomic testing of the endometrium is being studied as a diagnostic strategy for women suffering from RIF. A study by Galgani et al. suggested that patients with RIF exhibited an increase in pro-inflammatory markers such as resistin, leptin, and IL-22 on mid-secretory endometrial biopsies, as well as altered T-lymphocytes (76). Endometrial prostaglandin synthesis has also been proposed to be aberrant in women with RIF (77). Work has now begun to catalog the transcriptomic profile of the endometrium throughout the menstrual cycle, allowing for the potential molecular identification of receptive endometria (78–82). Dysregulation of 313 genes and 63 transcripts in mid-secretory endometria of women with RIF as compared to fertile controls has recently been demonstrated (83,84).

Transcriptomic studies suggest that the endometrial expression profile in patients with RIF is altered as compared to fertile control subjects (85). The group from IVI has developed an endometrial receptivity array examining 238 endometrial genes, which is reportedly capable of identifying a receptive endometrium in both natural and stimulated cycles (86). The array has been reported

to be more accurate than traditional histology and to be reproducible within the same patient up to 40 months after the first analysis (87). Based on this, the group proposes that in some patients, the window of implantation is either advanced or delayed, such that a "one-size-fits-all" approach to timing of embryo transfer may not benefit rare patients whose transcriptomic endometrial profiles are altered. In a preliminary study, 85 RIF patients and 25 controls underwent endometrial sampling and transfer guided by endometrial receptivity array (ERA) results. A total of 74.1% of patients in the RIF group had a "receptive" result on initial ERA, as compared to 88% of control subjects. In 15 of 22 RIF patients with "non-receptive" ERA results, a second ERA demonstrated a displaced implantation window; 8 of these 15 patients subsequently conceived following embryo transfer timed according to the window of implantation identified by the second ERA (88). These preliminary results will require further confirmative studies. Until then, the routine use of the ERA should not be recommended.

TUBAL PATHOLOGY

In individuals with RIF, tubal pathology must also be excluded. The mechanisms whereby hydrosalpinges adversely affect reproduction are potentially multifactorial: accumulated tubal fluid may exert a direct embryotoxic effect, may act to mechanically flush an embryo from the uterus, or may adversely alter endometrial receptivity (89). Evidence suggests that live birth rates in patients with hydrosalpinges undergoing IVF are reduced (90–92). A direct effect on the endometrium was suggested in a study by Seli et al., in which deranged expression of leukemia inhibitory factor (LIF), an endometrial cytokine, was restored to normal following salpingectomy (93). Avb3 integrin expression is similarly restored following salpingectomy (94). A multicenter prospective randomized trial revealed pregnancy rates of 23.9% and live birth rates of 16.3% in IVF patients in whom hydrosalpinges were left untreated as compared to 36.6% and 28.6%, respectively, when salpingectomy was performed prior to IVF (91). The greatest effect was noted in women in whom hydrosalpinges were evident on transvaginal ultrasound.

Given the negative impact of tubal pathology, it is advisable to exclude hydrosalpinges in women with RIF, regardless of the initial infertility diagnosis.

Techniques

Assisted hatching

Embryos subjected to *in vitro* culture may experience zona hardening and subsequent lower rates of hatching and blastocyst expansion than occurs *in vivo* (95,96). Cleaved embryos with reduced zona thickness have higher implantation rates than those with thick zonae. Thus, it was suggested that either artificially opening or thinning of the zona could facilitate the hatching process (97–99). A variety of techniques have subsequently been developed to aid in the hatching process, including mechanical partial zona dissection, chemical drilling using acid Tyrode's solution, enzymatic thinning, laser-assisted hatching, and piezo micromanipulation (100–105). It has been proposed that such techniques not only aid in mechanical hatching, but also could enhance transport of nutrients from incubating media by allowing for a two-way exchange of metabolites (106).

Early prospective randomized controlled trials undertaken at our center suggested maximal benefit from assisted hatching in individuals over the age of 38 years, and specifically for patients with thickened zonae (100). Subsequent studies examining the routine or targeted implementation of assisted hatching in IVF cycles have been mixed. While the data have not suggested a universal application for assisted hatching, individuals with RIF may preferentially benefit from the technique (103,107,108). Stein et al. reported that partial zona dissection resulted in a significant improvement in implantation and pregnancy rates in women older than 38 years of age who had a history of RIF (109). Petersen et al. similarly reported higher implantation rates when embryos underwent laser-assisted zona thinning, but only in individuals with at least two prior implantation failures (110). Magli et al. conducted a randomized controlled trial that included women who were >38 years old (45 cycles), patients with three or more prior failed attempts (70 cycles), and patients meeting both criteria (20 cycles). Clinical pregnancies per cycle were significantly elevated in patients undergoing assisted hatching where either age (31% vs. 10%) or repeated failure (36% vs. 17%) was the indication (111). Corroborating these reports, a meta-analysis examining data from five randomized controlled trials (561 patients) revealed a 73% improvement in clinical pregnancy (RR 1.73; 95% CI 1.37–2.17) when assisted hatching was employed in individuals with RIF (112). Unselected patients, however, do not seem to experience the same benefit (113).

The optimal technique for assisted hatching remains controversial. Hsieh et al. reported that hatching with a diode laser provided greater benefit than chemical-assisted hatching in older patients (114). Primi et al. similarly showed better results for patients with RIF when employing the diode laser (115). Conversely, Balaban et al. did not discern any appreciable difference when examining partial zona dissection, acid Tyrode's solution, diode laser, or pronase thinning (116). Others have argued that the optimal implementation of assisted hatching involves laser-assisted thinning of the zona, without a complete breach, limiting the procedure to only a quarter of the circumference of the embryo (117). Given the heterogeneity of techniques and the wide range of published evidence, no specific assisted hatching technique has been established as the gold standard for patients with RIF.

Endometrial "scratch"

Significant controversy exists over the benefit (or lack thereof) of "endometrial scratch" as a method for fostering implantation. The method is purported to induce a "healing process" that allows for release of cytokines and other

growth factors that facilitate implantation. Mechanical endometrial injury prior to controlled ovarian hyperstimulation has been proposed as a method to induce decidualization and attract cytokines, growth factors, LIF, and other immune modulators to the endometrium (118,119). Barash et al. in 2003 first suggested an association between endometrial biopsy and implantation in a study of 134 good responders who failed to conceive in one or more prior IVF cycles with at least three embryos transferred (120). In total, 54 of 134 subjects were subjected to repeated endometrial biopsy on days 8, 12, 21, and 26 of the cycle preceding IVF, with the data suggesting a significant improvement in subsequent implantation rates (27.7% vs. 14.3%) following repeated biopsies. Subsequent randomized controlled trials have employed a variety of inclusion criteria and frequencies/timings of biopsies, but overall have suggested an implantation benefit following the intervention (121–123).

Whereas initial studies seemed promising, subsequent data have been mixed, with some studies revealing a decrease in pregnancy rates in women undergoing biopsies prior to IVF (121,122,124–126). In a small randomized controlled trial of women with RIF involving a sham cervical biopsy for the control group, clinical pregnancy and live birth rates were lower in the experimental group (126). Two additional studies suggested no benefit of endometrial scratch in unselected populations undergoing IVF (124,127). In a sub-analysis of our own autologous co-culture program at Cornell, no improvement in implantation was seen for those patients having a co-culture biopsy in the luteal phase immediately preceding the IVF cycle (128). Similarly, endometrial disruption in 39 patients with a history of failed euploid embryo transfer did not improve implantation as compared to 251 control patients who did not undergo endometrial biopsies (129).

There exist only four small, heterogeneous randomized trials published in journals with low impact factors in a pool of 300 publications regarding endometrial scratch. Thus, significant questions remain regarding the benefit of the procedure, optimal frequency of biopsy, suitable timing of sampling, and what, if any, harm might exist (130). Additional well-designed, prospective randomized control trials are needed before this intervention for patients with RIF is adopted.

Co-culture

The *in vitro* culture conditions in mammalian IVF attempt to simulate *in vivo* conditions, but growth, biochemical synthetic activity, and reproductive competence may fall short during *in vitro* development. Co-culture of *in vitro*-derived embryos with either tubal epithelium, endometrial epithelium, granulosa, or cumulus cells has been proposed to foster more supportive culture conditions (131–135). Variable reported success rates with these techniques are likely attributable, at least in part, to differences in cell lines, maintenance of cells, and various environmental factors within each laboratory.

Vero cells (from monkey kidney epithelium) and bovine oviductal epithelium have both been noted to improve embryo quality and pregnancy rates in poor-prognosis patients (136,137). Co-culture of human embryos with buffalo rat liver cells also suggested a favorable trend (34% vs. 28%) towards improved pregnancy rates in patients with prior failures (138). Xeno-culture, however, poses both theoretical and practical infectious risks that make the use of various animal cells less than ideal for human embryos.

Because of these potential risks, investigators have focused on utilizing either homologous or autologous human cells in co-culture systems. Tubal cells from the ampullary portion of the fallopian tube have been harvested during hysterectomies or tubal ligations, and passaged several times to allow for use in multiple patients (131,139). Embryonic viability, morphological appearance, and number of blastomeres were enhanced when tubal epithelial co-culture was employed, with a second study revealing higher pregnancy, implantation, and embryo cryopreservation rates (131,139). However, the risk of transmission of infectious agents along with Creutzfeldt–Jakob disease limit the desirability of homologous techniques.

At the Center for Reproductive Medicine at Weill Cornell Medical College, we have developed and successfully applied a unique co-culture system that uses the patient's own endometrial cells to enhance embryo development (140,141). Patients undergo an endometrial biopsy in the mid-luteal phase of a cycle preceding their IVF treatment cycle, and endometrial glandular epithelial and stromal cells are separated by differential sedimentation and plated until a monolayer is achieved. The cells are then frozen and later thawed during the patient's treatment cycle. An equal mixture of glandular epithelial and stromal cells is seeded into a four-well tissue plate containing Ham's F-10 medium supplemented with 15% patient serum. Embryos are introduced into the co-culture system after fertilization and maintained with the autologous endometrial cells until the day of transfer.

Human endometrial co-culture has been noted to be beneficial to blastocyst development, presumably owing to a chemical cross-talk and paracrine signaling between embryo and endometrium (142–144). The use of autologous endometrial cells for co-culture in patients with RIF was first reported by Jayot et al. in 1995, with a pregnancy rate of 21% as compared to 8% in patients' previous cycles (145). Nieto et al. used predominantly endometrial epithelial cells and reported a decrease in fragmentation among day-3 embryos (132). Simon et al. achieved a 39.2% blastocyst formation rate, an 11.8% implantation rate, and a 20.2% pregnancy rate with an autologous endometrial co-culture system in 168 cycles among patients with three or more failed implantation cycles (146). Eyheremendy et al. similarly demonstrated benefit utilizing autologous endometrial cell co-culture with day-3 transfer in patients with RIF (147).

In our own experience, sibling oocytes from RIF patients undergoing endometrial co-culture exhibit lower fragmentation and more blastomeres at the time of transfer as

compared to traditionally cultured embryos (140). Further published studies revealed implantation and clinical pregnancy rates of 15% and 29%, respectively, in patients with prior IVF failures associated with poor embryo quality (141). We observe the best results when biopsies are performed in the mid-to-late luteal phase as opposed to the early luteal phase of the menstrual cycle (148). A meta-analysis of 17 studies has suggested an improvement in blastomere numbers and implantation and pregnancy rates with the utilization of co-culture (149). While the data suggests a distinct benefit to autologous endometrial co-culture for patients with RIF, such programs are difficult to maintain given the resources in personnel and time required. Moreover, as potentially better embryo incubation techniques such as time-lapse microscopy at low oxygen tensions emerge, the incremental benefit of endometrial coculture remains to be further defined for these difficult RIF patients.

CONCLUSION

Although treatment of patients with a history of RIF can be discouraging, techniques and methodologies striving to optimize IVF success in these patients continue to evolve. We must continue to investigate and elucidate factors that may prevent our patients from achieving live births. Further evaluation of embryo–endometrial crosstalk and of the ideal timing of transfer into a receptive endometrium may lead to new treatments for patients experiencing RIF. Likewise, improved embryo culture and embryo analytic techniques may offer finer discernment of embryos with the greatest implantation potential. The physician caring for a patient or couple with RIF must carefully review the prior diagnostic workup, complete the investigation with appropriate analytic techniques, and offer empathy and encouragement while providing accurate counsel on the likelihood of success.

REFERENCES

1. National Center for Chronic Disease Prevention and Health Promotion, Division of Reproductive Health, http://www.cdc.gov/art/artdata/index.html
2. Stern C, Pertile M, Norris H, Hale L, Baker HW. Chromosome translocations in couples with *in-vitro* fertilization implantation failure. *Hum Reprod* 1999; 14: 2097–101.
3. Raziel A et al. Increased frequency of female partner chromosomal abnormalities in patients with high-order implantation failure after *in vitro* fertilization. *Fertil Steril* 2002; 78: 515–9.
4. Telford NA, Watson AJ, Schultz GA. Transition from maternal to embryonic control in early mammalian development: A comparison of several species. *Mol Reprod Dev* 1990; 26: 90–100.
5. Demko ZP, Simon AL, McCoy RC., Petrov DA, Rabinowitz M. Effects of maternal age on euploidy rates in a large cohort of embryos analyzed with 24-chromosome single-nucleotide polymorphism-based preimplantation genetic screening. *Fertil Steril* 2016; 105: 1307–13.
6. Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995; 64: 382–91.
7. Franasiak JM et al. The nature of aneuploidy with increasing age of the female partner: A review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril* 2014; 101: 656–63, e651.
8. Mastenbroek S et al. *In vitro* fertilization with preimplantation genetic screening. *N Engl J Med* 2007; 357: 9–17.
9. Pinkel D, Albertson DG. Comparative genomic hybridization. *Annu Rev Genomics Hum Genet* 2005; 6: 331–54.
10. Pehlivan T et al. Impact of preimplantation genetic diagnosis on IVF outcome in implantation failure patients. *Reprod Biomed Online* 2003; 6: 232–7.
11. Gianaroli L et al. Will preimplantation genetic diagnosis assist patients with a poor prognosis to achieve pregnancy? *Hum Reprod* 1997; 12: 1762–7.
12. Greco E et al. Comparative genomic hybridization selection of blastocysts for repeated implantation failure treatment: A pilot study. *Biomed Res Int* 2014; 2014: 457913.
13. Gianaroli L, Magli MC, Ferraretti AP, Munne S. Preimplantation diagnosis for aneuploidies in patients undergoing *in vitro* fertilization with a poor prognosis: Identification of the categories for which it should be proposed. *Fertil Steril* 1999; 72: 837–44.
14. Blockeel C et al. Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. *Reprod Biomed Online* 2008; 17: 848–54.
15. Mastenbroek S, Twisk M, van der Veen F, Repping S Preimplantation genetic screening: A systematic review and meta-analysis of RCTs. *Hum Reprod Update* 2011; 17: 454–66.
16. Treff NR, Scott RT, Jr. Methods for comprehensive chromosome screening of oocytes and embryos: Capabilities, limitations, and evidence of validity. *J Assist Reprod Genet* 2012; 29: 381–90.
17. Treff NR et al. SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. *Mol Hum Reprod*2010; 16: 583–9.
18. Northrop LE, Treff NR, Levy B, Scott RT, Jr. SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. *Mol Hum Reprod* 2010; 16: 590–600.
19. Treff NR et al. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. *Fertil Steril* 2000; 97: 819–24.
20. Fragouli E et al. Comprehensive chromosome screening of polar bodies and blastocysts from

couples experiencing repeated implantation failure. *Fertil Steril* 2010; 94: 875–87.
21. Lee HL et al. *In vitro* fertilization with preimplantation genetic screening improves implantation and live birth in women age 40 through 43. *J Assist Reprod Genet* 2015; 32: 435–44.
22. Robinson L et al. The effect of sperm DNA fragmentation on miscarriage rates: A systematic review and meta-analysis. *Hum Reprod* 2012; 27: 2908–17.
23. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: Its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. 2002; *J Androl* 23: 25–43.
24. Bungum M et al. The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. *Hum Reprod* 2004; 19: 1401–8.
25. Bungum M et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. *Hum Reprod* 2007; 22: 174–9.
26. Duran EH, Morshedi M, Taylor S, Oehninger S. Sperm DNA quality predicts intrauterine insemination outcome: A prospective cohort study. *Hum Reprod* 2002; 17: 3122–8.
27. Muriel L et al. Value of the sperm chromatin dispersion test in predicting pregnancy outcome in intrauterine insemination: A blind prospective study. *Hum Reprod* 2006; 21: 738–44.
28. Absalan F et al. Value of sperm chromatin dispersion test in couples with unexplained recurrent abortion. *J Assist Reprod Genet* 2012; 29: 11–4.
29. Brahem S et al. Semen parameters and sperm DNA fragmentation as causes of recurrent pregnancy loss. *Urology* 2011; 78: 792–6.
30. Greco E et al. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. *Hum Reprod* 2005; 20: 226–30.
31. Steele EK, McClure N, Maxwell RJ, Lewis SE. A comparison of DNA damage in testicular and proximal epididymal spermatozoa in obstructive azoospermia. *Mol Hum Reprod* 1999; 5: 831–5.
32. Suganuma R, Yanagimachi R, Meistrich ML. Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. *Hum Reprod* 2005; 20: 3101–8.
33. Weissman A et al. Pregnancies and live births following ICSI with testicular spermatozoa after repeated implantation failure using ejaculated spermatozoa. *Reprod Biomed Online* 2008; 17: 605–9.
34. Jakab A et al. Intracytoplasmic sperm injection: A novel selection method for sperm with normal frequency of chromosomal aneuploidies. *Fertil Steril* 2005; 84: 1665–73.
35. Said TM et al. Advantage of combining magnetic cell separation with sperm preparation techniques. *Reprod Biomed Online* 2005; 10: 740–6.
36. Sakkas D, Alvarez JG. Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril* 2010; 93: 1027–36.
37. Bartoov B et al. Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. *Fertil Steril* 2003; 80: 1413–9.
38. Berkovitz A et al. How to improve IVF–ICSI outcome by sperm selection. *Reprod Biomed Online* 2006; 12: 634–8.
39. Nadalini M, Tarozzi N, Distratis V, Scaravelli G, Borini A. Impact of intracytoplasmic morphologically selected sperm injection on assisted reproduction outcome: A review. *Reprod Biomed Online* 2009; 19(Suppl 3): 45–55.
40. Oliveira JB et al. Pregnancy outcomes in women with repeated implantation failures after intracytoplasmic morphologically selected sperm injection (IMSI). *Reprod Biol Endocrinol* 2011; 9: 99.
41. Franco JG, Jr. et al. Significance of large nuclear vacuoles in human spermatozoa: Implications for ICSI. *Reprod Biomed Online* 2008; 17: 42–5.
42. Oliveira JB et al. Correlation between semen analysis by motile sperm organelle morphology examination and sperm DNA damage. *Fertil Steril* 2010; 94: 1937–40.
43. Perdrix A et al. Assessment of acrosome and nuclear abnormalities in human spermatozoa with large vacuoles. *Hum Reprod* 2011; 26: 47–58.
44. Boitrelle F et al. Large human sperm vacuoles observed in motile spermatozoa under high magnification: Nuclear thumbprints linked to failure of chromatin condensation. *Hum Reprod* 2011; 26: 1650–8.
45. El Khattabi L et al. Is intracytoplasmic morphologically selected sperm injection effective in patients with infertility related to teratozoospermia or repeated implantation failure? *Fertil Steril* 2013; 100: 62–8.
46. Burrello N et al. Lower sperm aneuploidy frequency is associated with high pregnancy rates in ICSI programmes. *Hum Reprod* 2003; 18: 1371–6.
47. Makrakis E, Pantos K. The outcomes of hysteroscopy in women with implantation failures after *in-vitro* fertilization: Findings and effect on subsequent pregnancy rates. *Curr Opin Obstet Gynecol* 2010; 22: 339–43.
48. Buttram VC, Jr., Reiter RC. Uterine leiomyomata: Etiology, symptomatology, and management. *Fertil Steril* 1981; 36: 433–45.
49. Donnez J, Jadoul P. What are the implications of myomas on fertility? A need for a debate? *Hum Reprod* 2002; 17: 1424–30.
50. Taylor E, Gomel V. The uterus and fertility. *Fertil Steril* 2008; 89: 1–16.
51. Metwally M, Farquhar CM, Li TC. Is another meta-analysis on the effects of intramural fibroids on reproductive outcomes needed? *Reprod Biomed Online* 2011: 23; 2–14.

52. Pritts EA, Parker WH, Olive DL. Fibroids and infertility: An updated systematic review of the evidence. *Fertil Steril* 2009; 91: 1215–23.
53. Sunkara SK, Khairy M, El-Toukhy T, Khalaf Y, Coomarasamy A. The effect of intramural fibroids without uterine cavity involvement on the outcome of IVF treatment: A systematic review and meta-analysis. *Hum Reprod* 2010; 25: 418–29.
54. Richlin SS, Ramachandran S, Shanti A, Murphy AA, Parthasarathy S. Glycodelin levels in uterine flushings and in plasma of patients with leiomyomas and polyps: Implications for implantation. *Hum Reprod* 2002; 17: 2742–7.
55. Shokeir TA, Shalan HM, El-Shafei MM. Significance of endometrial polyps detected hysteroscopically in eumenorrheic infertile women. *J Obstet Gynaecol Res* 2004; 30: 84–9.
56. Spiewankiewicz B et al. The effectiveness of hysteroscopic polypectomy in cases of female infertility. *Clin Exp Obstet Gynecol* 2003; 30: 23–5.
57. Varasteh NN, Neuwirth RS, Levin B, Keltz MD. Pregnancy rates after hysteroscopic polypectomy and myomectomy in infertile women. *Obstet Gynecol* 1999; 94: 168–71.
58. Perez-Medina T et al. Endometrial polyps and their implication in the pregnancy rates of patients undergoing intrauterine insemination: A prospective, randomized study. *Hum Reprod* 2005; 20: 1632–5.
59. Dawood A, Al-Talib A, Tulandi T. Predisposing factors and treatment outcome of different stages of intrauterine adhesions. *J Obstet Gynaecol Can* 2010; 32: 767–70.
60. Katz Z, Ben-Arie A, Lurie S, Manor M, Insler V. Reproductive outcome following hysteroscopic adhesiolysis in Asherman's syndrome. *Int J Fertil Menopausal Stud* 1996; 41: 462–5.
61. Pace S, Stentella P, Catania R, Palazzetti PL, Frega A. Endoscopic treatment of intrauterine adhesions. *Clin Exp Obstet Gynecol* 2003; 30; 26–8.
62. Yasmin H, Nasir A, Noorani KJ. Hystroscopic management of Ashermans syndrome. *J Pak Med Assoc* 2007; 57: 553–5.
63. Zikopoulos KA et al. Live delivery rates in subfertile women with Asherman's syndrome after hysteroscopic adhesiolysis using the resectoscope or the Versapoint system. *Reprod Biomed Online* 2004; 8: 720–5.
64. Strandell A, Waldenstrom U, Nilsson L, Hamberger L. Hydrosalpinx reduces *in-vitro* fertilization/embryo transfer pregnancy rates. *Hum Reprod* 1994; 9: 861–3.
65. Feghali J et al. Systematic hysteroscopy prior to *in vitro* fertilization. *Gynecol Obstet Fertil* 2003; 31: 127–31.
66. Johnston-MacAnanny EB et al. Chronic endometritis is a frequent finding in women with recurrent implantation failure after *in vitro* fertilization. *Fertil Steril* 2010; 93: 437–41.
67. Cicinelli E et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Hum Reprod* 2015; 30: 323–30.
68. Tremellen K, Russell P. Adenomyosis is a potential cause of recurrent implantation failure during IVF treatment. *Aust N Z J Obstet Gynaecol* 2011; 51: 280–3.
69. Maheshwari A, Gurunath S, Fatima F, Bhattacharya S. Adenomyosis and subfertility: A systematic review of prevalence, diagnosis, treatment and fertility outcomes. *Hum Reprod Update* 2012; 18: 374–92.
70. Sunkara SK, Khan KS. Adenomyosis and female fertility: A critical review of the evidence. *J Obstet Gynaecol* 2012; 32: 113–6.
71. Demirol A, Gurgan T. Effect of treatment of intrauterine pathologies with office hysteroscopy in patients with recurrent IVF failure. *Reprod Biomed Online* 2004; 8: 590–4.
72. Rama Raju GA, Shashi Kumari G, Krishna KM, Prakash, GJ, Madan K. Assessment of uterine cavity by hysteroscopy in assisted reproduction programme and its influence on pregnancy outcome. *Arch Gynecol Obstet* 2006; 274: 160–4.
73. Shokeir T, Abdelshaheed M. Sonohysterography as a first-line evaluation for uterine abnormalities in women with recurrent failed *in vitro* fertilization–embryo transfer. *Fertil Steril* 2009; 91: 1321–2.
74. Soares SR, Barbosa dos Reis MM, Camargos AF. Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine cavity diseases. *Fertil Steril* 2000; 73: 406–11.
75. Grimbizis GF et al. A prospective comparison of transvaginal ultrasound, saline infusion sonohysterography, and diagnostic hysteroscopy in the evaluation of endometrial pathology. *Fertil Steril* 2010; 94: 2720–5.
76. Galgani M et al. Regulatory T cells, inflammation, and endoplasmic reticulum stress in women with defective endometrial receptivity. *Fertil Steril* 2015; 103: 1579–86.e1.
77. Achache H, Tsafrir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing *in vitro* fertilization. *Fertil Steril* 2010; 94: 1271–8.
78. Riesewijk A et al. Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. *Mol Hum Reprod* 2003; 9: 253–64.
79. Ponnampalam AP, Weston GC, Trajstman AC, Susil B, Rogers PA. Molecular classification of human endometrial cycle stages by transcriptional profiling. *Mol Hum Reprod* 2004; 10: 879–93.
80. Talbi S et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* 2006; 147: 1097–121.

81. Horcajadas JA, Pellicer A, Simon C. Wide genomic analysis of human endometrial receptivity: New times, new opportunities. *Hum Reprod Update* 2007; 13: 77–86.
82. Ruiz-Alonso M, Blesa D, Simon C. The genomics of the human endometrium. *Biochim Biophys Acta* 2012; 1822: 1931–42.
83. Koler M et al. Disrupted gene pattern in patients with repeated *in vitro* fertilization (IVF) failure. *Hum Reprod* 2009; 24: 2541–8.
84. Bersinger NA, Wunder DM, Birkhauser MH, Mueller MD. Gene expression in cultured endometrium from women with different outcomes following IVF. *Mol Hum Reprod* 2008; 14: 475–84.
85. Tapia A et al. Differences in the endometrial transcript profile during the receptive period between women who were refractory to implantation and those who achieved pregnancy. *Hum Reprod* 2008; 23: 340–51.
86. Diaz-Gimeno P et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril* 2011; 95: 50–60, 60.e1–15.
87. Diaz-Gimeno P et al. The accuracy and reproducibility of the endometrial receptivity array is superior to histology as a diagnostic method for endometrial receptivity. *Fertil Steril* 2013; 99: 508–17.
88. Ruiz-Alonso M et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril* 2013; 100: 818–24.
89. Coughlan C et al. Recurrent implantation failure: Definition and management. *Reprod Biomed Online* 2014; 28: 14–38.
90. Camus E et al. Pregnancy rates after *in-vitro* fertilization in cases of tubal infertility with and without hydrosalpinx: A meta-analysis of published comparative studies. *Hum Reprod* 1999; 14: 1243–9.
91. Strandell A et al. Hydrosalpinx and IVF outcome: A prospective, randomized multicentre trial in Scandinavia on salpingectomy prior to IVF. *Hum Reprod* 1999; 14: 2762–9.
92. Zeyneloglu HB, Arici A, Olive DL. Adverse effects of hydrosalpinx on pregnancy rates after *in vitro* fertilization-embryo transfer. *Fertil Steril* 1998; 70: 492–9.
93. Seli E et al. Removal of hydrosalpinges increases endometrial leukaemia inhibitory factor (LIF) expression at the time of the implantation window. *Hum Reprod* 2005; 20: 3012–7.
94. Bildirici I., Bukulmez O, Ensari A, Yarali H, Gurgan T. A prospective evaluation of the effect of salpingectomy on endometrial receptivity in cases of women with communicating hydrosalpinges. *Hum Reprod* 2001; 16: 2422–6.
95. De Vos A, Van Steirteghem A. Zona hardening, zona drilling and assisted hatching: New achievements in assisted reproduction. *Cells Tissues Organs* 2000; 166: 220–7.
96. Fehilly CB, Cohen J, Simons RF, Fishel SB, Edwards RG. Cryopreservation of cleaving embryos and expanded blastocysts in the human: A comparative study. *Fertil Steril* 1985; 44: 638–44.
97. Cohen J, Inge KL, Suzman M, Wiker SR, Wright G. Videocinematography of fresh and cryopreserved embryos: A retrospective analysis of embryonic morphology and implantation. *Fertil Steril* 1989; 51: 820–7.
98. Malter HE, Cohen J. Blastocyst formation and hatching *in vitro* following zona drilling of mouse and human embryos. *Gamete Res* 1989; 24: 67–80.
99. Cohen J. Assisted hatching of human embryos. *J In Vitro Fert Embryo Transf* 1991; 8: 179–90.
100. Cohen J, Alikani M, Trowbridge J, Rosenwaks Z. Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. *Hum Reprod* 1992; 7: 685–91.
101. Khalifa EA, Tucker MJ, Hunt P. Cruciate thinning of the zona pellucida for more successful enhancement of blastocyst hatching in the mouse. *Hum Reprod* 1992; 7: 532–6.
102. Fong CY et al. Blastocyst transfer after enzymatic treatment of the zona pellucida: Improving *in-vitro* fertilization and understanding implantation. *Hum Reprod* 1998; 13: 2926–32.
103. Valojerdi MR, Eftekhari-Yazdi P, Karimian L, Ashtiani SK. Effect of laser zona pellucida opening on clinical outcome of assisted reproduction technology in patients with advanced female age, recurrent implantation failure, or frozen-thawed embryos. *Fertil Steril* 2008; 90: 84–91.
104. Laufer N et al. The efficacy and safety of zona pellucida drilling by a 193-nm excimer laser. *Fertil Steril* 1993; 59: 889–95.
105. Nakayama T et al. Clinical application of a new assisted hatching method using a piezo-micromanipulator for morphologically low-quality embryos in poor-prognosis infertile patients. *Fertil Steril* 1999; 71: 1014–8.
106. Hershlag A, Feng HL. Effect of prefreeze assisted hatching on postthaw survival of mouse embryos. *Fertil Steril* 2005; 84: 1752–4.
107. Antinori S et al. Zona opening of human embryos using a non-contact UV laser for assisted hatching in patients with poor prognosis of pregnancy. *Hum Reprod* 1996; 11: 2488–92.
108. Chao KH et al. Assisted hatching increases the implantation and pregnancy rate of *in vitro* fertilization (IVF)-embryo transfer (ET), but not that of IVF-tubal ET in patients with repeated IVF failures. *Fertil Steril* 1997; 67: 904–8.
109. Stein A et al. Assisted hatching by partial zona dissection of human pre-embryos in patients with recurrent implantation failure after *in vitro* fertilization. *Fertil Steril* 1995; 63: 838–41.
110. Petersen CG et al. Implantation failures: Success of assisted hatching with quarter-laser zona thinning. *Reprod Biomed Online* 2005; 10: 224–9.

111. Magli MC et al. Rescue of implantation potential in embryos with poor prognosis by assisted zona hatching. *Hum Reprod* 1998; 13: 1331–5.
112. Martins WP, Rocha IA, Ferriani RA, Nastri CO. Assisted hatching of human embryos: A systematic review and meta-analysis of randomized controlled trials. *Hum Reprod Update* 2011; 17: 438–53.
113. Das S, Blake D, Farquhar C, Seif MM. Assisted hatching on assisted conception (IVF and ICSI). *Cochrane Database Syst Rev* 2009; 2: CD001894.
114. Hsieh YY et al. Laser-assisted hatching of embryos is better than the chemical method for enhancing the pregnancy rate in women with advanced age. *Fertil Steril* 2002; 78: 179–82.
115. Primi MP et al. A European multicentre prospective randomized study to assess the use of assisted hatching with a diode laser and the benefit of an immunosuppressive/antibiotic treatment in different patient populations. *Hum Reprod* 2004; 19: 2325–33.
116. Balaban B et al. A comparison of four different techniques of assisted hatching. *Hum Reprod* 2002; 17: 1239–43.
117. Blake DA, Forsberg AS., Johansson BR, Wikland M. Laser zona pellucida thinning—An alternative approach to assisted hatching. *Hum Reprod* 2001; 16: 1959–64.
118. Finn CA, Pope M Vascular and cellular changes in the decidualized endometrium of the ovariectomized mouse following cessation of hormone treatment: A possible model for menstruation. *J Endocrinol* 1984; 100: 295–300.
119. Gnainsky Y et al. Local injury of the endometrium induces an inflammatory response that promotes successful implantation. *Fertil Steril* 2010; 94: 2030–6.
120. Barash A et al. Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing *in vitro* fertilization. *Fertil Steril* 2003; 79: 1317–22.
121. Karimzadeh MA, Ayazi Rozbahani M, Tabibnejad N. Endometrial local injury improves the pregnancy rate among recurrent implantation failure patients undergoing *in vitro* fertilisation/intra cytoplasmic sperm injection: A randomised clinical trial. *Aust N Z J Obstet Gynaecol* 2009; 49: 677–80.
122. Narvekar SA et al. Does local endometrial injury in the nontransfer cycle improve the IVF-ET outcome in the subsequent cycle in patients with previous unsuccessful IVF? A randomized controlled pilot study. *J Hum Reprod Sci* 2010; 3: 15–9.
123. Nastri CO, Ferriani RA., Raine-Fenning N, Martins WP. Endometrial scratching performed in the nontransfer cycle and outcome of assisted reproduction: A randomized controlled trial. *Ultrasound Obstet Gynecol* 2013; 42: 375–82.
124. Yeung TW et al. The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing *in vitro* fertilization: A randomized controlled trial. *Hum Reprod* 2014; 29: 2474–81.
125. Gibreel A, Badawy A, El-Refai W, El-Adawi N. Endometrial scratching to improve pregnancy rate in couples with unexplained subfertility: A randomized controlled trial. *J Obstet Gynaecol Res* 2013; 39: 680–4.
126. Baum M et al. Does local injury to the endometrium before IVF cycle really affect treatment outcome? Results of a randomized placebo controlled trial. *Gynecol Endocrinol* 2012; 28: 933–6.
127. Dain L et al. Effect of local endometrial injury on pregnancy outcomes in ovum donation cycles. *Fertil Steril* 2014; 102: 1048–54.
128. Melnick AP et al. Autologous endometrial coculture biopsy: Is timing everything? *Fertil Steril* 2015; 104: 104–9.e1.
129. Werner MD et al. Endometrial disruption does not improve implantation in patients who have failed the transfer of euploid blastocysts. *J Assist Reprod Genet* 2015; 32: 557–62.
130. Simon C, Bellver J. Scratching beneath 'The Scratching Case': Systematic reviews and meta-analyses, the back door for evidence-based medicine. *Hum Reprod* 2014; 29: 1618–21.
131. Yeung WS, Lau EY, Chan ST, Ho PC. Coculture with homologous oviductal cells improved the implantation of human embryos—A prospective randomized control trial. *J Assist Reprod Genet* 1996; 13: 762–7.
132. Nieto FS, Watkins WB, Lopata A, Baker HW, Edgar DH. The effects of coculture with autologous cryopreserved endometrial cells on human *in vitro* fertilization and early embryo morphology: A randomized study. *J Assist Reprod Genet* 1996; 13: 386–9.
133. Plachot M et al. Granulosa cells improve human embryo development *in vitro*. *Hum Reprod* 1993; 8: 2133–40.
134. Quinn P, Margalit R Beneficial effects of coculture with cumulus cells on blastocyst formation in a prospective trial with supernumerary human embryos. *J Assist Reprod Genet* 1996; 13: 9–14.
135. Sakkas D, Jaquenoud N, Leppens G, Campana A. Comparison of results after *in vitro* fertilized human embryos are cultured in routine medium and in coculture on Vero cells: A randomized study. *Fertil Steril* 1994; 61: 521–5.
136. Wiemer KE et al. Beneficial aspects of co-culture with assisted hatching when applied to multiple-failure *in-vitro* fertilization patients. *Hum Reprod* 1996; 11: 2429–33.
137. Wiemer KE et al. Embryonic morphology and rate of implantation of human embryos following coculture on bovine oviductal epithelial cells. *Hum Reprod* 1993; 8: 97–101.
138. Hu Y, Maxson WS, Hoffman DI, Eager S, Dupre J. Coculture of human embryos with buffalo rat liver

cells for women with decreased prognosis in *in vitro* fertilization. *Am J Obstet Gynecol* 1997; 177: 358–62, discussion 362–353.
139. Bongso A et al. Improved quality of human embryos when co-cultured with human ampullary cells. *Hum Reprod* 1989; 4: 706–13.
140. Barmat LI et al. Human preembryo development on autologous endometrial coculture versus conventional medium. *Fertil Steril* 1998; 70: 1109–13.
141. Barmat LI et al. Autologous endometrial co-culture in patients with repeated failures of implantation after *in vitro* fertilization–embryo transfer. *J Assist Reprod Genet* 1999; 16: 121–7.
142. Cross JC, Werb Z, Fisher SJ. Implantation and the placenta: Key pieces of the development puzzle. *Science* 1994; 266: 1508–18.
143. Liu HC et al. Human endometrial stromal cells improve embryo quality by enhancing the expression of insulin-like growth factors and their receptors in cocultured human preimplantation embryos. *Fertil Steril* 1999; 71: 361–7.
144. Tazuke SI, Giudice LC. Growth factors and cytokines in endometrium, embryonic development, and maternal: Embryonic interactions. *Semin Reprod Endocrinol* 1996; 14: 231–45.
145. Jayot S et al. Coculture of embryos on homologous endometrial cells in patients with repeated failures of implantation. *Fertil Steril* 1995; 63: 109–14.
146. Simon C et al. Coculture of human embryos with autologous human endometrial epithelial cells in patients with implantation failure. *J Clin Endocrinol Metab* 1999; 84: 2638–46.
147. Eyheremendy V et al. Beneficial effect of autologous endometrial cell coculture in patients with repeated implantation failure. *Fertil Steril* 2010; 93: 769–73.
148. Spandorfer SD et al. Importance of the biopsy date in autologous endometrial cocultures for patients with multiple implantation failures. *Fertil Steril* 2002; 77: 1209–13.
149. Kattal N, Cohen J, Barmat LI. Role of coculture in human *in vitro* fertilization: A meta-analysis. *Fertil Steril* 2008; 90: 1069–76.

Ultrasonography in assisted reproduction

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LAUREL STADTMAUER, KAY WAUD, DAVID P. COHEN, and ILAN TUR-KASPA

INTRODUCTION

Can we imagine assisted reproduction technology (ART) today without imaging? Ultrasound has become the most widely used and important tool in the diagnosis and treatment of infertility. When a patient presents with infertility, ultrasound evaluation is the key part of the exam performed to evaluate the ovaries, uterus, and fallopian tubes. The saline sonogram is used most commonly as an evaluation of the uterine cavity before ART and can identify both congenital and acquired anomalies as well as tubal patency and the presence of hydrosalpinges. This initial ultrasound exam of the ovaries includes an antral follicle count (AFC) for ovarian reserve and a diagnosis of polycystic-appearing ovaries, endometriosis, and other adnexal pathologies. When ART treatment begins, ultrasound is used for monitoring of follicular development and endometrial response and is critical in the success of the cycle. Ultrasound-guided procedures for oocyte retrieval and embryo transfer (ET) are standard practice, and ultrasound guidance is helpful in the treatment of Asherman's syndrome and congenital anomalies such as a large septate uterus. Of course, the goal of ART is a singleton viable pregnancy, and early ultrasound monitoring can evaluate the location and viability of the pregnancy and the existence of multiples or vanishing twins.

The quality of the new ultrasound machines and the use of three-dimensional (3D) ultrasound allow better imaging as well as more accurate diagnosis of pathology and preoperative preparation. 3D ultrasound has become a gold standard for the diagnosis of uterine anomalies and may assist in more accurate follicular monitoring measurements. Doppler modalities of ultrasound allow identification of the direction and magnitude of blood flow and calculation of velocity, which are useful in separating pathology from normal.

This chapter is aimed to review how 2D and 3D ultrasound are used to maximize ART outcome, concentrating more on the use of 3D. When we see better, we do ART better.

ULTRASOUND AND THE OVARY

The ovaries are composed of germ cells, stromal cells, and epithelium. The ovaries are visualized with a variety of growing follicles. During puberty, the ovaries enlarge as the follicles grow. Changes in the sizes of the follicles are due to secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The ovaries contain several subtypes of follicles: the primordial follicles, primary follicles, secondary follicles, pre-antral follicles, and antral follicles (AFs; >2 mm diameter). The AFs are visible as small cysts and are the smallest follicles that are visible on ultrasound. Follicles grow in two stages—the gonadotropin-independent and gonadotropin-dependent stages—and the recruitment occurs over three months. AFs are gonadotropin dependent and best evaluated on cycle days 2 or 3. In the early follicular phase, the AFs that measure from 2 to 10 mm represent the pool of follicles that may be recruited in the follicular phase for ovulation. In a natural cycle, the dominant follicles reach a diameter of 17–24 mm prior to ovulation. Ovarian blood flow in an ovulatory cycle is constant up to the point of ovulation. Ovarian flow velocity tends to increase at and immediately after ovulation (1,2). After ovulation, a corpus luteum (CL) is frequently seen during the secretory phase of the cycle. It is well vascularized and may have the appearance of a "ring of fire" from the vascularity as seen by power Doppler (3).

A normal CL has a variety of sonographic appearances. Most commonly, the CL appears as a round anechoic cystic mass with a homogeneous, thick, moderately echogenic wall. The cyst is highly vascular with low impedance blood flow and a low-resistance arterial waveform. Hemorrhage into a CL can create a sonographic pattern of internal echoes (Figure 52.1). CL cysts and hemorrhagic cysts with layers that jiggle and rupture of the cyst can result in hemorrhage or clot surrounding the ovary or within the peritoneal cavity.

Ovarian reserve

Ovarian reserve can be indirectly measured by counting the number of AFs (measuring 2–10 mm) in each ovary. Age, previous surgery, chemotherapy, and genetics can all affect ovarian reserve, as women are born with a fixed number of oocytes and oocyte loss can be accelerated by the above. The peak number of 5 million primordial follicles occurs prior to birth at about 20 weeks' gestation, and the decrease in the number of oocytes is the result of atresia and is associated with a decrease in oocyte quality (4). At birth, there are about 1–2 million oocytes, and at puberty approximately 250,000 oocytes. The exponential loss of follicles accelerates at 37–38 years of age (only about 25,000 oocytes remain), leading to full depletion of the oocytes at menopause at the average age of 51 years (5). About 400 follicles will achieve preovulatory maturation and ovulate between menarche and menopause. Retrieval of 10–100 of oocytes with multiple *in vitro* fertilization (IVF) cycles does not seem to significantly affect this age-related follicular loss.

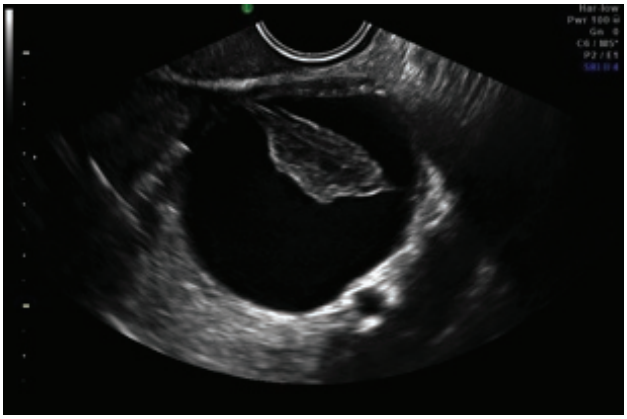

Figure 52.1 Hemorrhagic cyst resolving.

Blood tests for estimating ovarian reserve include day–3 FSH and estradiol (E2) levels, as well as anti-Mullerian hormone (AMH) and inhibin B levels (6–8). 3D ultrasound for AFC and ovarian volumes is more reproducible and accurate than 2D (9). However, this method is not universally used, as 3D technology is not freely available for all reproductive endocrinologists and increases the cost. Figure 52.2 shows a 3D AFC and volumes. Both AFC and AMH are the superior methods for predicting ovarian reserve and response to treatment (10). These methods are equal in predicting the number of oocytes retrieved in an IVF stimulated cycle (11). Unlike biochemical parameters, ultrasound is the only method so far that allows a direct assessment of each ovary separately. In addition, the size of the AFs on day 2 is important as the larger follicles (>6 mm) are more likely to rapidly grow and lead to atretic or poor-quality oocytes. Determination of the pretreatment AFC and the number of stimulation-selectable follicles help physicians determine patients who are likely to respond poorly during IVF treatment. Those couples can be counseled regarding cycle cancelation and lower chances of success. On the other end of the spectrum, identification of those at a high risk of hyperstimulation allows adjustment of the dose and use of protocols to reduce the corresponding risks and avoid cycle cancelation. Several meta-analyses showed that the AFC correlated with oocyte number and hypo- and hyper-response better than other parameters such as FSH or age (12,13). Direct comparison of AFC to AMH levels has shown equivalent predictive values for ovarian response. However, AFC and AMH correlate less well with pregnancy outcomes, which is the more important outcome for the patient than oocyte number. The validity of AFC for ovarian reserve comes from studies demonstrating a direct correlation with the number of non-growing follicles viewed on histological sections (14). Other ultrasound parameters such as ovarian volume, vascularity, and perfusion had no significant value in predicting poor ovarian response and are inferior to AFC (15). In women with low AFC, especially at a young age, there is a decrease in quantity but not in quality of the oocytes. Therefore, AFC is highly predictive of the ovarian reserve and is strongly associated with the serum AMH level (16). The estimation of the ovarian response by ultrasound is simple and reliable.

The 3D AFC may be a better predictor of poor ovarian response (ovarian hyperstimulation syndrome [OHSS]) and the number of oocytes collected than 2D AFC. Standardization of AFC may improve with the 3D automated identification of the follicles via *post-hoc* image analysis (17). Although there are limited studies, automated imaging can reduce intra- and inter-observer

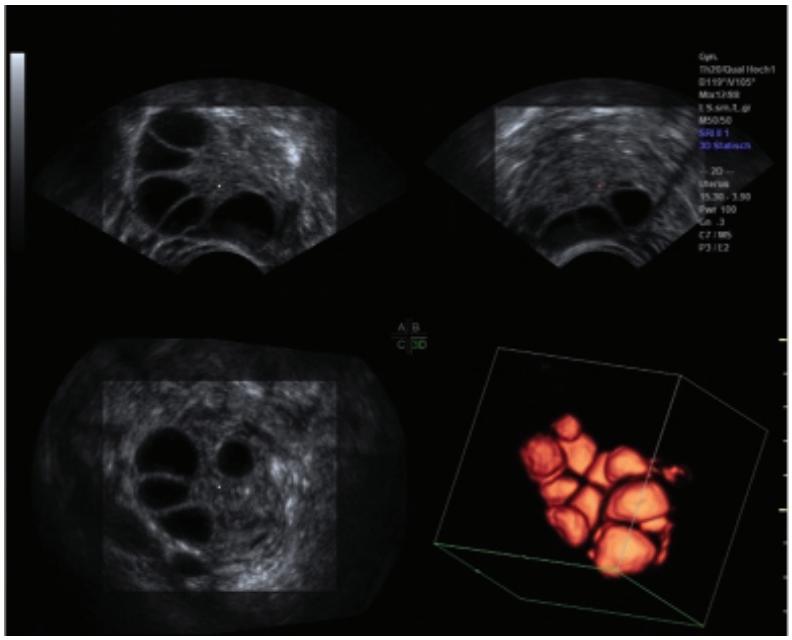

Figure 52.2 Three-dimensional antral follicle count using inverse mode.

variability and can be reviewed later. Scheffer et al. compared healthy volunteers with proven fertility to patients visiting an infertility clinic (18). For each patient, 2D or 3D transvaginal sonography (TVS) were conducted for AFC (2–10 mm) and inter-observer reliability was calculated. Both techniques were equivalent when only a few follicles were present; however, when higher AFCs occurred, the reproducibility decreased with the 2D technique. In addition to this report, studies by the group of Raine-Fenning demonstrated an improvement of inter-observer/intra-observer reliability by the application of 3D methods (particularly for automated systems such as sonography-based automated volume calculation [SonoAVC]) (19). In addition, they measured the examination time, including time for post-processing of the ultrasound scans, and still found less time to be required for 3D methods.

Ovarian cysts: Normal and pathology

Ultrasound is the best method for evaluating the ovaries for cysts, and it is a mandatory step in the initial evaluation of the infertile woman. The most common ovarian cysts seen in infertility patients are simple functional cysts, hemorrhagic cysts, endometriomas, and dermoid cysts (20). Functional follicular or luteal cysts are the most common cystic structures seen in the reproductive age group and they tend to resolve spontaneously within a few months. If they are small (<3 cm) and not hormonally active, they do not need to be treated before ART. However, patients with low ovarian reserve and large simple ovarian cysts may have lower response to stimulation. Ovarian cyst aspiration under ultrasound guidance with local or intravenous sedation immediately prior to ovarian stimulation has been shown to be beneficial (21). An endometrioma is also a common finding in the infertile patient and is a sign of the presence of endometriosis in other areas (22). The typical endometrioma is a unilocular cyst with homogeneous low-level internal echogenicity (ground glass echogenicity) of the cyst fluid (Figure 52.3) An ultrasound diagnosis can avoid surgery in the asymptomatic patient and lead to a decision to move to IVF earlier. During IVF stimulation, one should avoid aspiration of an endometrioma because of an increased risk of infection, compared with aspiration of a simple cyst. Studies show that the presence of an endometrioma is associated with lower response to ovarian stimulation; however, removing the endometrioma prior to stimulation can also affect ovarian response and may significantly diminish ovarian reserve (23–25). A recent study of 112,475 IVF cycles from published U.S. Centers for Disease Control and Prevention (CDC) data showed that endometriosis cycles had decreased oocyte yield and higher cancellation rates, but no differences in pregnancy or live birth rates compared with male factor patients (26).

Ovarian surgery for endometriosis does not result in improved ART outcome, but, on the contrary, may compromise ovarian reserve (22–26). Therefore, in the asymptomatic woman, the recommendation is not to intervene prior to IVF. If surgery is performed, more conservative treatment of partial removal and burning of the base may be preferential to a full ovarian cystectomy with laparoscopic stripping of an endometrioma. This recommendation is in line with results from our group (27).

Dermoid cysts or ovarian teratomas are a common ovarian neoplasm in young women of reproductive age and can present as solid hyperechoic heterogeneous masses with a mixed pattern of solid and cystic areas (Figure 52.4). They contain different elements and may contain calcifications, fat, and hair, giving a variable appearance, but commonly the tip of the iceberg sign. They should be removed prior to IVF if they are causing pain or if there is a question of malignancy. Puncture during oocyte retrieval should be avoided due to the high risk of peritonitis. Dermoids should be removed if they are >4 cm as they can rupture or torse with increased pain during pregnancy (28).

Ultrasound and polycystic ovary

Ultrasound is one of the criteria for the diagnosis of polycystic ovary syndrome (PCOS) based on the Rotterdam Consensus conference. Current data suggest that polycystic ovaries detected by transvaginal ultrasonography may be found in approximately 75% of women with a clinical diagnosis of PCOS (29–31). The most commonly used criteria today are those proposed by Dewailly and colleagues with a string of pearls pattern, and these criteria

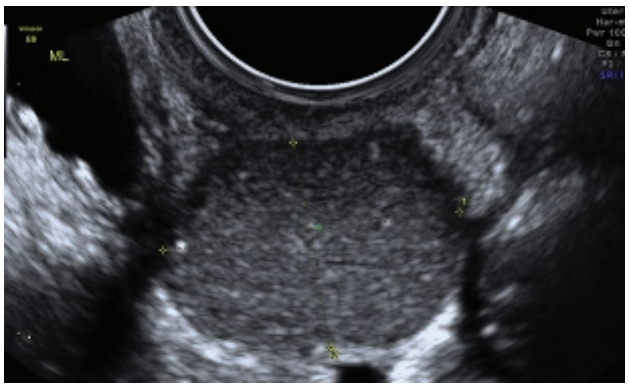

Figure 52.3 Endometrioma with typical ground-glass appearance.

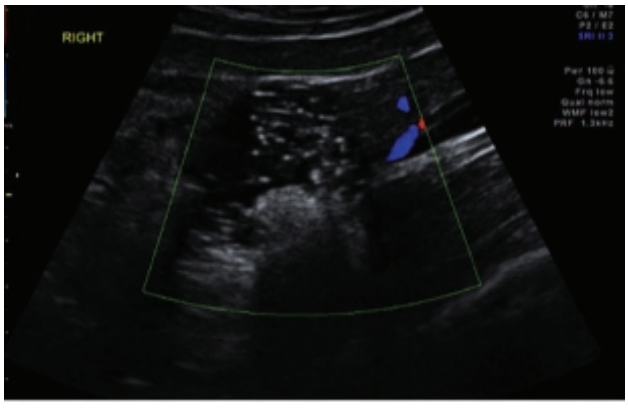

Figure 52.4 Dermoid cyst with hair and fat.

have been reaffirmed in the Rotterdam 2003 consensus (32–34). However, not all women with polycystic ovaries will demonstrate the clinical and biochemical features of PCOS, oligomenorrhea, and/or hyperandrogenism (35). Polycystic ovaries independently, without the full syndrome, constitute a risk factor for the development of OHSS, and the stimulation protocol chosen should reflect this and should be aimed at reducing the risk of OHSS.

Transvaginal ultrasound (TVUS) is a highly sensitivity method for identification of PCOS, and an AFC of >12 in one or both ovaries is mandatory for the definition of polycystic ovaries (36). The 3D ultrasound and the use of color and pulsed Doppler ultrasound showing increased ovarian blood flow are techniques that further enable the identification of PCOS, but are not mandatory for the diagnosis (37). The polycystic ovary definition is established when one ovary (minimum) demonstrates an ovarian volume of greater than 10 cm^3 with no follicles measuring over 10 mm in diameter, or 12 or more follicles measuring 2–9 mm in diameter (small AFs) arranged either peripherally or diffusely with a dense increased volume of ovarian stroma (Figure 52.5). The preferred stimulation protocol in PCOS patients is the antagonist cycle with gonadotropin-releasing hormone (GnRH) agonist trigger to significantly reduce the risk of OHSS.

Ovarian stimulation for IVF: 2D and 3D SonoAVC

Transvaginal sonography has a vital role in monitoring the follicular growth rate in women receiving ovulation induction medications. Ultrasound monitoring of follicle growth during gonadotropin stimulation was first performed in 1978 (38). Gonadotropins cause growth of the cohort of follicles at various stages of development, and monitoring with serial ultrasound and serum E2 is routine with the hope of reducing the risks of OHSS and multiple births. However, data from the Cochrane Database indicate that there is no evidence from randomized trials to support cycle monitoring by ultrasound plus serum E2; it is not more efficacious than cycle monitoring by ultrasound only when measuring outcomes of live birth and pregnancy rates (39). Follicle size in 2D is best estimated by calculating the mean of the maximum follicular diameter in three planes, but is more commonly done in two planes. Follicular growth of 1–3 mm per day is expected once the dominant follicle(s) measure greater that 12 mm. The aim of the use of gonadotropins for controlled ovarian stimulation during an IVF cycle is to obtain the maximum number of mature and good-quality oocytes, as the success improves with numbers. Both nuclear and cytoplasmic maturity are critical and the number of days of stimulation is also a consideration in the formula. Common IVF protocols include the use of either GnRH agonists or antagonists, and in both protocols human chorionic gonadotropin (hCG) is administered when three dominant follicles reach a diameter of 17 mm or greater. Over- or under-stimulation can affect the quality of the oocyte. Use of 3D ultrasound for measuring follicle volumes instead of diameters is being studied to see if there is an ideal follicular volume to time the trigger, and whether outcomes can be improved with more precise measuring of the follicles (40).

SonoAVC (GE Medical Systems, Zipf, Austria) is one of the 3D methods developed for follicular monitoring during controlled ovarian stimulation and may increase the reproducibility of the results (41). First, the multiplanar view is used to ensure the ovary is centrally placed and the render mode is selected to generate a 3D volume of interest box. After SonoAVC is implemented, the individual follicles identified are automatically displayed with a specific color and shown together with their dimensions and relative sizes. Post-processing is required to manually identify those AFs that have been missed in the initial automated

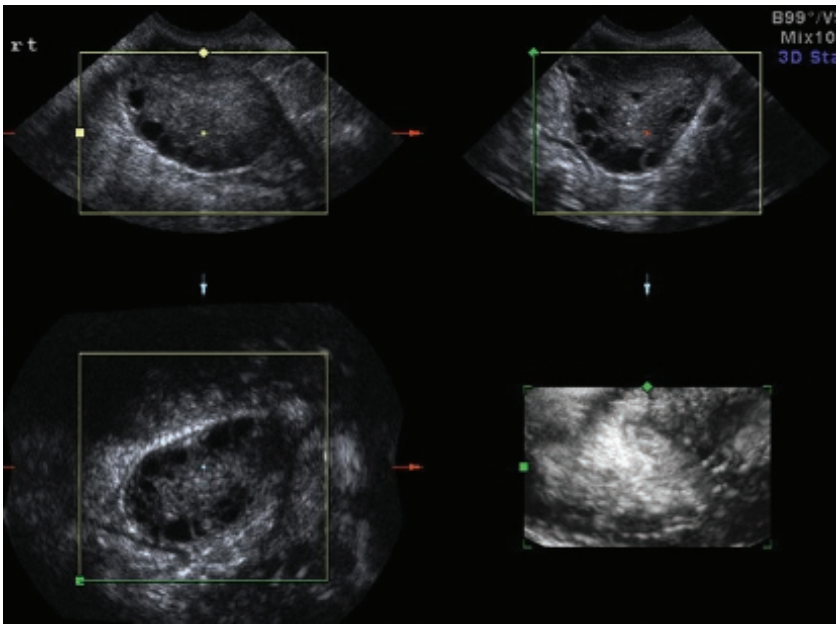

Figure 52.5 Polycystic-appearing ovary in three dimensions.

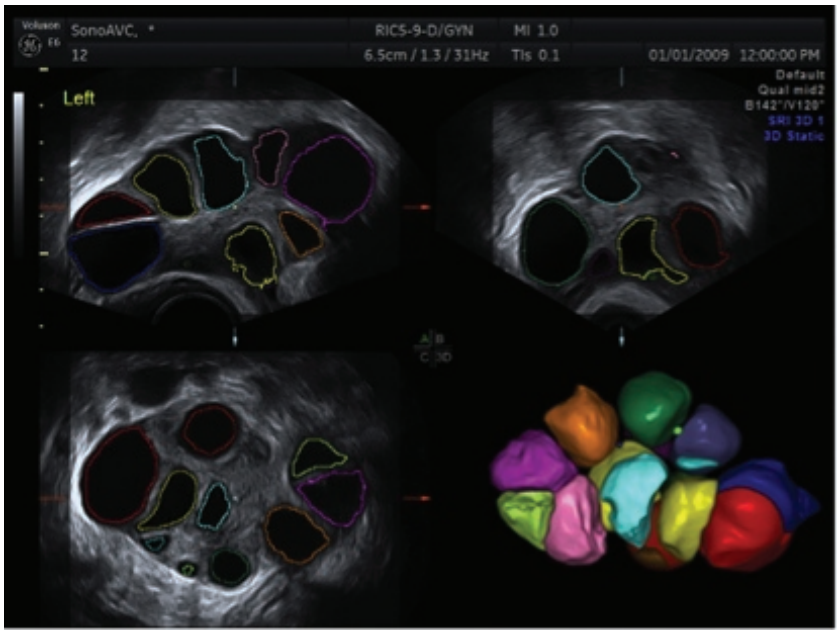

Figure 52.6 Automated follicular monitoring and follicle volumes using the relaxed sphere technique.

analysis, and these are then added (Figure 52.6). There have been improvements in this technology so that false positives and negatives are minimized. The total number of follicles is recorded together with the mean follicular diameter, the volume, and the diameter of each follicle calculated using the relaxed sphere technique (19,42). The volume calculation is based on a voxel count defined by the axes x, y, and z of the follicles (43). The application of SonoAVC for IVF was first described by Raine-Fenning et al. in 2008 (42). Deutch et al. in our practice and Rousian et al. verified the SonoAVC technique using an ultrasound phantom, showing <0.02 mL error comparing the spheres of known volume with a hyperechoic matrix (44,45). Rousian et al. showed that the SonoAVC system underestimated the volume by a mean difference of -0.63 mL in their study, which used larger volumes of spheres than the previous study. There is a correlation between the number and size of follicles in stimulated ovaries with SonoAVC and true follicle volume, showing the accuracy of the system for the stimulated ovary. There is also a relationship between the follicular volume calculation and final oocyte maturation and likelihood of collecting mature eggs (43,46). However, although SonoAVC leads to standardization of follicle measurements, no studies have shown differences in IVF outcomes using this technique. This has not been extensively studied and there is a need for larger randomized studies. Rodriguez-Fuentes et al. in their study found a correlation between follicular volume (determined by SonoAVC), day of hCG administration, and retrieval of mature oocytes (47). They postulated that there is a higher likelihood of obtaining mature oocytes when the follicular volume is ≥ 0.6 mL. However, Raine-Fenning et al. did not find differences in results in a randomized study (46). In this study, they used diameter rather than volume-based criteria for determining the timing of hCG injection. Even with similar results, the advantages of SonoAVC may be an ultrasound exam time decrease as the ovarian volumes are saved, leading to less discomfort for the patients. Rodriguez-Fuentes et al. found a time saving of four minutes per case after including the post-processing time. Their study has shown that SonoAVC provides more accurate results than those of 2D ultrasound imaging when the size of the follicle is larger (47). A comprehensive review of SonoAVC is provided by Vandekerckhove et al., reviewing multiple studies and demonstrating the time-saving feature of the SonoAVC studies (48).

ULTRASOUND OF UTERUS ENDOMETRIAL THICKNESS AND ART MONITORING

Ultrasound assessment of the endometrium and the myometrium of the uterus is important in order to maximize implantation, both in natural pregnancies and in IVF. Endometrial thicknesses and patterns vary throughout the menstrual cycle and are the parameters reviewed in most studies (49). The endometrium is thin immediately after menstruation (2–5 mm), thickens during the proliferative phase, is trilaminar before ovulation, and is thick and echogenic in the secretory phase of the cycle (Figure 52.7). TVUS parameters of the endometrium have long been considered implantation markers in IVF, and abnormalities in the uterus explain many causes for recurrent implantation failure (50–52). A small amount of endometrial fluid may be seen at the end of stimulation in the middle of the cavity. This is thought to be mucus, and can be seen to frequently disappear. However, significant endometrial fluid at the time of ET, usually visible with hydrosalpinges, is associated with poor prognosis, and freezing all the embryos should be considered.

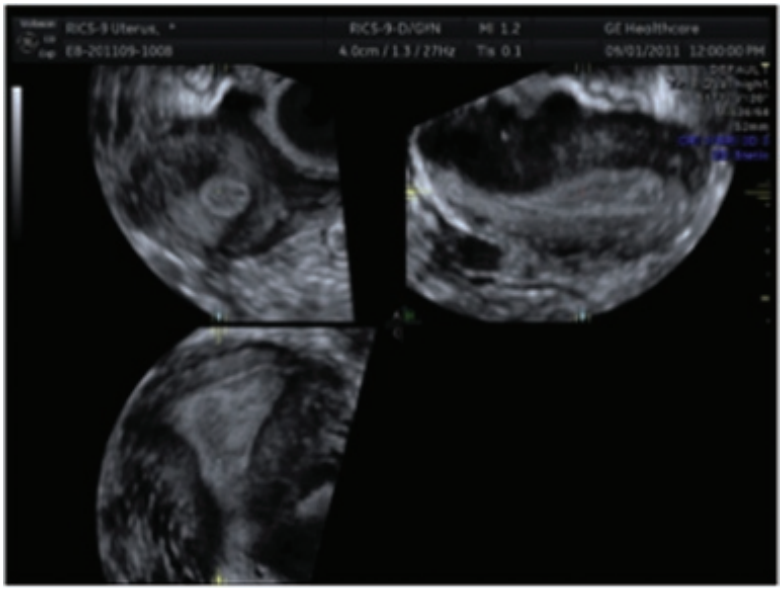

Figure 52.7 Three-dimensional normal endometrium.

Other assessment of the uterus besides the endometrium includes obtaining the size and position of the uterus and the presence of uterine fibroids or adenomyosis. Assessment of the uterine cavity for fibroids, polyps, adhesions, and Mullerian anomalies is mandatory before ART. Uterine imaging is therefore essential in the diagnosis of infertility. These techniques include conventional hysterosalpingography (HSG), 2D ultrasound, 3D ultrasound, and sonohysterography (SHG). Magnetic resonance imaging (MRI) may be considered only rarely for special cases.

Ultrasound examination of the endometrium in a natural or mock cycle supplemented with estrogen and progesterone provides a noninvasive way to evaluate endometrial development and receptivity before ET treatment. Synchronization between the endometrial and embryo development is essential for successful implantation.

SHG and the uterus

SHG, hysterosonogram, or saline infusion sonography are different names for a minimally invasive office technique used for the evaluation of intrauterine abnormalities. By injecting saline into the uterus, the fluid contrast enhances the visualization of the expanded uterine cavity, and filling defects such as polyps, submucosal fibroids, adhesions, and uterine anomalies are more easily visualized (Figures 52.8a and 52.8b).

Randolph et al. were the first to perform a transabdominal ultrasound scan during saline infusion (53). The main uses for SHG are for abnormal uterine bleeding, screening of the uterine cavity prior to ART, and habitual abortion (54–58). Contrast media may also be used for injection, such as saline mixed with air or Echovist® (Schering AG, Germany). The American College of Obstetricians and Gynecologists (ACOG) published a bulletin in conjunction with the American Institute of Ultrasound in Medicine (AIUM), the Society for Reproductive Endocrinology and Infertility (SREI), and with the American College of Radiology (ACR) describing the technique of SHG (59).

Initially, the conventional B-mode transvaginal scan is done to assess the uterus, ovaries, and pouch of Douglas. After gaining consent, the patient is placed into the dorsal lithotomy position. A speculum is inserted into the vagina and the cervix is cleaned with an aseptic solution. A balloon or soft catheter can be used to cannulate the cervix. Once the catheter is in place, the speculum is removed (open sided is easier) and the TVUS probe is inserted to confirm placement. The contrast medium or saline should be injected slowly to decrease bubbles, along with real-time sonographic imaging. Spieldoch et al. in a randomized controlled trial showed that cervical placement of the catheter was less painful than intrauterine placement (60). Intracervical catheter placement resulted in significantly less pain during SHG and also requires half the saline volume to perform. Therefore, intracervical balloon placement should be preferred for SHG. Tubal patency can be assessed if contrast or agitated saline is used to demonstrate flow along the entirety of the tube and spill around the ovary. In most cases, contrast fluid can be seen moving from the cornual end distally with spill into the pouch of Douglas. A detailed examination of the uterus is performed by scanning slowly and systematically from cervix to fundus. A 3D image is helpful and can be processed to review any lesions such as fibroids or polyps affecting the cavity (Figure 52.9) (61). SHG had been described with gel instillation as well (62,63). Evaluating the pelvic anatomy with 3D pelvic ultrasound by saline intraperitoneal sonogram has also been described recently (64). Studies comparing findings on SHG with 2D and 3D ultrasound, HSG, and hysteroscopy show excellent predictive value of SHG and a benefit to the 3D image (56–58). A prospective study of 65 infertile women up to 43 years of age compared the diagnostic accuracy of 2D SHG with that of HSG and 2D

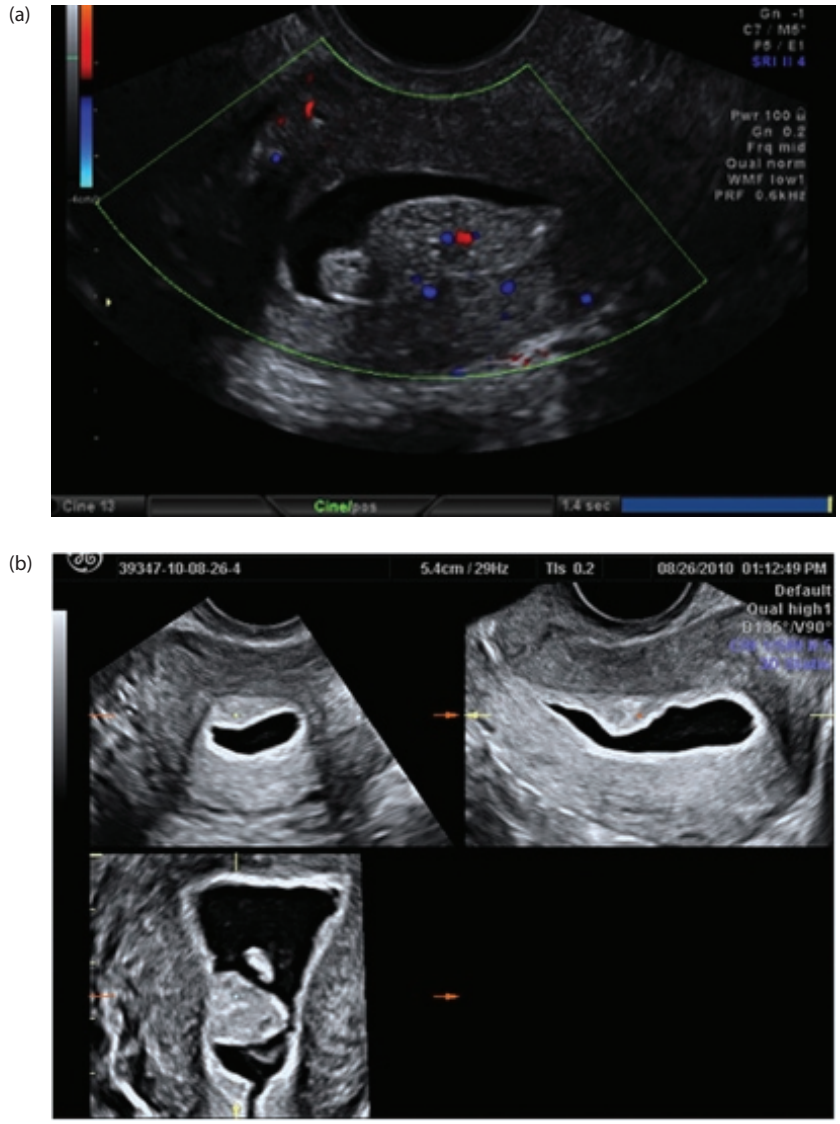

Figure 52.8 Saline sonogram of uterine polyp. (a) Color Doppler showing feeding vessel into polyp; (b) Pre-operative evaluation—multiplanar view of the uterine polyp.

TVS using hysteroscopy as the gold standard for intracavitary abnormalities (65). Although polyps and fibroids were detected on TVS accurately, intrauterine adhesions (IUAs) were not. SHG and HSG had similar sensitivities of 75% in the detection of IUAs and respective positive predictive values (PPVs) of 42.9% and 50%, while TVS showed a sensitivity and PPV of 0% for this diagnosis. Therefore, for adhesions, SHG had limited accuracy, similar to that obtained by HSG, with a high false-positive diagnosis rate, but better than 2D alone (65). SHG was more accurate for polyps. In a recent study comparing 2D with 3D SHG, Kowalczyk et al. reported 3D SHG to be superior to hysteroscopy to detect uterine anomalies with a sensitivity of 72% and specificity of 96% compared with a sensitivity and specificity of 83% and 99% and a diagnostic precision similar to the results achieved by hysteroscopy (66). In another study, Makris et al. compared 3D SHG diagnostic hysteroscopy in 242 women with abnormal uterine bleeding (67). In their study, there was a similar specificity of 99.4% but a higher sensitivity for hysteroscopy compared to 3D SHG (98.7% vs. 93.5%, respectively). The two techniques were in agreement for eight cases of adhesions and in 165 cases of normal endometrium. Therefore, hysterosonography is an accurate screening test.

Uterine abnormalities are very common both in infertility and abnormal bleeding patients. In a prospective study of 600 infertility patients by Tur-Kaspa et al., 20% were found to have cavitary abnormalities, including arcuate uterus (15%), polyps (13%), submucosal fibroids (3%), and adhesions (1%) (68). This prospective study compared the incidence of uterine cavity anomalies in patients referred for infertility or abnormal bleeding. More patients in the bleeding group had intracavitary abnormalities such as polyps, fibroids, and adhesions, as well as intramural abnormalities, and the infertility group had more congenital uterine anomalies.

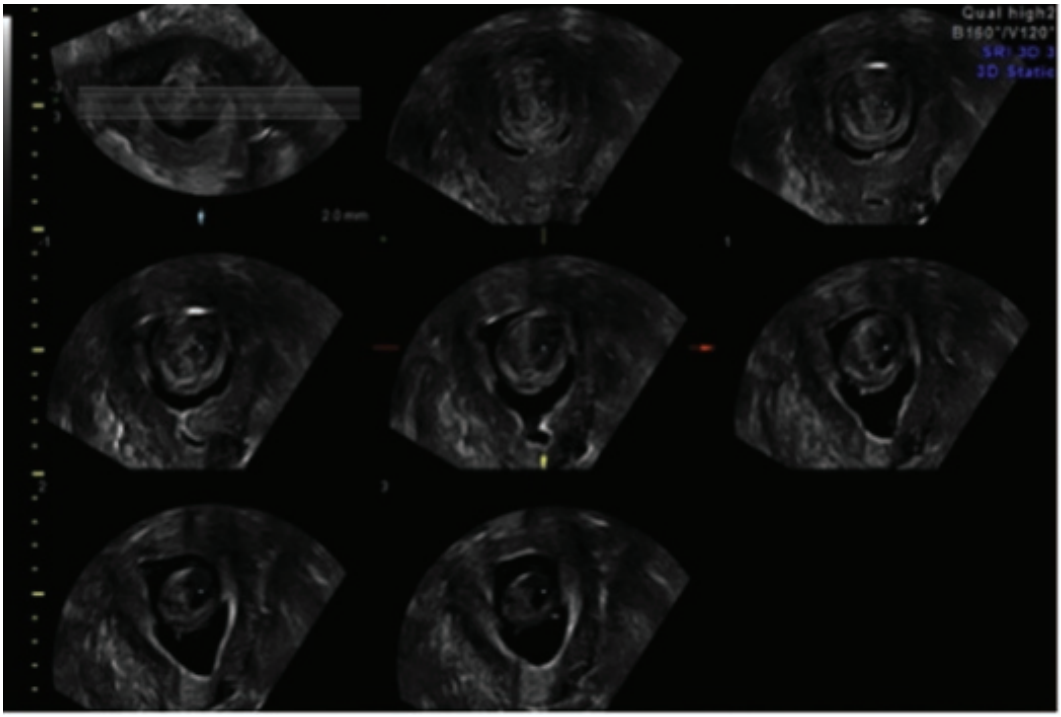

Figure 52.9 Saline sonogram of submucosal fibroid tomographic ultrasound image (TUI).

In another study by Alborzi et al., SHG (compared with HSG) showed higher specificity and negative predictive values (NPVs) for detection of uterine and tubal abnormalities (69). They reported a sensitivity, specificity, PPV, and NPV of 78.2%, 93.1%, 82.7%, and 91%, respectively, compared to 76.3%, 81.8%, 90.9%, and 59.2%, respectively, for HSG. SHG is very accurate for pathology with less pain and no exposure to radiation, and 3D is desirable to evaluate the entire cavity by using section places in order to scroll through. There are further advantages of SHG over HSG, such as the ovaries and other non-cavitary lesions being able to be seen at the same time.

Acquired uterine abnormalities

Multiple studies support the hypothesis that intramural fibroids have a detrimental impact on pregnancy and on implantation rates in IVF, especially when the fibroids are large (70). This includes cesarean sections, preterm delivery, preterm rupture of membranes, and hemorrhage. The mean gestational age at delivery for women with fibroids larger than 5 cm is 36 weeks, significantly earlier than women with smaller fibroids or no fibroids (71). Assessment of uterine fibroids has been most commonly achieved using ultrasonography. The large uterus may require abdominal ultrasound as well, but in general TVUS is satisfactory. For intramural and submucosal fibroids, 3D ultrasound, especially in the coronal view, is a way to map the position and distance from the endometrial cavity. The addition of saline infusion can help with the type of surgical approach chosen to remove submucosal fibroids and to subtype the fibroid. SHG is better at showing the cavity involvement and the percentage of the fibroid in the cavity and in the myometrium (72). The 3D multiplanar display is also useful in some cases for differentiating adnexal lesions close to the uterus from lesions within or originating from the uterus. MRI is superior in selected cases where the fibroids are large and outside of the pelvis, leading to shadowing on TVUS (73,74). An unpublished study by the authors on the imaging of uterine fibroids reveals that the number of fibroids is underestimated by ultrasound compared to those found at surgery and MRI, even when 3D imaging is used (75). Use of 3D ultrasound and 3D SHG for determining the position of the fibroids can be seen in Figure 52.9.

Another method that may be helpful to identify fibroids is the use of color Doppler. Since the fibroid is surrounded by a rich vascular supply, a myoma will usually demonstrate a "ring of fire." It is important to make the diagnosis of fibroids and to identify the women that will benefit from a myomectomy. A recent systematic review confirmed that ART outcomes are decreased in women with submucosal fibroids, and hysteroscopic removal improves the outcome significantly (76). A meta-analysis study showed a significantly lower pregnancy rate if submucosal fibroids are present (Relative risk [RR] 0.32, 95% confidence interval [CI] 0.12–0.85), and that removal of submucosal fibroids improves clinical pregnancy rates with an RR of 2.03 (95% CI 1.08–3.83) (77). Subserosal fibroids do not affect fertility, and removal does not confer a benefit. Intramural fibroids appear to decrease fertility and implantation rates with IVF, but the results of myomectomy are unclear. The distance from the uterine cavity and the size of the fibroids may be relevant. Two randomized controlled trials failed to demonstrate a clear benefit (76). Other non-surgical

treatments of fibroids such as uterine artery embolization and magnetic resonance-guided focused ultrasound procedures are options, and pregnancies have been reported (78), although they both carry a higher risk for complications in pregnancy. For uterine artery embolization, MRI is recommended prior to the embolization procedure for best results. The Exablate magnetic resonance-guided focused ultrasound (MRgFUS) treatment uses a "sonication" process in which a focused ultrasound concentrates a high-energy beam on a specific point, raising its temperature and destroying fibroid tissues by coagulation necrosis. Review of the literature on pregnancy outcome after MRgFUS revealed 88 pregnancy cases. The mean time to conception was eight months after treatment. Of those 88 pregnancies, there were 45 (51%) total deliveries, 67% spontaneous vaginal deliveries, and 33% cesarean sections. However, 19 of those 88 (22%) women experienced spontaneous abortions. Rabinovici et al. noted that uterine rupture, preterm labor, placental abruption, and abnormal placentation leading to fetal growth restriction were not observed in any of the cases, unlike the uterine artery embolization (UAE) procedures (79).

Adenomyosis may displays several appearances by ultrasound, making the diagnosis uncertain in some cases. Adenomyosis meets traditional radiological criteria such as the presence of an enlarged "globular" uterus in the absence of fibroids, asymmetric thickening of the anterior or posterior myometrial wall, heterogeneous poorly circumscribed areas within the myometrium, anechoic myometrial blood-filled lacunae or cysts of varying sizes, increased echo-texture of the endometrium, and sub-endometrial linear striations (80). On ultrasound, it appears as an asymmetry and thickening of the uterine walls with loss of endometrium–myometrium border and hypoechogenic nodules in the myometrium (Figure 52.10). However, adenomyosis may appear hyperechoic, hypoechoic, or the signal may be mixed. Adenomyosis can enlarge or shrink throughout a menstrual cycle, depending on the hormonal response. In some cases, adenomyosis forms a nodular myometrial mass that is readily identified by ultrasound,

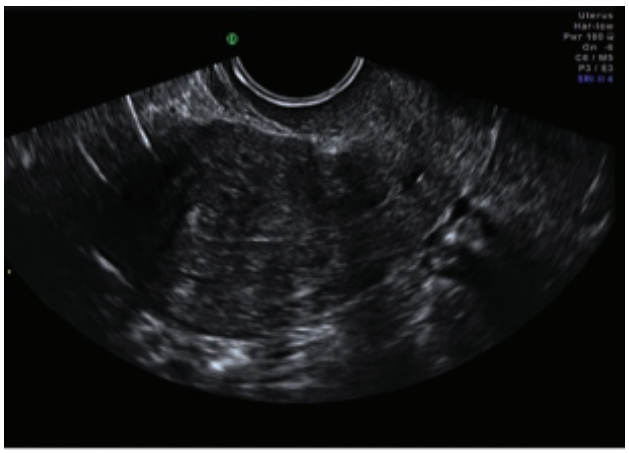

Figure 52.10 Adenomyosis with the "venetian blind" shadowing appearance.

which is called an adenomyoma. Adenomyosis can also be a diffuse condition affecting a large segment of the myometrium, with the only ultrasound finding being a subtle uterine enlargement. Sometimes, adenomyosis and uterine fibroids have a remarkably similar appearance with ultrasound, and some women have both conditions. Color Doppler studies are helpful to distinguish uterine fibroids from adenomyosis, since vascular flow is peripheral with fibroids and more homogeneously affects adenomyosis lesions. Fibroids must be differentiated from adenomyosis, especially when surgery is considered, since resection of adenomyosis and repair of the defect can be difficult (81,82). Whether adenomyosis in itself is a cause of infertility is controversial. However, in a meta-analysis of published data, women with adenomyosis had a 28% reduction in the likelihood of a clinical pregnancy with IVF/intracytoplasmic sperm injection (ICSI) (83).

Endometrial polyps are the most common endometrial anomaly and may be found in about 15% of infertile women (68). TVUS is the imaging method of choice, and saline sonography, especially 3D, may be helpful in locating the exact location of the polyp (84). Endometrial polyps appear as ovoid echogenic masses that project into the endometrial lumen without myometrial involvement and are best seen in the follicular phase when the endometrium is thinnest and the least echogenic. Doppler ultrasound shows a feeding vessel in many cases (Figure 52.8a); however, a polyp may present as a diffuse thickening of the endometrium, which poses a difficulty to the ability to detect polyps (72). Salim et al. reported that, for TVUS, the sensitivity is 19%–96%, specificity is 53%–100%, PPV is 75%–100%, and NPV is 87%–97% when compared with hysteroscopy with guided biopsy (72). 3D may improve the results, as Kupesic and Kurjak reported that 3D ultrasound has sensitivity of 100%, specificity of 99%, PPV of 99%, and NPV of 100% for diagnosing endometrial polyps when compared to hysteroscopy with biopsy (85).

It is controversial whether endometrial polyps contribute to infertility or miscarriages. Some studies show it depends on the location and size of the polyps. Therefore, automatic surgical correction is not indicated when polyps are found, since it depends on the polyps' sizes and locations (86,87). However, surgery should be performed for submucous fibroids and endometrial polyps when there is recurrent implantation failure or recurrent pregnancy loss. Following surgery, there is no need to wait, as IVF success is no different in the cycle after hysteroscopy compared to waiting a month (88).

Tiras et al. investigated the impact of endometrial polyps on pregnancy rates in 8359 ICSI patients (89). The study included all fresh ICSI cycles performed in the Anatolia IVF Center between 2005 and 2009. All patients diagnosed with an endometrial polyp by TVUS before the ICSI cycle underwent hysteroscopic polyp resection. Localization of the polyp (upper, middle, or lower third of the uterine cavity) or polyp size (4–14 mm) did not seem to affect pregnancy rates. They concluded that endometrial polyps less than 1.4 cm found during ovarian stimulation did not

affect pregnancy rates, miscarriage rates, and live birth rates in ICSI cycles, and that patients with an endometrial polyp detected before ICSI treatment and resected by hysteroscopy had similar pregnancy rates compared with patients with no endometrial polyps (90). However, there are other studies showing the benefit of polypectomy, leading to higher pregnancy rates after hysteroscopy and polyp biopsy (90,91). In addition, in small studies, even polyps of <1.5 cm removed hysteroscopically during stimulation led to pregnancies without cycle cancelation (92,93). The interval between polyp resection and ET was 2–16 days. Four patients achieved pregnancy (two twins and two singletons), four patients were unsuccessful, and one pregnancy was a blighted ovum. Based on the evidence, the management of polyps seen during the course of IVF should be individualized after consideration of the number of embryos created, the previous reproductive history of the patient, and the individual clinic's success rate for its frozen ART program. Hysteroscopic polypectomy just before ET should be kept to a minimum, as recent studies failed to prove any deleterious effect of small endometrial polyps <1.5 cm on pregnancy rates and pregnancy outcomes in ICSI cycles, and there is a fear of disturbing the endometrial cavity too close to the time of transfer. In conclusion, further studies are required to identify the most appropriate management of endometrial polyps found during IVF stimulation.

Intrauterine adhesions (IUA) also present as acquired uterine anomalies and are, in most cases, the result of retained products of conception after pregnancy and repeat curettage for incomplete abortions. Myomectomy for intracavitary fibroids and uterine artery embolization are also causes. As mentioned previously, adhesions are not seen very clearly with basic ultrasonography. There have been reports of MRI appearances in four cases of Asherman's syndrome in which the diagnosis was confirmed by hysteroscopy. However, the full range of MRI appearances in Asherman's syndrome has not been established, and there has been only one case reported in the literature (94). Figure 52.11 shows IUAs using a multiplanar view after SHG. The study by Knopman and Copperman showed that 3D imaging was very accurate in Asherman's syndrome in a case series of 54 infertile patients with thin endometrial linings (95). IUAs were diagnosed on 3D ultrasound and HSG in all cases and confirmed by hysteroscopy. They reported 100% sensitivity with 3D ultrasound for correctly grading the extent of IUAs compared to only 66.7% for HSG. HSG overdiagnosed the extent of the Asherman's segment outflow obstruction. For the surgical treatment of Asherman's syndrome, ultrasound guidance may aid in the hysteroscopic lysis of dense scar tissue and difficult entry into the cervix. Importantly, an obliterated cavity may require multiple hysteroscopic treatments (96,97). In the largest study involving 6680 hysteroscopies with hysteroscopic adhesiolyses in 75 patients, 94.6% functional restoration and 93.3% anatomic resolution, with pregnancy rates ranging from 28.7% to 53.6%, was achieved (98). At the two-month follow-up, the uterine cavity was completely restored in 70 cases, while in four cases a second surgical treatment was necessary.

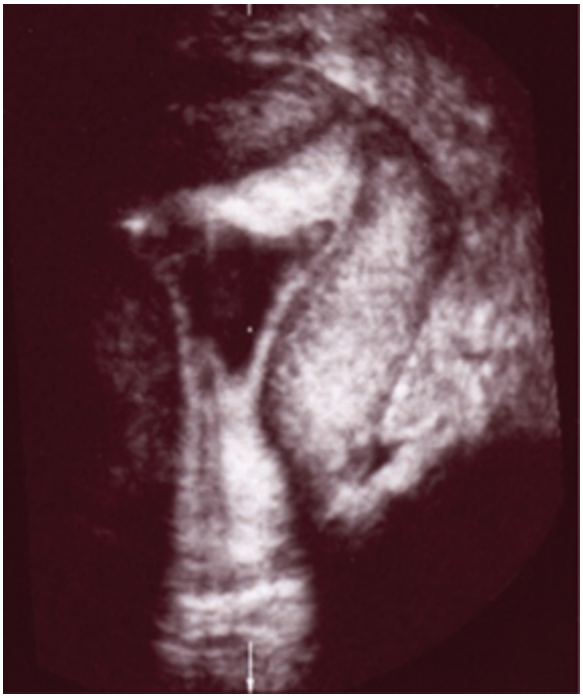

Figure 52.11 Intrauterine adhesions after saline sonogram.

Congenital uterine anomalies

Mullerian anomalies are congenital defects in the development of the uterus and upper vagina. It has been demonstrated that conventional 2D ultrasound imaging is a good screening tool for the detection of congenital uterine anomalies and has a high sensitivity for some anomalies (99). However, 3D ultrasound, especially the coronal view, is superior to 2D and has been accepted as the first-line test and shown to be as accurate as MRI in the detection of congenital anomalies but with difficulty visualizing the vagina (100). New grading systems have been published based on 3D ultrasound. Precise classification of a uterine anomaly is of clinical importance because the need for surgical intervention and the type of intervention depend on this distinction.

Again, the mid-coronal view is the most important view for congenital anomalies. With 3D ultrasound, a volume of ultrasonographic data is acquired and stored. The stored data can be reformatted and analyzed in numerous ways; navigation through the saved volume can demonstrate innumerable arbitrary planes. The optimal time to examine patients for the presence of uterine anomalies is the luteal phase of the cycle, when the endometrium is thick and echogenic and the cavity can be clearly differentiated from the surrounding myometrium. The most important advantage of 3D ultrasound over HSG is the ability to visualize both the uterine cavity and myometrium. It provides complete information about the nature and extent of uterine masses and congenital anomalies. A number of studies have shown complete agreement and accuracy between the congenital uterine anomaly seen on 3D ultrasound with that of HSG or hysteroscopy and laparoscopy as the gold standards (101,102). Controversy exists over whether

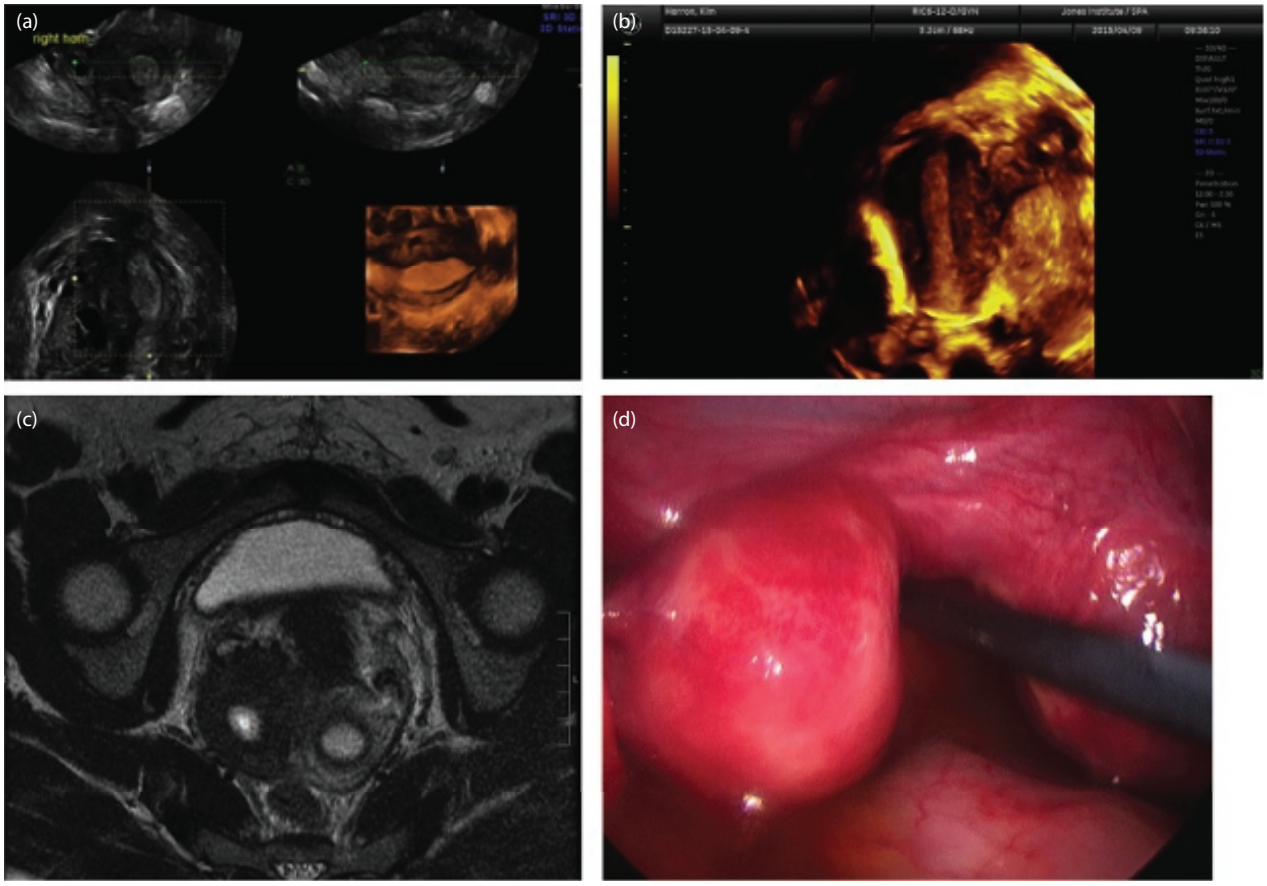

Figure 52.12 (a) Three-dimensional (3D) manipulation of unicornuate uterus. (b) Unicornuate uterus non-communicating horn in 3D. (c) Magnetic resonance imaging of unicornuate uterus with non-communicating horn. (d) Laparoscopy of unicornuate uterus with horn.

an arcuate uterus, defined by an endometrial dip <1 cm, carries any clinical significance, as many studies show a poorer obstetrical outcome and an increase in infertility associated with an arcuate uterus. There is a large difference in the incidence of uterine anomalies in the population of infertile women, varying from 6% in some studies to 66% in others (101–103). Figures 52.12 and 52.13 show the 3D visualization of a unicornuate uterus, a unicornuate uterus with a non-communicating horn via MRI, and findings at surgery.

Monitoring endometrial thickness during the ART cycle

The endometrium is the innermost glandular layer of the uterus and the location for embryo implantation. Morphology and thickness as well as volume of the endometrium can be visualized by ultrasound. The endometrial thickness and pattern are noted during ovarian stimulation in IVF and ovulation induction cycles, and have been noted to affect pregnancy rates. Endometrial pattern can be classified as type A, a multilayered triple-line endometrium consisting of a hyperechogenic outer and central lines, as shown in Figure 52.7; type B, an intermediate isoechogenic pattern with a non-prominent central line; and type C, an entirely homogeneous endometrium. Most commonly, the endometrium is described as "triple-line" or "homogeneous." The best pattern is the trilaminar with a central echogenic line, inner hypoechoic regions, and hyperechogenic outer walls compared with the homogeneous pattern (104). There is controversy regarding the value of measuring endometrial thickness to predicting pregnancy during IVF treatment. Endometrial thickness is measured from outside to outside in an anterior–posterior view at the widest point. Patients with a thin endometrium following ovarian stimulation have a significantly lower pregnancy rate but have yielded a high percentage of false-positive results (50). Low-dose aspirin, vaginal sildenafil (Viagra), and pentoxifylline have been used to treat patients with thin endometrium (105,106). The underlying assumption is that patients with thin endometrium have suboptimal endometrial blood flow and may have scar tissue, and aspirin or Viagra increase the endometrial blood flow and endometrial development (107). However, studies do not consistently show increased uterine receptivity and IVF success with these agents and are based on small numbers. No consensus has been reached with regard to the minimum endometrial thickness required for successful pregnancy. In one study, pregnancies did not occur when the endometrial thickness was less than 7 mm (108). Other studies found that a minimum endometrial thickness of

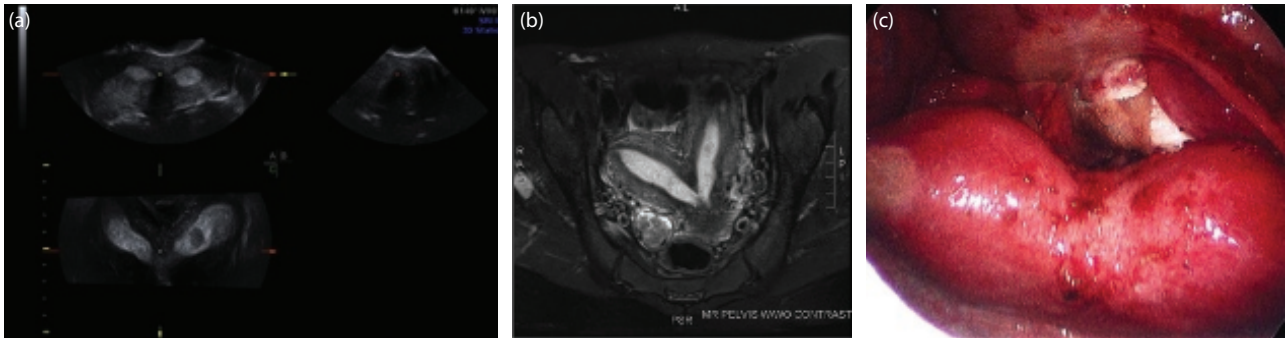

Figure 52.13 (a) Three-dimensional view of bicornuate uterus. (b) Magnetic resonance imaging of bicornuate uterus. (c) Laparoscopy of bicornuate uterus.

6 mm is acceptable for implantation (109–112). In a recent study, the thinnest endometrial lining for successful ongoing pregnancy was 5.8 mm, and the maximum number of conceptions occurred when the thickness was 8–10 mm (113). With increasing endometrial thickness (>14 mm), a high miscarriage rate was reported by Weissman et al. (114). An excessively thick endometrium may start in a previous cycle, so ovarian stimulation should not be started following menstruation if the endometrial thickness is greater than 6 mm. Increased preclinical or biochemical miscarriages are also seen when the endometrial thickness is 6–8 mm verses 9 mm or greater (115). These findings correlate well with the recent report of increased pregnancy loss with low endometrial volume on the day of the first pregnancy test 14–18 days after oocyte retrieval (116). The thinnest reported lining in a successful pregnancy was with an endometrial thickness of 4 mm, so this still remains controversial (117). In another 2001 study, it was reported that implantation is unlikely when the endometrial thickness is <5 mm (118). Despite this first study, the majority of studies show a deleterious effect of thin endometrium. There is a high consensus to recommend embryo cryopreservation in cases of thin and non-trilaminar endometrium because the likelihood of implantation is low.

Assessment of endometrial blood flow in IVF treatment with Doppler and 3D imaging has been studied to see if it is predictive of implantation. Importantly, Doppler studies of uterine arteries do not reflect the actual blood flow to the endometrium. Endometrial and sub-endometrial blood flows may be more objectively and reliably measured with 3D power Doppler ultrasound. Doppler can measure the pulsatility index of the uterine arteries, and elevated levels are associated with low implantation and pregnancy rates in one study, but not in others (51). The absence of color Doppler mapping at endometrial and sub-endometrial levels can be associated with a significant decrease in pregnancy and implantation rates, while flow-through vessels at the endometrial and sub-endometrial levels are associated with increased rates. The use of 3D ultrasound for calculation of the endometrial volume has also been studied. Some studies show that endometrial volume can better predict implantation rates over endometrial thickness (119). Endometrial volume of <2.5 mL is predictive of a low pregnancy rate or pregnancy loss, but it is not found to be predictive of pregnancy if the endometrium attains at least 2.5 mL or 1 mL (120). With the addition of Doppler, it was found that the endometrial and sub-endometrial vascularity were significantly lower for patients with low-volume endometrium when compared with those with normal-volume endometrium, but these did not correlate with the endometrial thickness. Merce et al. concluded that 3D ultrasound and power Doppler angiography are useful exams to assess endometrial receptivity in IVF/ICSI cycles (51). Doppler in 2D, however, has not been shown to benefit fertility at this time in studies with large numbers (51). There has been much attention on the progesterone levels at the time of hCG administration. Several studies have suggested that a premature secretory endometrial pattern is caused by the advanced progesterone rise, and this premature conversion has an adverse effect on pregnancy rates. The reason that the no-triple-line endometrial pattern is observed prior to ovulation in some women is not known and cannot be explained by higher progesterone levels. In the study by Ng et al., three patients with calcifications in the uterus and two with fluid in the endometrial cavity on the day of hCG did not conceive (121). Other poor prognostic factors include fluid in the endometrial cavity or calcifications in the uterus. In these cases, freezing all the embryos until an evaluation of the uterine cavity can be done may be recommended.

In conclusion, although characteristics of the human endometrium including thickness (volume), morphology, endometrial blood flow, and vascularization can be readily and noninvasively monitored by ultrasound, there still is not a clear correlation between the patterns and successful implantation. However, the 3D studies show better correlations than 2D ones.

ULTRASOUND OF THE FALLOPIAN TUBES

Fallopian tube patency

Normal fallopian tubes are usually not visualized by ultrasound. Evaluating tubal patency is a crucial step in the work-up for infertility, and making a diagnosis of hydrosalpinx is important prior to IVF. HSG has been the standard treatment for assessment of the fallopian tubes, but has high false-positive rates. When compared to laparoscopy

with chromopertubation, HSG has a false-positive rate of 20% and has disadvantages of exposure to radiation and pain (122). Tubal occlusion—unilateral or bilateral—is seen in approximately 20% of women with infertility (123).

Richman et al. were the first to describe in 1984 the use of transabdominal ultrasound for the diagnosis of tubal patency with saline contrast (124). After transcervical installation of saline, the cul-de-sac was evaluated for the appearance of free fluid. During the preliminary ultrasound, the posterior cul-de-sac and pelvis were evaluated for the presence of free fluid. If none was present before injection of fluid and it was present after fluid injection, then it was concluded that at least one tube was patent, but which tube this indicated was not clear. Since the development of this first technique, significant advances have been made in ultrasound technology, including the advent of transvaginal sonography, 3D volume sonography, and other contrast agents (125).

For infertility patients, the advantage of the use of ultrasound is the ability to see the adnexal structures, the uterus for polyps, fibroids, or congenital anomalies, as well as the presence of hydrosalpinges. Additional contrast material or a small amount of air is injected with the fluid with concurrent real-time sonographic imaging in the cornual plane of the adnexae and cul-de-sac to assess tubal patency. Recent studies demonstrate a good correlation with HSG. The ultrasonographic evaluation of tubal patency is referred to as hysterosalpingo-contrast sonography (HyCoSy). HyCoSy can be performed using a negative contrast agent such as saline or a positive contrast agent such as Echovist 200 (126). HyCoSy in the ultrasound is usually performed by injecting a small amount of saline into the uterus via an intrauterine balloon catheter, as the contrast agent is not U.S. Food and Drug Administration (FDA) approved. During the installation process, a TVUS is performed to assess the tubal flow of contrast material and/or the accumulation of contrast material in the pouch of Douglas (53). Two meta-analyses and reviews have been published showing that 2D HyCoSy is a sensitive and specific procedure to evaluate tubal patency, with a more than 80% agreement with chromopertubation (127). A 3D evaluation has been published and a recent systematic review comparing 3D with 2D HyCoSy shows 3D superiority (128).

Agitated saline is used in lieu of commercially manufactured contrast material in the ultrasound. Agitated saline is produced by placing 19 cc of saline and 1 cc of air in a 20-mL syringe. The syringe is then vigorously shaken and the mixture is injected into the uterus using a balloon catheter (129). Sonographic criteria for tubal patency were bubbles entering the fallopian tube without production of a hydrosalpinx or exit of bubbles into the peritoneal cavity. The results showed tubal patency was confirmed in 89% of the tubes (Figure 52.14).

The disadvantages of HyCoSy include the difficulty at times of following the passage of contrast through the entire length of the fallopian tube and the difficulty of visualizing the tube in a single plane. Therefore, 2D HyCoSy requires significant skill on the part of the ultrasonographer (130). If positive contrast media are used, it can be challenging to differentiate the echogenicity of the contrast material from the surrounding bowel. Therefore, the visualization of true spill from the fimbriated end of the fallopian tube and visualization of the fimbria remain difficult. Tubal pathology such as mucosal folds or salpingitis isthmica nodosa cannot be evaluated using HyCoSy. Still, from the meta-analysis, 3D HyCoSy has been shown to be an accurate test for diagnosing tubal occlusion in women with infertility.

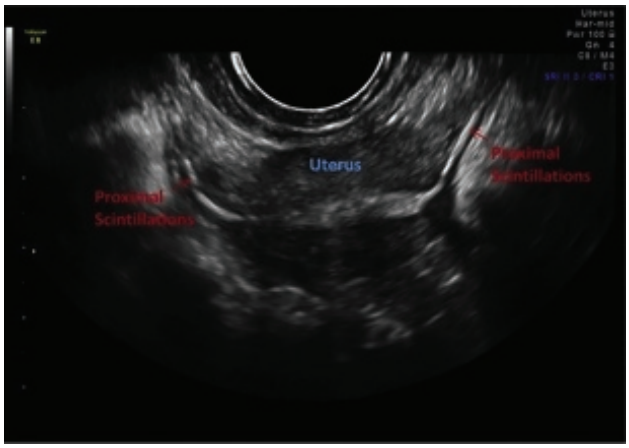

Figure 52.14 Transverse two-dimensional view of uterus with installation of fluid.

Doppler and 3D ultrasound

3D HyCoSy with color power Doppler has been shown to increase the ability to depict true tubal patency by free spillage of contrast material from the fimbriated end of the fallopian tube. In addition, it more accurately differentiates free fluid of echogenic contrast from the bowel. One study demonstrated that free spill of contrast material was seen 91% of the time with 3D HyCoSy and only 46% of the time with 2D HyCoSy (131). In addition, 3D HyCoSy with color power Doppler seems to be accurate, as it was found to agree with laparoscopy with chromopertubation 99% of the time. Blood flow and Doppler are additional modalities that can be employed in conjunction with HyCoSy (132).

The use of color Doppler with 2D HyCoSy has been shown to increase the ability to diagnose true tubal occlusion and to help differentiate between the contrast material that is spilling out of the tube and the surrounding bowel. Agitated saline can be seen flowing through the tubes by using the blood flow technique of Doppler. The accuracy of color Doppler mapping for tubal patency had a sensitivity of 76%, a specificity of 81%, a PPV of 66%, and an NPV of 89% (133). This technique was never incorporated into routine evaluation of infertile women.

For 3D HyCoSy, ultrasound should be used to sweep the region of interest along the entire tubal length. As a result, 3D HyCoSy can visualize the volume of the tube and is less operator dependent and easier to perform. 3D color power Doppler can also be used to depict the flow of contrast material through the entire length of the fallopian tube, so it has clear advantages over the use of HyCoSy alone. It has been shown that visualization of distal tubal

spill occurs twice as often when 3D color power Doppler is employed (133). Since the procedure relies on the technical ability of the clinician performing the procedure, it is still not routinely performed.

Hydrosalpinges and ART outcome

Hydrosalpinges are common causes of infertility, decreasing IVF pregnancy rates by 50% (134). Studies have shown embryotoxic fluid in the cavity, and prophylactic salpingectomy, or tubal ligation, is recommended to improve pregnancy rates. Even hysteroscopic tubal occlusion had been suggested for some patients (135). Studies show that the hydrosalpinx fluid may affect endometrial development and contraction (136). If a hydrosalpinx appears during stimulation, ultrasound-guided aspiration of hydrosalpinges at oocyte collection can be an option. A randomized, blinded study showed that 30% of the fallopian tubes in the aspiration group re-accumulated by 14 days after the aspiration (137). This study implies that the window of opportunity may be present at oocyte aspiration, but not significantly earlier, and even then there may be fluid re-accumulation by the time of transfer. Aboulghar et al. reported that aspiration one month prior to retrieval did not improve pregnancy rates (138). If a hydrosalpinx develops during stimulation, an alternative option is to freeze all embryos and to perform a salpingectomy later (Figure 52.15).

Ultrasound-guided IVF procedures: Oocyte retrieval and ET

Laparoscopy was the first technique used for oocyte retrieval. The ultrasound-guided follicular aspiration was first described in the early 1980s by a Danish group, Lenz and Lauritsen, using abdominal ultrasound (139). TVUS-guided oocyte retrieval has been the current standard of care since the late 1980s, and is associated with a low complication risk of injury to bladder, bowel, or bleeding from blood vessels. It is usually performed under sedation with a 17-gauge needle. Thinner needles and lower pressures should be used for the *in vitro* maturation technique of aspiration of immature eggs and smaller follicles. The tip of the needle is echogenic and can be visualized at all times and is aligned with the ultrasound beam. The current standard of care for oocyte retrieval is transvaginal aspiration under ultrasound guidance, and there are no randomized controlled trials comparing techniques of transabdominal versus transvaginal approaches. Flushing of follicles has not been shown to make a difference in oocyte yield, takes more time, and should not be routinely performed.

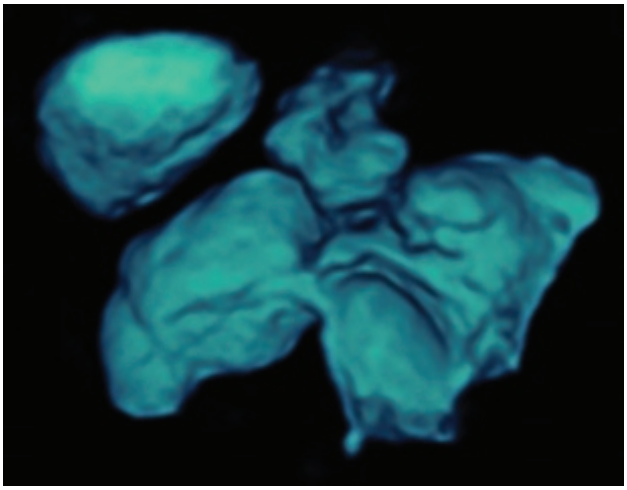

Figure 52.15 Three-dimensional rendered view of hydrosalpinx.

ET is a critical step in ART cycles and can be performed with or without ultrasound guidance. However, recent Cochrane reviews demonstrated a significant difference between "ultrasound-guided" and "clinical touch" methods, so utilizing ultrasound during ET has become the standard of care. The ultrasound-guided ET significantly improved clinical pregnancy rate (odds ratio 1.49, 95% CI 1.29–1.72, $p < 0.00001$) (140–144). There were no statistically significant differences for other clinical outcomes such as ectopic pregnancy, miscarriage, or multiple gestation rates. Ultrasound guidance of the transfer catheter resulted in higher pregnancy rates in all but one of the studies identified; this difference was significant in five of the eight studies, and the preferred use of the soft catheters was shown. The one study that did not show any difference had a single, very experienced operator. A Cochrane review also compared the incidence of retained embryos for ultrasound versus clinical touch (3.2%–10.0%) (145). The most recent Cochrane review showed that ultrasound-guided embryo transfer was associated with improvement in clinical pregnancy rates, live birth rates with odds ratio 1.47 (1.30–1.65) compared with clinical touch. The majority of programs are using ultrasound guidance and our experience has shown improvement. The ultrasound is ideal in training programs with the use of simulation prior to performing real transfers.

The advantages of ultrasound guidance are that the physician can avoid touching the fundus, which has been shown to be deleterious, and can reduce the incidence of difficult transfers by allowing the direction of the catheter to follow the contour of the cavity and ensuring embryos are placed properly (146). Several transfer catheters with echogenic tips have been produced, making it easier to visualize placement, but no significant increase in pregnancy rates has been demonstrated. A few studies comparing 2D versus 3D ultrasound guidance show a possible advantage of 3D ultrasound for monitoring catheter placement, but this is not commonly used (147,148).

Ultrasound for the diagnosis and treatment of ART complications and outcome

Ultrasound for the diagnosis and treatment of OHSS

OHSS is a serious iatrogenic condition that arises in women undergoing ovulation induction with fertility medication, and occurs during the luteal phase of the ovulatory cycle after hCG trigger, usually peaking

three to seven days later or during early pregnancy. The incidence of severe OHSS ranges from 0.5% to 5%, with increased risks in women with PCOS, thin body habitus, young age, the use of long GnRH agonist protocols, and high E2 levels (149). Preventing OHSS is crucial in ART treatment and strategies which have been used in order to minimize the risk include the use of the GnRH antagonist protocol with the GnRH agonist trigger. It is characterized by VEGF over-expression, ovarian enlargement, and pelvic discomfort. In more severe cases, abdominal distention, nausea, vomiting, and ascites may also occur (150). In severe cases, the third spacing of fluid into the peritoneal and pleural cavities leads to respiratory compromise, hypotension, increased intra-abdominal pressure, and renal compromise related to decreased perfusion The ovaries can enlarge to more than 5–10 cm in diameter, predisposing them to torsion. Sonographic findings in patients with OHSS include markedly enlarged multicystic ovaries. Doppler evaluation should always be performed in symptomatic patients to help assess for torsion, although the presence of blood flow does not exclude the diagnosis. Drainage of the ascites for improvement of symptoms is done by the abdominal or vaginal approach under ultrasound guidance, and a catheter can be left in the abdominal cavity for drainage at home to avoid hospitalization if there are no electrolyte or renal anomalies (151).

Early pregnancy ultrasound and pregnancy of unknown locations

Ultrasound is essential for the diagnosis of clinical pregnancy, position of the pregnancy, and number of sacs and fetuses. Recent emphasis has been on reducing the number of embryos transferred to reduce the risk of multiple births. In a normally developing pregnancy, a blastocyst implants by 23 days of menstrual age. The first structure identified by TVUS is the gestational sac (GS), appearing as a spherical, fluid-filled cavity surrounded by an echogenic rim. A double decidual sac sign is a reliable signal of an intrauterine pregnancy. There is a correlation between sac size and hCG level and gestational age, but there is variability and it is helpful to monitor sequential sonographic milestones. As development progresses, the first structure inside the GS is the yolk sac, followed by the embryo. The yolk sac is a spherical, echogenic, ring-like formation with a sonolucent center, and its presence confirms a true intrauterine pregnancy with 100% PPV. The confirmation of a yolk sac is necessary by 37–40 menstrual days or six weeks of gestation. Fetal heart beat is visible from six weeks and two days of gestation based on ET dates. It is seen as a linear echodensity next to the yolk sac. The embryo or fetal pole is measured along its long axis and is called the "crown–rump length" (CRL). Subchorionic hematoma, a fluid collection between the chorionic membrane and deciduae, is very common with ART pregnancies and is associated with abnormal placentation and a higher risk of miscarriage. Discriminatory values should be used with caution as they are a range rather than a specific cutoff value. In a recent study, the discriminatory levels at which structures would be seen 99% of the time were 3510 mIU/mL for a GS, 17,716 mIU/mL for a yolk sac, and 47,685 mIU/mL for a fetal pole (152). However, threshold values are much lower at 390, 1094, and 1394 mIU/mL for the GS, yolk sac, and fetal pole, respectively. When the ultrasound reveals an intrauterine pregnancy but neither an embryonic pole nor fetal heart activity are identified, the pregnancy is classified as an intrauterine pregnancy of unknown viability based on the new criteria. A mean sac diameter $\geq$16–17 mm with an empty GS is highly suggestive and a mean sac diameter $>$25 mm is definitive of a failed pregnancy. A CRL $\geq$5–6 mm with absence of fetal cardiac activity is highly suggestive and a CRL $>$7 mm is definitive of a failed pregnancy (153). In terms of time, there is definitely a failed pregnancy if more than two weeks pass after a GS is seen without a yolk sac and $>$10 days pass after a GS and yolk sac are identified but an embryo is not (153). In addition, approximately 90% of incomplete abortions and 50% of missed abortions can be expected to spontaneously abort within two weeks of initial presentation and ultrasound (154).

When compared to natural conception, ART increases the chance of multiple gestations. Twinning should be classified as either monozygotic (a single ovum divides into two embryos) or dizygotic (two separate ova) and dichorionic/diamniotic, monochorionic/diamniotic, or monochorionic/monoamniotic. The type of twinning affects the incidence of maternal and fetal morbidity and mortality (Figure 52.16).

A pregnancy of unknown location may require serial ultrasounds. Ultrasonography is the primary diagnostic modality for ectopic pregnancy. The visualization of a fluid-filled sac outside the uterine cavity that contains an embryo or a yolk sac is definitive of ectopic pregnancy. An adnexal mass with the "tubal ring" is also highly predictive. A pregnancy outside the endometrial cavity can be visualized best in the coronal view (Figure 52.17). The presence of an intrauterine pregnancy in an asymptomatic patient conceived by IVF should not exclude the diagnosis of a concurrent ectopic pregnancy, called a heterotopic pregnancy, so evaluation of the adnexa should be done in

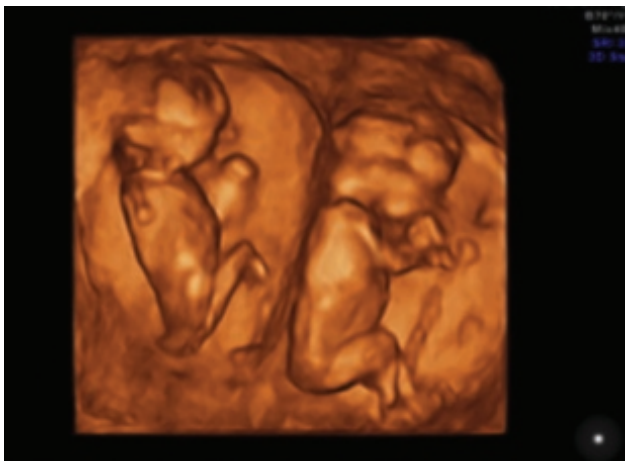

Figure 52.16 Three-dimensional view of dichorionic and diamniotic twin pregnancy.

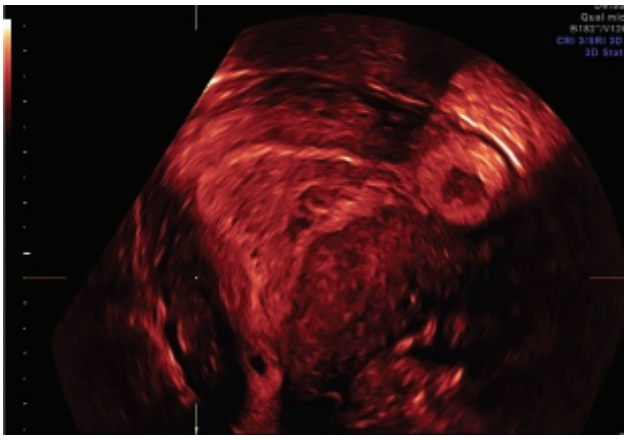

Figure 52.17 Ectopic pregnancy cornual.

all circumstances. In the case of heterotopic pregnancy, methotrexate injection is unacceptable and laparoscopic surgery or aspiration of the GS and injection with potassium chloride under transvaginal sonography can be done.

CONCLUSIONS

Modern ART and infertility treatments cannot be imagined today without ultrasound imaging, and advances in both fields have occurred simultaneously. The use of 3D visualization of the pelvic structures is the most striking advancement in the use of ultrasound in ART. As costs decrease, accessibility will increase. The future will bring smaller and portable ultrasounds for increased access in underserved communities, as well as more standardization and increased automation with savings in time and possible improved outcomes. The reduction in OHSS and in multiple gestations is one of the key concerns of ART treatments, and with recent advances in IVF as well as ultrasound guidance, success of mild stimulation and an elective single ET can be maximized. With modern ultrasound usage, we can see better, and use ART better.

REFERENCES

1. Matijevic R, Grgic O. Predictive values of ultrasound monitoring of the menstrual cycle. *Curr Opin Obstet Gynecol* 2005; 17: 405–10.
2. Jokubkiene L, Sladkevicius P, Rovas L, Valentin L. Assessment of changes in volume and vascularity of the ovaries during the normal menstrual cycle using three-dimensional power Doppler ultrasound. *Hum Reprod* 2006; 21: 2661–8.
3. Durfee SM, Frates MC. Sonographic spectrum of the corpus luteum in early pregnancy: Gray-scale, color, and pulsed Doppler appearance. *J Clin Ultrasound* 1999; 27: 55–9.
4. Nelson SM. Biomarkers of ovarian response: Current and future applications. *Fertil Steril* 2013; 99: 963–9.
5. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: Implications for forecasting menopause. *Hum Reprod* 1992; 7: 1342–6.
6. Muasher SJ, Oehninger S, Simonetti S et al. The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and *in vitro* fertilization outcome. *Fertil Steril* 1988; 50: 298–307.
7. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006 12: 685–718.
8. Broekmans FJ, Knauff EA, te Velde ER, Macklon NS, Fauser BC. Female reproductive ageing: Current knowledge and future trends. *Trends Endocrinol Metab* 2007; 18: 58–65.
9. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: Practical recommendations for better standardization. *Fertil Steril* 2010; 94: 1044–51.
10. Riggs RM, Duran EH, Baker MW et al. Assessment of ovarian reserve with anti-Mullerian hormone: A comparison of the predictive value of anti-Mullerian hormone, follicle-stimulating hormone, inhibin B, and age. *Am J Obstet Gynecol* 2008; 199: 202.e1–8.
11. Hendriks DJ, Mol BW, Bancsi LF, Te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after *in vitro* fertilization: A meta-analysis and comparison with basal follicle-stimulating hormone level. *Fertil Steril* 2005; 83: 291–301.
12. La Marca A, Sighinolfi G, Radi D et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum Reprod Update* 2010; 16: 113–30.
13. Lukaszuk K, Kunicki M, Liss J, Lukaszuk M, Jakiel G. Use of ovarian reserve parameters for predicting live births in women undergoing *in vitro* fertilization. *Eur J Obstet Gynecol Reprod Biol* 2013; 168: 173–7.
14. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. *Fertil Steril* 2011; 95: 170–5.
15. Jayaprakasan K, Al-Hasie H, Jayaprakasan R et al. The three-dimensional ultrasonographic ovarian vascularity of women developing poor ovarian response during assisted reproduction treatment and its predictive value. *Fertil Steril* 2009; 92: 1862–9.
16. Almog B, Shehata F, Shalom-Paz E, Tan SL, Tulandi T. Age-related normogram for antral follicle count: McGill reference guide. *Fertil Steril* 2011; 95: 663–6.
17. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Mullerian hormone and antral follicle count as biomarkers of ovarian response. *Hum Reprod Update* 2015; 21: 698–710.
18. Scheffer GJ, Broekmans FJ, Looman CW et al. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. *Hum Reprod* 2003; 18: 700–6.

19. Raine-Fenning N, Jayaprakasan K, Chamberlain S, Devlin L, Priddle H, Johnson I. Automated measurements of follicle diameter: A chance to standardize? *Fertil Steril* 2009; 91: 1469–72.
20. Stadtmauer LA, Kovac A, Tur-Kaspa I. Ovarian reserve and ovarian cysts. In: *Ultrasound Imaging in Reproductive Medicine: Advances in Infertility Work-up, Treatment, and ART*. Stadtmauer LA, Tur-Kaspa I (eds). New York, NY: Springer, 2014, 63–74.
21. Firouzabadi RD, Sekhavat L, Javedani M. The effect of ovarian cyst aspiration on IVF treatment with GnRH. *Arch Gynecol Obstet* 2010; 281: 545–9.
22. Raine-Fenning N, Jayaprakasan K, Deb S. Three-dimensional ultrasonographic characteristics of endometriomata. *Ultrasound Obstet Gynecol* 2008; 31: 718–24.
23. Gupta S, Agarwal A, Agarwal R, Loret de Mola JR. Impact of ovarian endometrioma on assisted reproduction outcomes. *Reprod Biomed Online* 2006; 13: 349–60.
24. Matalliotakis IM, Cakmak H, Mahutte N, Fragouli Y, Arici A, Sakkas D. Women with advanced-stage endometriosis and previous surgery respond less well to gonadotropin stimulation, but have similar IVF implantation and delivery rates compared with women with tubal factor infertility. *Fertil Steril* 2007; 88: 1568–72.
25. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on *in vitro* fertilization. *Fertil Steril* 2002; 77: 1148–55.
26. Kawwass JF, Crawford S, Session DR, Kissin DM, Jamieson DJ, National ART Surveillance System Group. Endometriosis and assisted reproductive technology: United States trends and outcomes 2000–2011. *Fertil Steril* 2015; 103: 1537–43.
27. Wong BC, Gillman NC, Oehninger S, Gibbons WE, Stadtmauer LA. Results of *in vitro* fertilization in patients with endometriomas: Is surgical removal beneficial? *Am J Obstet Gynecol* 2004; 191: 597–606; discussion 607.
28. Caspi B, Weissman A, Zalel Y, Barash A, Tulandi T, Shoham Z. Ovarian stimulation and *in vitro* fertilization in women with mature cystic teratomas. *Obstet Gynecol* 1998;92: 979–81.
29. Alborzi S, Khodaee R, Parsanejad ME. Ovarian size and response to laparoscopic ovarian electro-cauterization in polycystic ovarian disease. *Int J Gynaecol Obstet* 2001; 74: 269–74.
30. Amer SA, Li TC, Bygrave C, Sprigg A, Saravelos H, Cooke ID. An evaluation of the inter-observer and intra-observer variability of the ultrasound diagnosis of polycystic ovaries. *Hum Reprod* 2002; 17: 1616–22.
31. Ardaens Y, Robert Y, Lemaitre L, Fossati P, Dewailly D. Polycystic ovarian disease: Contribution of vaginal endosonography and reassessment of ultrasonic diagnosis. *Fertil Steril* 1991; 55: 1062–8.
32. Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C, Dewailly D. Ultrasound examination of polycystic ovaries: Is it worth counting the follicles? *Hum Reprod* 2003; 18: 598–603.
33. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004; 81: 19–25.
34. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; 19: 41–7.
35. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *Br Med J (Clin Res Ed)* 1986; 293: 355–9.
36. Fox R, Corrigan E, Thomas PA, Hull MG. The diagnosis of polycystic ovaries in women with oligo-amenorrhoea: Predictive power of endocrine tests. *Clin Endocrinol (Oxf)* 1991; 34: 127–31.
37. Kyei-Mensah A, Maconochie N, Zaidi J, Pittrof R, Campbell S, Tan SL. Transvaginal three-dimensional ultrasound: Reproducibility of ovarian and endometrial volume measurements. *Fertil Steril* 1996; 66: 718–22.
38. Hackeloer BJ, Robinson HP. [Ultrasound examination of the growing ovarian follicle and of the corpus luteum during the normal physiologie menstrual cycle (author's transl)]. *Geburtshilfe Frauenheilkd* 1978; 38: 163–8.
39. Kwan I, Bhattacharya S, McNeil A, van Rumste MM. Monitoring of stimulated cycles in assisted reproduction (IVF and ICSI). *Cochrane Database Syst Rev* 2008: 2: CD005289.
40. Revelli A, Martiny G, Delle Piane L, Benedetto C, Rinaudo P, Tur-Kaspa I. A critical review of bi-dimensional and three-dimensional ultrasound techniques to monitor follicle growth: Do they help improving IVF outcome? *Reprod Biol Endocrinol* 2014; 12: 107.
41. Deb S, Jayaprakasan K, Campbell BK, Clewes JS, Johnson IR, Raine-Fenning NJ. Intraobserver and interobserver reliability of automated antral follicle counts made using three-dimensional ultrasound and SonoAVC. *Ultrasound Obstet Gynecol* 2009; 33: 477–83.
42. Raine-Fenning N, Jayaprakasan K, Clewes J et al. SonoAVC: A novel method of automatic volume calculation. *Ultrasound Obstet Gynecol* 2008; 31: 691–6.
43. Ata B, Tulandi T. Ultrasound automated volume calculation in reproduction and in pregnancy. *Fertil Steril* 2011; 95: 2163–70.
44. Deutch TD, Joergner I, Matson DO et al. Automated assessment of ovarian follicles using a novel three-dimensional ultrasound software. *Fertil Steril* 2009; 92: 1562–8.

45. Rousian M, Verwoerd-Dikkeboom CM, Koning AH et al. Early pregnancy volume measurements: Validation of ultrasound techniques and new perspectives. *BJOG* 2009; 116: 278–85.
46. Raine-Fenning N, Deb S, Jayaprakasan K, Clewes J, Hopkisson J, Campbell B. Timing of oocyte maturation and egg collection during controlled ovarian stimulation: A randomized controlled trial evaluating manual and automated measurements of follicle diameter. *Fertil Steril* 2010; 94: 184–8.
47. Rodriguez-Fuentes A, Hernandez J, Garcia-Guzman R, Chinea E, Iaconianni L, Palumbo A. Prospective evaluation of automated follicle monitoring in 58 *in vitro* fertilization cycles: Follicular volume as a new indicator of oocyte maturity. *Fertil Steril* 2010; 93: 616–20.
48. Vandekerckhove F, Bracke V, De Sutter P. The value of automated follicle volume measurements in IVF/ICSI. *Front Surg* 2014; 1: 18.
49. Casper RF. It's time to pay attention to the endometrium. *Fertil Steril* 2011; 96: 519–21.
50. Friedler S, Schenker JG, Herman A, Lewin A. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: A critical review. *Hum Reprod Update* 1996; 2: 323–35.
51. Merce LT, Barco MJ, Bau S, Troyano J. Are endometrial parameters by three-dimensional ultrasound and power Doppler angiography related to *in vitro* fertilization/embryo transfer outcome? *Fertil Steril* 2008; 89: 111–7.
52. Killick SR. Ultrasound and the receptivity of the endometrium. *Reprod Biomed Online* 2007; 15: 63–7.
53. Randolph JF, Jr., Ying YK, Maier DB, Schmidt CL, Riddick DH. Comparison of real-time ultrasonography, hysterosalpingography, and laparoscopy/hysteroscopy in the evaluation of uterine abnormalities and tubal patency. *Fertil Steril* 1986; 46: 828–32.
54. Parsons AK, Lense JJ. Sonohysterography for endometrial abnormalities: Preliminary results. *J Clin Ultrasound* 1993; 21: 87–95.
55. Kim AH, McKay H, Keltz MD, Nelson HP, Adamson GD. Sonohysterographic screening before *in vitro* fertilization. *Fertil Steril* 1998; 69: 841–4.
56. Cullinan JA, Fleischer AC, Kepple DM, Arnold AL. Sonohysterography: A technique for endometrial evaluation. *Radiographics* 1995; 15: 501–14; discussion 15-6.
57. de Kroon CD, de Bock GH, Dieben SW, Jansen FW. Saline contrast hysterosonography in abnormal uterine bleeding: A systematic review and meta-analysis. *BJOG* 2003; 110: 938–47.
58. Brown SE, Coddington CC, Schnorr J, Toner JP, Gibbons W, Oehninger S. Evaluation of outpatient hysteroscopy, saline infusion hysterosonography, and hysterosalpingography in infertile women: A prospective, randomized study. *Fertil Steril* 2000; 74: 1029–34.
59. American College of Obstetricians and Gynecologists. ACOG Technology Assessment in Obstetrics and Gynecology No. 5: Sonohysterography. *Obstet Gynecol* 2008; 112: 1467–9.
60. Spieldoch RL, Winter TC, Schouweiler C, Ansay S, Evans MD, Lindheim SR. Optimal catheter placement during sonohysterography: A randomized controlled trial comparing cervical to uterine placement. *Obstet Gynecol* 2008; 111: 15–21.
61. Tur-Kaspa I, Stadtmauer LA. Sonohysterography (SHG) in reproductive medicine. In: *Ultrasound Imaging in Reproductive Medicine : Advances in Infertility Work-up, Treatment, and ART*. Stadtmauer LA, Tur-Kaspa I (eds). New York, NY: Springer, 2014, p. xvii.
62. Werbrouck E, Veldman J, Luts J et al. Detection of endometrial pathology using saline infusion sonography versus gel instillation sonography: A prospective cohort study. *Fertil Steril* 2011; 95: 285–8.
63. de Ziegler D. Contrast ultrasound: A simple-to-use phase-shifting medium offers saline infusion sonography-like images. *Fertil Steril* 2009; 92: 369–73.
64. Shah AA, Walmer DK. A feasibility study to evaluate pelvic peritoneal anatomy with a saline intraperitoneal sonogram (SIPS). *Fertil Steril* 2010; 94: 2766–8.
65. Soares SR, Barbosa dos Reis MM, Camargos AF. Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine cavity diseases. *Fertil Steril* 2000; 73: 406–11.
66. Kowalczyk D, Guzikowski W, Wiecek J, Sioma-Markowska U. Clinical value of real time 3D sonohysterography and 2D sonohysterography in comparison to hysteroscopy with subsequent histopathological examination in perimenopausal women with abnormal uterine bleeding. *Neuro Endocrinol Lett* 2012; 33: 212–6.
67. Makris N, Skartados N, Kalmantis K, Mantzaris G, Papadimitriou A, Antsaklis A. Evaluation of abnormal uterine bleeding by transvaginal 3-D hysterosonography and diagnostic hysteroscopy. *Eur J Gynaecol Oncol* 2007; 28: 39–42.
68. Tur-Kaspa I, Gal M, Hartman M, Hartman J, Hartman A. A prospective evaluation of uterine abnormalities by saline infusion sonohysterography in 1,009 women with infertility or abnormal uterine bleeding. *Fertil Steril* 2006; 86: 1731–5.
69. Alborzi S, Parsanezhad ME, Mahmoodian N, Alborzi S, Alborzi M. Sonohysterography versus transvaginal sonography for screening of patients with abnormal uterine bleeding. *Int J Gynaecol Obstet* 2007; 96: 20–3.
70. Shavell VI, Thakur M, Sawant A et al. Adverse obstetric outcomes associated with sonographically identified large uterine fibroids. *Fertil Steril* 2012; 97: 107–10.
71. Klatsky PC, Tran ND, Caughey AB, Fujimoto VY. Fibroids and reproductive outcomes: A systematic literature review from conception to delivery. *Am J Obstet Gynecol* 2008; 198: 357–66.

72. Salim R, Lee C, Davies A, Jolaoso B, Ofuasia E, Jurkovic D. A comparative study of three-dimensional saline infusion sonohysterography and diagnostic hysteroscopy for the classification of submucous fibroids. *Hum Reprod* 2005; 20: 253–7.
73. Mayer DP, Shipilov V. Ultrasonography and magnetic resonance imaging of uterine fibroids. *Obstet Gynecol Clin North Am* 1995; 22: 667–725.
74. Sheth S, Macura K. Sonography of the uterine myometrium: myomas and beyond. *Ultrasound Clin* 2007; 2: 267–95.
75. Stadtmauer L, Oehninger S. Abstract Presented at the Global Congress of Minimally Invasive Gynecology, the 39th Annual Meeting of the AAGL. November 2010. Las Vegas, Nevada, USA. *J Minim Invasive Gynecol*. Nov–Dec 2010;17(Suppl).
76. Pritts EA, Parker WH, Olive DL. Fibroids and infertility: An updated systematic review of the evidence. *Fertil Steril* 2009; 91: 1215–23.
77. Pritts EA. Fibroids and infertility: A systematic review of the evidence. *Obstet Gynecol Surv* 2001; 56: 483–91.
78. Rajan DK, Margau R, Kroll RR et al. Clinical utility of ultrasound versus magnetic resonance imaging for deciding to proceed with uterine artery embolization for presumed symptomatic fibroids. *Clin Radiol* 2011; 66: 57–62.
79. Rabinovici J, David M, Fukunishi H, Morita Y, Gostout BS, Stewart EA et al. Pregnancy outcome after magnetic resonance-guided focused ultrasound surgery (MRgFUS) for conservative treatment of uterine fibroids. *Fertil Steril* 2010; 93: 199–209.
80. Meredith SM, Sanchez-Ramos L, Kaunitz AM. Diagnostic accuracy of transvaginal sonography for the diagnosis of adenomyosis: Systematic review and metaanalysis. *Am J Obstet Gynecol* 2009; 201: 107.e1–6.
81. Marshburn PB, Hurst BS. *Disorders of Menstruation*. Chichester, U.K.; Hoboken, NJ: Wiley-Blackwell, 2011.
82. Dueholm M. Transvaginal ultrasound for diagnosis of adenomyosis: A review. *Best Pract Res Clin Obstet Gynaecol* 2006; 20: 569–82.
83. Galliano D, Bellver J, Diaz-Garcia C, Simon C, Pellicer A. ART and uterine pathology: How relevant is the maternal side for implantation? *Hum Reprod Update* 2015; 21: 13–38.
84. American Association of Gynecologic Laparoscopists. AAGL practice report: Practice guidelines for the diagnosis and management of endometrial polyps. *J Min Invasive Gynecol* 2012; 19: 3–10.
85. Kupesic S, Kurjak A. Septate uterus: Detection and prediction of obstetrical complications by different forms of ultrasonography. *J Ultrasound Med* 1998; 17: 631–6.
86. Lass A, Williams G, Abusheikha N, Brinsden P. The effect of endometrial polyps on outcomes of *in vitro* fertilization (IVF) cycles. *J Assist Reprod Genet* 1999; 16: 410–5.
87. Heinonen PK, Saarikoski S, Pystynen P. Reproductive performance of women with uterine anomalies. An evaluation of 182 cases. *Acta Obstet Gynecol Scand* 1982; 61: 157–62.
88. Pereira N, Amrane S, Estes JL et al. Does the time interval between hysteroscopic polypectomy and start of *in vitro* fertilization affect outcomes? *Fertil Steril* 2016; 105: 539–44.e1.
89. Tiras B, Korucuoglu U, Polat M, Zeyneloglu HB, Saltik A, Yarali H. Management of endometrial polyps diagnosed before or during ICSI cycles. *Reprod Biomed Online* 2012; 24: 123–8.
90. Kirsop R, Porter R, Torode H, Smith D, Saunders D. The role of hysteroscopy in patients having failed IVF/GIFT transfer cycles. *Aust N Z J Obstet Gynaecol* 1991; 31: 263–4.
91. Dicker D, Ashkenazi J, Feldberg D, Farhi J, Shalev J, Ben-Rafael Z. The value of repeat hysteroscopic evaluation in patients with failed *in vitro* fertilization transfer cycles. *Fertil Steril* 1992; 58: 833–5.
92. Batioglu S, Kaymak O. Does hysteroscopic polypectomy without cycle cancellation affect IVF? *Reprod Biomed Online* 2005; 10: 767–9.
93. Madani T, Ghaffari F, Kiani K, Hosseini F. Hysteroscopic polypectomy without cycle cancellation in IVF cycles. *Reprod Biomed Online* 2009; 18: 412–5.
94. Bacelar AC, Wilcock D, Powell M, Worthington BS. The value of MRI in the assessment of traumatic intra-uterine adhesions (Asherman's syndrome). *Clin Radiol* 1995; 50: 80–3.
95. Knopman J, Copperman AB. Value of 3D ultrasound in the management of suspected Asherman's syndrome. *J Reprod Med* 2007; 52: 1016–22.
96. Kodaman PH, Arici A. Intra-uterine adhesions and fertility outcome: How to optimize success? *Curr Opin Obstet Gynecol* 2007; 19: 207–14.
97. Diamond MP, Freeman ML. Clinical implications of postsurgical adhesions. *Hum Reprod Update* 2001; 7: 567–76.
98. Pace S, Stentella P, Catania R, Palazzetti PL, Frega A. Endoscopic treatment of intrauterine adhesions. *Clin Exp Obstet Gynecol* 2003; 30: 26–8.
99. Valdes C, Malini S, Malinak LR. Ultrasound evaluation of female genital tract anomalies: A review of 64 cases. *Am J Obstet Gynecol* 1984; 149: 285–92.
100. Sakhel K, Benson CB, Platt LD, Goldstein SR, Benacerraf BR. Begin with the basics: Role of 3-dimensional sonography as a first-line imaging technique in the cost-effective evaluation of gynecologic pelvic disease. *J Ultrasound Med* 2013; 32: 381–8.
101. Balen FG, Allen CM, Gardener JE, Siddle NC, Lees WR. 3-dimensional reconstruction of ultrasound images of the uterine cavity. *Br J Radiol* 1993; 66: 588–91.
102. Jurkovic D, Gruboeck K, Tailor A, Nicolaides KH. Ultrasound screening for congenital uterine anomalies. *Br J Obstet Gynaecol* 1997; 104: 1320–1.

103. Jurkovic D. Three-dimensional ultrasound in gynecology: Acritical evaluation. *Ultrasound Obstet Gynecol* 2002; 19: 109–17.
104. Smith B, Porter R, Ahuja K, Craft I. Ultrasonic assessment of endometrial changes in stimulated cycles in an *in vitro* fertilization and embryo transfer program. *J In Vitro Fert Embryo Transf* 1984; 1: 233–8.
105. Weckstein LN, Jacobson A, Galen D, Hampton K, Hammel J. Low-dose aspirin for oocyte donation recipients with a thin endometrium: Prospective, randomized study. *Fertil Steril* 1997; 68: 927–30.
106. Sher G, Fisch JD. Effect of vaginal sildenafil on the outcome of *in vitro* fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. *Fertil Steril* 2002; 78: 1073–6.
107. Hsieh Y-Y, Tsai H-D, Chang C-C, Lo H-Y, Chen C-L. Gynecology: Low-dose aspirin for infertile women with thin endometrium receiving intraeuterine insemination: a prospective, randomized study. *J Assist Reprod Genet* 2000; 17: 174–7.
108. Oliveira JB, Baruffi RL, Mauri AL, Petersen CG, Borges MC, Franco JG, Jr. Endometrial ultrasonography as a predictor of pregnancy in an *in-vitro* fertilization programme after ovarian stimulation and gonadotrophin-releasing hormone and gonadotrophins. *Hum Reprod* 1997; 12: 2515–8.
109. Gonen Y, Casper RF. Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for *in vitro* fertilization (IVF). *J In Vitro Fert Embryo Transf* 1990; 7: 146–52.
110. Gonen Y, Casper RF, Jacobson W, Blankier J. Endometrial thickness and growth during ovarian stimulation: A possible predictor of implantation in *in vitro* fertilization. *Fertil Steril* 1989; 52: 446–50.
111. Shapiro H, Cowell C, Casper RF. The use of vaginal ultrasound for monitoring endometrial preparation in a donor oocyte program. *Fertil Steril* 1993; 59: 1055–8.
112. Coulam CB, Bustillo M, Soenksen DM, Britten S. Ultrasonographic predictors of implantation after assisted reproduction. *Fertil Steril* 1994; 62: 1004–10.
113. Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, Awartani K. The correlation between endometrial thickness and outcome of *in vitro* fertilization and embryo transfer (IVF-ET) outcome. *Reprod Biol Endocrinol* 2008; 6: 37.
114. Weissman A, Gotlieb L, Casper RF. The detrimental effect of increased endometrial thickness on implantation and pregnancy rates and outcome in an *in vitro* fertilization program. *Fertil Steril* 1999; 71: 147–9.
115. Dickey RP, Olar TT, Taylor SN, Curole DN, Matulich EM. Relationship of endometrial thickness and pattern to fecundity in ovulation induction cycles: Effect of clomiphene citrate alone and with human menopausal gonadotropin. *Fertil Steril* 1993; 59: 756–60.
116. Zohav E, Orvieto R, Anteby EY, Segal O, Meltcer S, Tur-Kaspa I. Low endometrial volume may predict early pregnancy loss in women undergoing *in vitro* fertilization. *J Assist Reprod Genet* 2007; 24: 259–61.
117. Sundstrom P. Establishment of a successful pregnancy following *in-vitro* fertilization with an endometrial thickness of no more than 4 mm. *Hum Reprod* 1998; 13: 1550–2.
118. Vlaisavljevic V, Reljic M, Gavric-Lovrec V, Kovacic B. Subendometrial contractility is not predictive for *in vitro* fertilization (IVF) outcome. *Ultrasound Obstet Gynecol* 2001; 17: 239–44.
119. Raga F, Bonilla-Musoles F, Casan EM, Klein O, Bonilla F. Assessment of endometrial volume by three-dimensional ultrasound prior to embryo transfer: Clues to endometrial receptivity. *Hum Reprod* 1999; 14: 2851–4.
120. Yaman C, Ebner T, Sommergruber M, Polz W, Tews G. Role of three-dimensional ultrasonographic measurement of endometrium volume as a predictor of pregnancy outcome in an IVF-ET program: A preliminary study. *Fertil Steril* 2000; 74: 797–801.
121. Ng EH, Chan CC, Tang OS, Yeung WS, Ho PC. The role of endometrial and subendometrial vascularity measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during frozen–thawed embryo transfer cycles. *Hum Reprod* 2006; 21: 1612–7.
122. Tur-Kaspa I. Fear no pain: Uterine cavity and tubal patency assessment tests should be pain free. *Ultrasound Obstet Gynecol* 2012; 39: 247–51.
123. Radic V, Canic T, Valetic J, Duic Z. Advantages and disadvantages of hysterosonosalpingography in the assessment of the reproductive status of uterine cavity and fallopian tubes. *Eur J Radiol* 2005; 53: 268–73.
124. Richman TS, Viscomi GN, deCherney A, Polan ML, Alcebo LO. Fallopian tubal patency assessed by ultrasound following fluid injection. Work in progress. *Radiology* 1984; 152: 507–10.
125. Balen FG, Allen CM, Siddle NC, Lees WR. Ultrasound contrast hysterosalpingography—Evaluation as an outpatient procedure. *Br J Radiol* 1993; 66: 592–9.
126. Dietrich M, Suren A, Hinney B, Osmers R, Kuhn W. Evaluation of tubal patency by hysterocontrast sonography (HyCoSy, Echovist) and its correlation with laparoscopic findings. *J Clin Ultrasound* 1996; 24: 523–7.
127. Deichert U, Schleif R, van de Sandt M, Juhnke I. Transvaginal hysterosalpingo-contrast-sonography (Hy-Co-Sy) compared with conventional tubal diagnostics. *Hum Reprod* 1989; 4: 418–24.
128. Alcázar JL, Martinez-Astorquiza Corral T, Orozco R, Dominguez-Piriz J, Juez L, Errasti T. Three-dimensional hysterosalpingo-contrast-sonography for the assessment of tubal patency in women with infertility: A systematic review with meta-analysis. *Gynecol Obstet Invest*. 2016;81(4):289–95.

129. Chenia F, Hofmeyr GJ, Moolla S, Oratis P. Sonographic hydrotubation using agitated saline: A new technique for improving fallopian tube visualization. *Br J Radiol* 1997; 70: 833–6.
130. Saunders RD, Shwayder JM, Nakajima ST. Current methods of tubal patency assessment. *Fertil Steril* 2011; 95: 2171–9.
131. Sladkevicius P, Ojha K, Campbell S, Nargund G. Three-dimensional power Doppler imaging in the assessment of Fallopian tube patency. *Ultrasound Obstet Gynecol* 2000; 16: 644–7.
132. Chan CC, Ng EH, Tang OS, Chan KK, Ho PC. Comparison of three-dimensional hysterosalpingo-contrast-sonography and diagnostic laparoscopy with chromopertubation in the assessment of tubal patency for the investigation of subfertility. *Acta Obstet Gynecol Scand* 2005; 84: 909–13.
133. Kupesic S, Plavsic BM. 2D and 3D hysterosalpingo-contrast-sonography in the assessment of uterine cavity and tubal patency. *Eur J Obstet Gynecol Reprod Biol* 2007; 133: 64–9.
134. Johnson NP, Mak W, Sowter MC. Surgical treatment for tubal disease in women due to undergo *in vitro* fertilisation. *Cochrane Database Syst Rev* 2004; 3: CD002125.
135. Mijatovic V, Veersema S, Emanuel MH, Schats R, Hompes PG. Essure hysteroscopic tubal occlusion device for the treatment of hydrosalpinx prior to *in vitro* fertilization–embryo transfer in patients with a contraindication for laparoscopy. *Fertil Steril* 2010; 93: 1338–42.
136. Sills ES, Walsh DJ, Jones CA, Wood SH. Endometrial fluid associated with Essure implants placed before *in vitro* fertilization: Considerations for patient counseling and surgical management. *Clin Exp Reprod Med* 2015; 42: 126–9.
137. Hammadieh N, Coomarasamy A, Ola B, Papaioannou S, Afnan M, Sharif K. Ultrasound-guided hydrosalpinx aspiration during oocyte collection improves pregnancy outcome in IVF: A randomized controlled trial. *Hum Reprod* 2008; 23: 1113–7.
138. Aboulghar MA, Mansour RT, Serour GI. Spontaneous intrauterine pregnancy following salpingectomy for a unilateral hydrosalpinx. *Hum Reprod* 2002; 17: 1099–100.
139. Lenz S, Lauritsen JG. Ultrasonically guided percutaneous aspiration of human follicles under local anesthesia: A new method of collecting oocytes for *in vitro* fertilization. *Fertil Steril* 1982; 38: 673–7.
140. Mirkin S, Jones EL, Mayer JF, Stadtmauer L, Gibbons WE, Oehninger S. Impact of transabdominal ultrasound guidance on performance and outcome of transcervical uterine embryo transfer. *J Assist Reprod Genet* 2003; 20: 318–22.
141. Anderson RE, Nugent NL, Gregg AT, Nunn SL, Behr BR. Transvaginal ultrasound-guided embryo transfer improves outcome in patients with previous failed *in vitro* fertilization cycles. *Fertil Steril* 2002; 77: 769–75.
142. Buckett WM. A meta-analysis of ultrasound-guided versus clinical touch embryo transfer. *Fertil Steril* 2003; 80: 1037–41.
143. Brown JA, Buckingham K, Abou-Setta A, Buckett W. Ultrasound versus "clinical touch" for catheter guidance during embryo transfer in women. *Cochrane Database Syst Rev* 2007; 1: CD006107.
144. Derks RS, Farquhar C, Mol BW, Buckingham K, Heineman MJ. Techniques for preparation prior to embryo transfer. *The Cochrane Database of Systematic Reviews* 2009; 4: CD007682.
145. Brown J, Buckingham K, Buckett W, Abou-Setta AM. Ultrasound versus 'clinical touch' for catheter guidance during embryo transfer in women. The Cochrane database of systematic reviews. *Cochrane Database Syst Rev.* March 17, 2016; 3: CD006107.
146. Confino EJR, Tur-Kaspa I. Ultrasound role in embryo transfers. In: *Ultrasound Imaging in Reproductive Medicine: Advances in Infertility Work-up, Treatment, and ART*. Stadtmauer LA, Tur-Kaspa I (eds). New York, NY: Springer, 2014, p. xvii.
147. Letterie GS. Three-dimensional ultrasound-guided embryo transfer: a preliminary study. *Am J Obstet Gynecol* 2005; 192: 1983–7; discussion 1987–8.
148. Gergely RZ, DeUgarte CM, Danzer H, Surrey M, Hill D, DeCherney AH. Three dimensional/four dimensional ultrasound-guided embryo transfer using the maximal implantation potential point. *Fertil Steril* 2005; 84: 500–3.
149. Delvigne A, Dubois M, Battheu B, Bassil S, Meuleman C, De Sutter P et al. The ovarian hyperstimulation syndrome in *in-vitro* fertilization: A Belgian multicentric study. II. Multiple discriminant analysis for risk prediction. *Hum Reprod* 1993; 8: 1361–6.
150. Aboulghar MA, Mansour RT. Ovarian hyperstimulation syndrome: Classifications and critical analysis of preventive measures. *Hum Reprod Update* 2003; 9: 275–89.
151. Shrivastav P, Nadkarni P, Craft I. Day care management of severe ovarian hyperstimulation syndrome avoids hospitalization and morbidity. *Hum Reprod* 1994; 9: 812–4.
152. Connolly A, Ryan DH, Stuebe AM, Wolfe HM. Reevaluation of discriminatory and threshold levels for serum beta-hCG in early pregnancy. *Obstet Gynecol* 2013; 121: 65–70.
153. Doubilet PM, Benson CB, Bourne T, Blavias M, Society of Radiologists in Ultrasound Multispecialty Panel on Early First Trimester Diagnosis of Miscarriage and Exclusion of a Viable Intrauterine Pregnancy. Diagnostic criteria for nonviable pregnancy early in the first trimester. *N Engl J Med* 2013; 369: 1443–51.
154. Whitworth M, Bricker L, Neilson JP, Dowswell T. Ultrasound for fetal assessment in early pregnancy. *Cochrane Database Syst Rev* 2010: 4: CD007058.

Sperm recovery techniques

Clinical aspects

53

HERMAN TOURNAYE, PATRICIO DONOSO, and FRANCISCO OSORIO

In the past 10–20 years, several changes have taken place in clinical andrology. Gradually, empirical treatments have been replaced by techniques of assisted reproduction (i.e., intrauterine insemination, *in vitro* fertilization [IVF], and intracytoplasmic sperm injection [ICSI]). In particular, the introduction of ICSI in 1992 (1,2) has completely changed the clinical approach towards male infertility by offering a novel opportunity for parenthood to azoospermic men. A single spermatozoon can be injected into an oocyte and result in normal fertilization, embryonic development, and implantation. Not only ejaculated spermatozoa can be used, but epididymal or testicular spermatozoa can also be used for ICSI. Testicular spermatozoa can be retrieved in some patients with non-obstructive azoospermia (NOA) because of the persistence of isolated foci of active spermatogenesis. The first pregnancies using epididymal and testicular spermatozoa in men with obstructive azoospermia (OA) and NOA were published in 1993 and 1995, respectively (3–6). Surgical retrieval of spermatozoa for ICSI has become a routine technique in clinical andrology. Several techniques are available to retrieve epididymal or testicular spermatozoa. Although there is no real method of choice, some guidelines may be given in order to make the best choice for a specific clinical setting. ICSI has also reinforced the role of non-surgical techniques to retrieve sperm in men suffering from anejaculation.

AZOOSPERMIA: WHAT IS IN A NAME?

Most azoospermic patients suffer from primary testicular failure (60%) (7,8). Because these patients do not show any clinical sign of obstruction, often they are referred to as patients with NOA. However, in a few cases, azoospermia without any obstruction is the result of a hypogonadotropic hypogonadism (i.e., a lack of adequate hormonal stimulation to support spermatogenesis). These patients have an early maturation arrest in spermatogenesis. Treatment with follicle-stimulating hormone (FSH) and human chorionic gonadotropin will restore spermatogenesis, and these patients do not, in the first instance, need assisted reproduction (9). Thus, these patients are not to be referred to as suffering from NOA and primarily do not need surgical sperm recovery.

Azoospermic patients with primary testicular failure show either a germ cell aplasia (Sertoli cell-only), a maturation arrest, or tubular sclerosis and atrophy at their testicular histopathology.

Germ cell aplasia may be iatrogenic, as it may result from irradiation or chemotherapy, or it may be congenital because of a genetic disorder such as Klinefelter's syndrome or a deletion on the long arm of the Y chromosome. In many cases, however, the cause of germ cell aplasia remains unknown. Many patients with primary testicular dysfunction, however, are now assumed to have testicular dysgenesis syndrome, a congenital developmental disorder causing spermatogenic failure, maldescent of the testis (cryptorchidism), and eventually hypospadias in more severe forms of this disorder (10). They also have a higher risk of developing testis carcinoma (10).

Men with NOA may also show maturation arrest at testicular histology. Maturation arrest may be caused by viral orchitis, irradiation, and/or chemotherapy and Yq deletions. Other causes include systemic illness or exposure to gonadotoxins, but here, too, idiopathic maturation arrest is most common. Again, testicular dysgenesis syndrome may explain some of these cases.

Fewer men with NOA will show tubular sclerosis and atrophy at their testicular histology. This may be the result of testicular torsion, vascular injury, or infection, but is also a common finding in Klinefelter's syndrome patients.

Many studies on assisted reproduction technology (ART) with testicular spermatozoa or spermatids use inadequate definitions often based on the absence or presence of clinical signs of obstruction. According to World Health Organization (WHO) guidelines for ART, the diagnosis of "NOA" should be made according to the histopathological findings, rather than on the basis of clinical indicators such as FSH levels or testicular size (11,12). Testicular failure is found in a third of normogonadotropic azoospermic men with normally sized testes; on the other hand, small testicular size or elevated FSH does not preclude normal spermatogenesis. Whenever testicular biopsy shows a normal spermatogenesis or a mild hypospermatogenesis, an obstruction of the excretory ducts is present. In a substantial subgroup of these men, however, no clinical signs of obstruction will be present. An accurate distinction between these two types of azoospermia is particularly relevant since spermatozoa can be retrieved in almost all patients with OA and mild hypospermatogenesis, but only in up to 50% of unselected patients with NOA when no preliminary selection of patients on the basis of histopathology has been performed (13).

DOES MY PATIENT NEED SURGICAL SPERM RECOVERY?

In patients with OA, fertility can be restored by surgical correction (i.e., vasoepididymostomy, vasovasostomy, or perurethral prostatic resection). When surgery has failed or is not indicated (e.g., patients with congenital bilateral

absence of the vas deferens [CBAVD]), surgical sperm recovery procedures are indicated.

Most methods described for surgical sperm recovery are simple techniques. However, in some patients with azoospermia, even these simple techniques are not indicated. When after appropriate analysis the diagnosis of azoospermia is made, an appropriate clinical work-up is necessary in order to define the exact cause of the azoospermia and to define the best treatment option. If azoospermia is the result of a primary testicular failure caused by hormonal deficiency, such as hypogonadotropic hypogonadism, then hormone-replacement therapy must be proposed.

Often the diagnosis of azoospermia is made without centrifuging the semen. Centrifugation at 1800 × *g* for at least five minutes may reveal spermatozoa in the pellet, which may be used for ICSI (14). In 2007, a national survey conducted by Swanton et al. including all 70 IVF units in the U.K. revealed that 91% of these centers routinely performed extended sperm preparation (ESP). In the same communication, the Oxford Fertility Unit presented a series of 87 azoospermic men in which ESP identified sperm in 22% of the cases. This percentage rises to 30% excluding patients after attempted vasectomy reversal (15). In cases of NOA, it may therefore be worthwhile to perform centrifugation of an ejaculate before embarking on a surgical recovery procedure to retrieve spermatozoa. Only when no spermatozoa are found in the pellet after centrifugation or when only immotile, non-viable spermatozoa are found is surgical sperm recovery indicated in order to avoid performing ICSI with spermatozoa with DNA damage.

ANEJACULATION DOES NOT EQUAL AZOOSPERMIA

Surgical sperm retrieval methods have been proposed as means for obtaining spermatozoa for assisted reproduction in men with anejaculation (i.e., the absence of antegrade or retrograde ejaculation). However, given the efficiency of assisted ejaculation in these men, surgical methods are only to be considered when penile vibratory stimulation (PVS) or electroejaculation (EEJ) have failed.

Epididymal or testicular sperm recovery procedures are often proposed to anejaculatory patients because no PVS or EEJ is available (16). When these first-line recovery methods are unavailable, it is even preferable to refer anejaculatory patients, especially patients with spinal cord injuries, to specialized services where assisted ejaculation can be performed and semen can be cryopreserved. Vibro- or electro-stimulation are noninvasive techniques that may be performed without any anesthesia in paraplegic men. EEJ is now a well-established method for procuring sperm from spinal cord-injured men (17). Since scrotal hematoma may take a long time to heal in such men, surgical sperm retrieval techniques are indicated only where these noninvasive techniques fail to produce an ejaculate that may be used for ICSI. Even here, vas deferens aspiration may be preferable because of its low risk of iatrogenic obstruction (18,19). The ejaculate, even in cases of oligoasthenoteratozoospermia, can be cryopreserved for later use. Testicular sperm retrieval must be considered only where primary testicular failure is present in an anejaculatory patient or when techniques of assisted ejaculation have failed to produce an ejaculate that can be used for ICSI. It is preferable in such patients to refrain from epididymal sperm aspiration techniques because of their higher risk of iatrogenic epididymal obstruction.

Psychogenic anejaculation may be encountered unexpectedly during treatments with ARTs. In these patients, assisted ejaculation combined with ART can be an effective approach, achieving acceptable fertilization, pregnancy, and live birth rates (20).

Patients facing IVF treatment can also suffer from erectile dysfunction. If treatment with sildenafil citrate has failed, assisted ejaculation has a role in order to obtain spermatozoa (21). In some anejaculatory patients, prostatic massage—a simple, alternative, noninvasive method—can be used in order to obtain spermatozoa for ART (22).

EJACULATION INDUCED BY PVS AND EEJ

Anejaculation may be psychogenic or may result from spinal cord injury or retroperitoneal lymph node dissection. These three causes represent almost 95% of etiologies. Diabetic neuropathy, multiple sclerosis, Parkinson's disease, and aorto-iliac, colorectal, or bladder neck surgery are less commonly encountered causes. Occasionally, anejaculation is drug associated: antidepressive, antipsychotic, and antihypertensive medication may induce anejaculation.

Given the low efficiency of medical treatments for inducing ejaculation in anejaculatory men, PVS (Figure 53.1 and protocol 2 in Appendix) and EEJ (Figure 53.2 and protocol 1 in Appendix) may be considered as the first-line treatments for anejaculation (23).

PVS is recommended because it is still less invasive and less expensive than EEJ and because semen quality has been reported to be much better after PVS than after EEJ, especially in men with spinal cord injury (24). PVS can

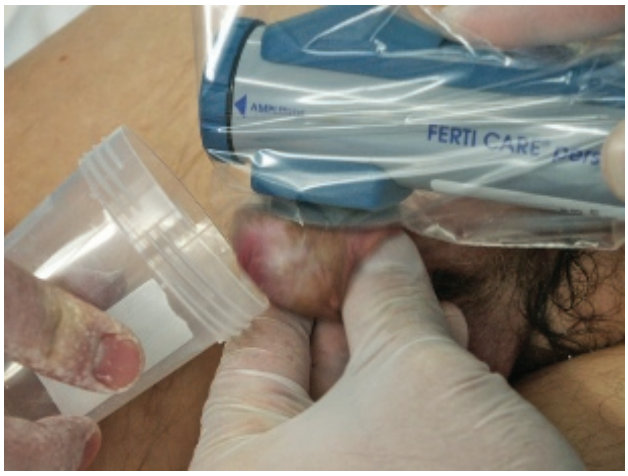

Figure 53.1 Penile vibratory stimulation. The vibrator should deliver a high peak-to-peak amplitude of at least 2.5 mm and a frequency of about 100 Hz. The vibrating part is applied to the posterior glans penis and frenulum.

Figure 53.2 Electroejaculation. The patient is in lateral decubitus and a stimulatory probe is gently introduced in the rectum with the electrodes facing the prostate.

restore ejaculation in half of anejaculatory patients when it is properly used (24). In fact, new PVS devices are showing up to 77% of ejaculatory success without major adverse side effects (25).

Each patient scheduled for PVS should undergo a complete neurological and uro-andrological examination. PVS needs an intact spinal cord up to the lumbosacral level. In spinal cord-injured men, PVS is less successful in cases of lower cord lesions. When the patient has a transection at T6 or higher, an increase in blood pressure because of autonomic dysreflexia may occur during a PVS procedure. Close monitoring of blood pressure is thus indicated. Whenever acute hypertension develops, 10–20 mg nifedipine should be administered sublingually. In spinal cord-injured patients with a history of autonomic dysreflexia, 10 mg nifedipine should be given preventively about 15 minutes before starting PVS.

The patient is instructed to drink 500 mL water containing 600 mg sodium bicarbonate on the morning of the procedure in order to alkalinize the urine. After emptying, the bladder is washed with a buffered sperm preparation medium. About 50 mL of this medium is left in the bladder. The vibrating part of the vibrator is placed on the posterior glans penis and frenulum. The position can be slowly changed in order to find a reactive trigger-point. When no ejaculation is obtained within 10 minutes, the procedure should be discontinued. Although less frequently than with EEJ, retrograde ejaculation may occur during PVS. Flushing, goose skin, and spasms of the abdominal muscles and legs may indicate ejaculation. In general, spermatozoa can be obtained in approximately 55% of men; however, in spinal cord-injured men with lesions above T11, the retrieval rate reaches 88% (16).

High-amplitude penile vibrostimulators have become affordable and therefore couples that are infertile because of anejaculation can use PVS at home for attempting pregnancy (26) or to improve semen quality by regular ejaculation. Home-use penile vibrostimulators may be not indicated in spinal cord-injured men with lesions above T6 because of the risk of autonomic dysreflexia.

EEJ is the treatment of choice if PVS fails. EEJ is a technique initially introduced to obtain spermatozoa from endangered species. In the late 1980s, the technique was introduced successfully in the clinic, too (27) Patients should receive the same work-up and preparation as for PVS.

For EEJ, patients with no spinal cord injury or patients with incomplete spinal cord lesions need general anesthesia. Sympathicolytic agents should not be used during anesthesia. As for PVS, spinal cord-injured men with lesions at T6 or above must be closely monitored for autonomic dysreflexia and pretreated whenever indicated (see above).

The patient is placed in lateral decubitus. Because of the risk of rectal burning due to the heating of the EEJ probe, it is recommended to use equipment with a built-in temperature sensor.

The EEJ probe is introduced in the rectum with the electrodes facing the prostate. In spinal cord-injured men, it may be recommended to perform a preliminary digital rectal examination and an anoscopy. A repetitive electrical stimulus of a maximum 5 V is applied for about two to four seconds each stimulus. When no ejaculation, either antegrade or retrograde, is obtained, the voltage may be gradually increased. With a few exceptions, ejaculation occurs at voltages lower than 20 V. During the stimulation, an assistant collects the antegrade fraction. After the procedure, anoscopy is repeated to ensure no rectal lesions occurred. The patient is placed in lithotomy position and the bladder is washed in order to recover any retrograde fraction. In 80%–95% of patients, spermatozoa can be recovered (16,27). According to the quality of the specimen obtained, either intrauterine insemination or assisted reproduction by ICSI can be performed. In anejaculatory men, and especially in spinal cord-injured men, both semen quality and sperm function may be deteriorated because of accumulation of reactive oxygen species, denervation, male accessory gland infection, post-infectious partial obstruction, or post-infectious primary testicular failure. Therefore, the introduction of ICSI has dramatically changed the perspective of patients suffering from anejaculation (16,28). Indeed, in combination with ICSI, spinal cord-injured men can father their children who are genetically their own, even when sperm quality is limited. In a small retrospective study, prostatic massage, EEJ, and testicular sperm extraction (TESE) were compared in terms of establishing a pregnancy by ICSI. It was shown that the three techniques resulted in similar pregnancy and live birth rates (22). A recent study showed that spinal cord-injured men who had ICSI with sperm obtained either after PVS or after EEJ had similar take-home baby rates compared to non-spinal cord-injured men (29).

METHODS FOR RETRIEVING EPIDIDYMAL OR TESTICULAR SPERMATOZOA

If no motile spermatozoa can be obtained from the ejaculate after centrifugation, a sperm retrieval procedure has

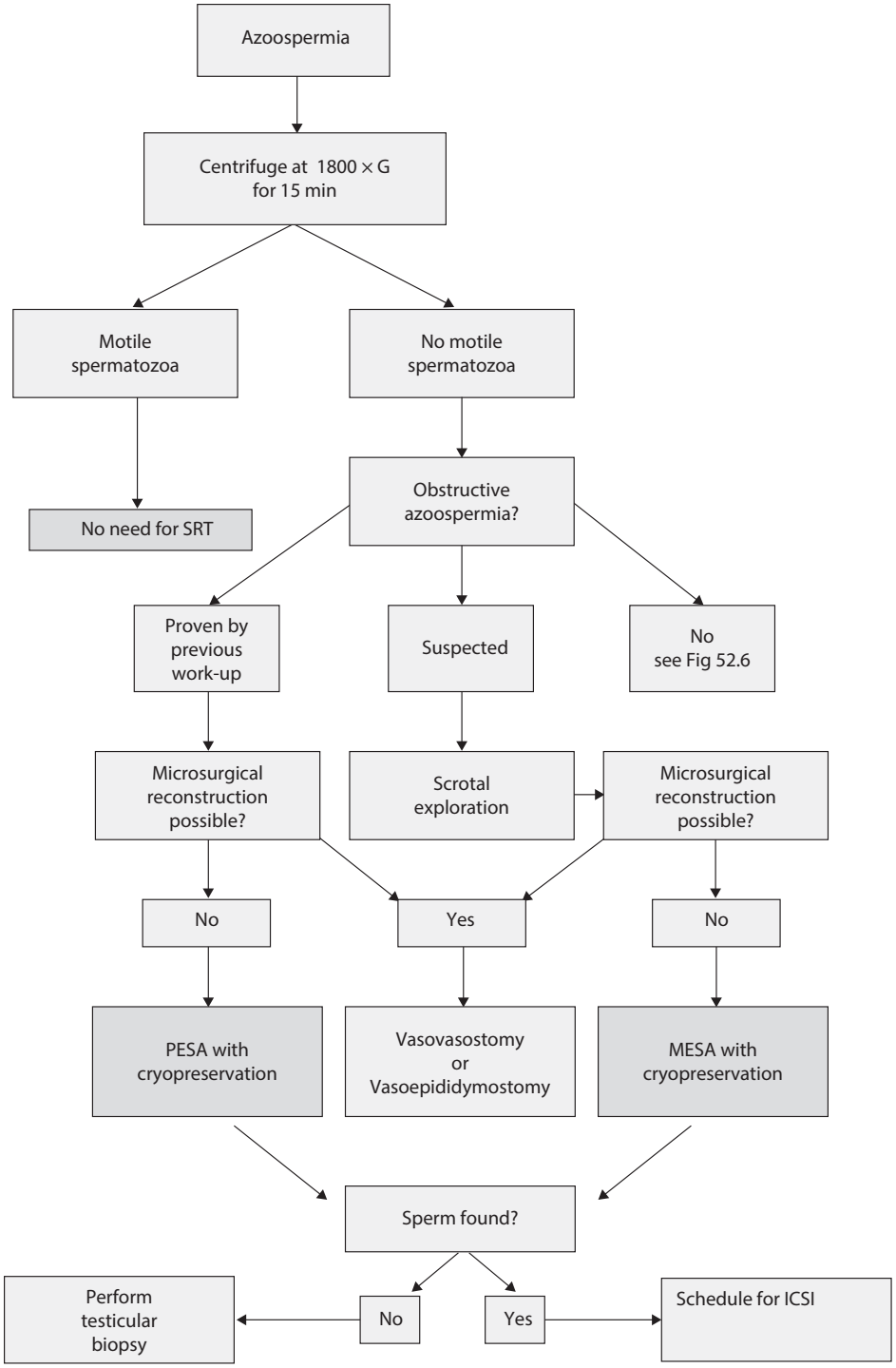

Figure 53.3 Treatment algorithm for patients with obstructive azoospermia. *Abbreviations*: ICSI, intracytoplasmic sperm injection; MESA, microsurgical epididymal sperm aspiration; PESA, percutaneous epididymal sperm aspiration; SRT, sperm recovery technique.

to be performed. At present, different methods are available to obtain spermatozoa from the vas deferens, epididymis, or testicular mass (30,31). The method of choice will depend on the type of azoospermia (non-obstructive or obstructive), surgical skills, and the techniques available in a given setting. If sperm has to be retrieved on an outpatient basis, techniques should be adopted that are compatible with local or locoregional anesthesia.

In case of OA, several methods are available. Figure 53.3 shows the algorithm currently used in our setting. If OA is expected but either the cause or the site of the obstruction is unknown, a scrotal exploration can be performed. This may not only reveal the cause and site of the obstruction and confirm the diagnosis of OA, but may also provide the possibility of performing reconstructive surgery. If no surgical correction is feasible, then microsurgical epididymal

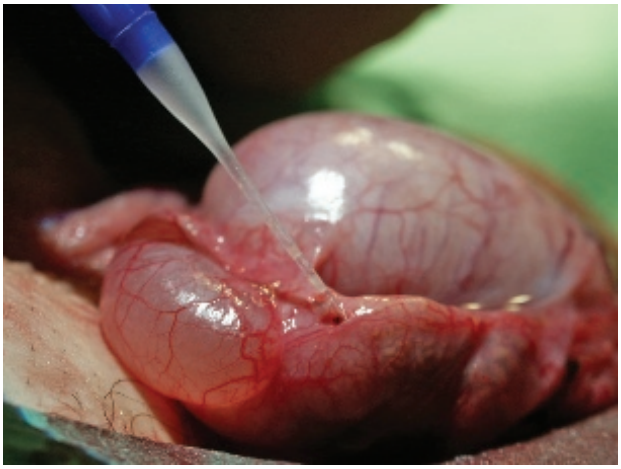

Figure 53.4 Microsurgical epididymal sperm aspiration. The epididymis is exposed and epididymal fluid is collected after a microincision in a dilated tubule.

sperm aspiration (MESA) is performed during scrotal exploration, expecting a more than 90% sperm retrieval rate using this technique (Figure 53.4, protocol 7 in Appendix) (32). The epididymal spermatozoa that are obtained can be easily cryopreserved for later use (33). A meta-analysis showed no difference in fertilization (Relative risk [RR] 1.02; 95% confidence interval [CI] 0.96–1.08) or implantation rates (RR 1.17; 95% CI 0.86–1.59) after fresh versus frozen–thawed epididymal sperm were used. Although a significantly higher clinical pregnancy rate was observed (RR 1.20; 95% CI 1.0 –1.42), no difference was found in ongoing pregnancy rates (RR 1.17; 95% CI 0.96–1.43) (34).

If, however, previous work-up has shown that microsurgical reconstruction is not possible, then a percutaneous epididymal sperm aspiration (PESA) may be performed (Figure 53.5, protocol 3 in Appendix). Although there may be some concerns that this blind method may cause epididymal damage and fibrosis, this issue is not important when reconstruction is not possible. When epididymal sperm are to be used for ICSI, spermatozoa with low levels of DNA damage (i.e., motile spermatozoa) are to be obtained in order not to jeopardize the success rate of the coincident ICSI cycle. Epididymal sperm may accumulate DNA damage over time: the study by Ramos et al. reported that about 17% of sperm obtained from the epididymis by MESA shows DNA damage as demonstrated by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. The DNA damage rate was only 9.3% for sperm recovered from the testis and 6.2% in fresh sperm obtained from sperm donors (35). However, in the motile fractions of the surgically recovered sperm, the final DNA damage rate was less than 1% and comparable to that of donor sperm (35). When motile spermatozoa are used, no differences in fertilization rates or live birth rates are observed between epididymal and testicular sperm used for ICSI (34). PESA has a high sperm retrieval rate, being able to provide motile sperm for ICSI in more than 80% of the patients with OA (36,37). In cases of PESA failure, testicular spermatozoa may be obtained, although live birth rates after ICSI using epididymal sperm are higher than after using testis sperm (38).

In men with OA, testicular sperm can be obtained either by fine-needle aspiration (FNA) or by open testicular biopsy of the testis (Figure 53.6, protocols 4 and 5 in Appendix). Both methods are similar in terms of outcome in OA, but the number of sperm obtained after open biopsies is much higher (39). For this reason, testicular biopsy may be preferred whenever cryopreservation is desired. Alternative methods of testicular aspiration have been described yielding higher numbers of spermatozoa (31,40). In these aspiration techniques, either needles with a larger diameter are used in order to obtain tissue cylinders or seminiferous tubules are pulled out by microforceps after puncturing or incising the tunica albuginea

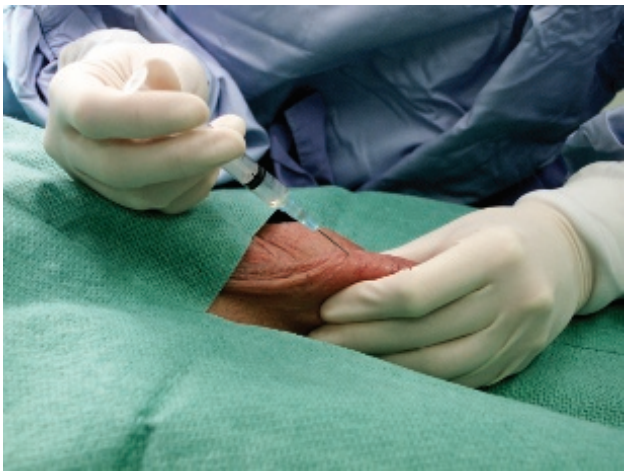

Figure 53.5 Percutaneous epididymal sperm aspiration. The epididymis is palpated and epididymal fluid is collected after a blind percutaneous puncture with a 19- or 21-gauge needle.

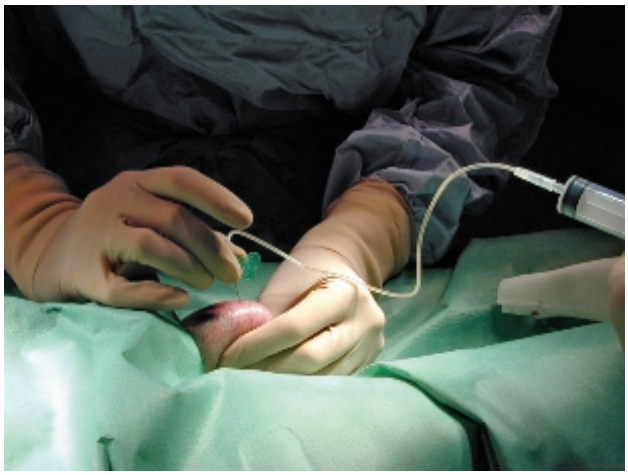

Figure 53.6 Fine-needle aspiration of the testis. Using a fine 21-gauge butterfly needle filled with a minute volume of sperm preparation medium, the testicular mass is punctured and an aspirate is collected.

(31). Compared with FNA, these alternative methods are less patient friendly and need local or locoregional anesthesia. Sometimes they even need to be combined with a small incision by a sharp blade in the scrotal skin. Their main advantage is that cryopreservation is easy and efficient because of the higher numbers of sperm obtained.

For men with OA who need surgical sperm recovery for ICSI, both patient and surgeon can decide which approach will be used. When cryopreservation is required, then PESA is the method of choice, followed by TESE whenever the former approach fails to recover motile epididymal sperm. These two techniques yield high numbers of sperm necessary for cryopreservation.

When a minimally invasive technique is preferred ("no-scar technique"), then again PESA is the first-choice method, followed by FNA whenever PESA fails to recover motile spermatozoa. However, with FNA the numbers of spermatozoa are limited, hampering cryopreservation.

Figure 53.7 shows our current algorithm for patients with NOA willing to undergo ICSI treatment combining ability to freeze and patient friendliness.

TESE is the most frequently used technique for NOA, with an average sperm retrieval rate of 50% (41). Although testicular volume and serum FSH are routinely assessed in azoospermic men, their predictive power remains limited and is subject to the heterogeneity of the

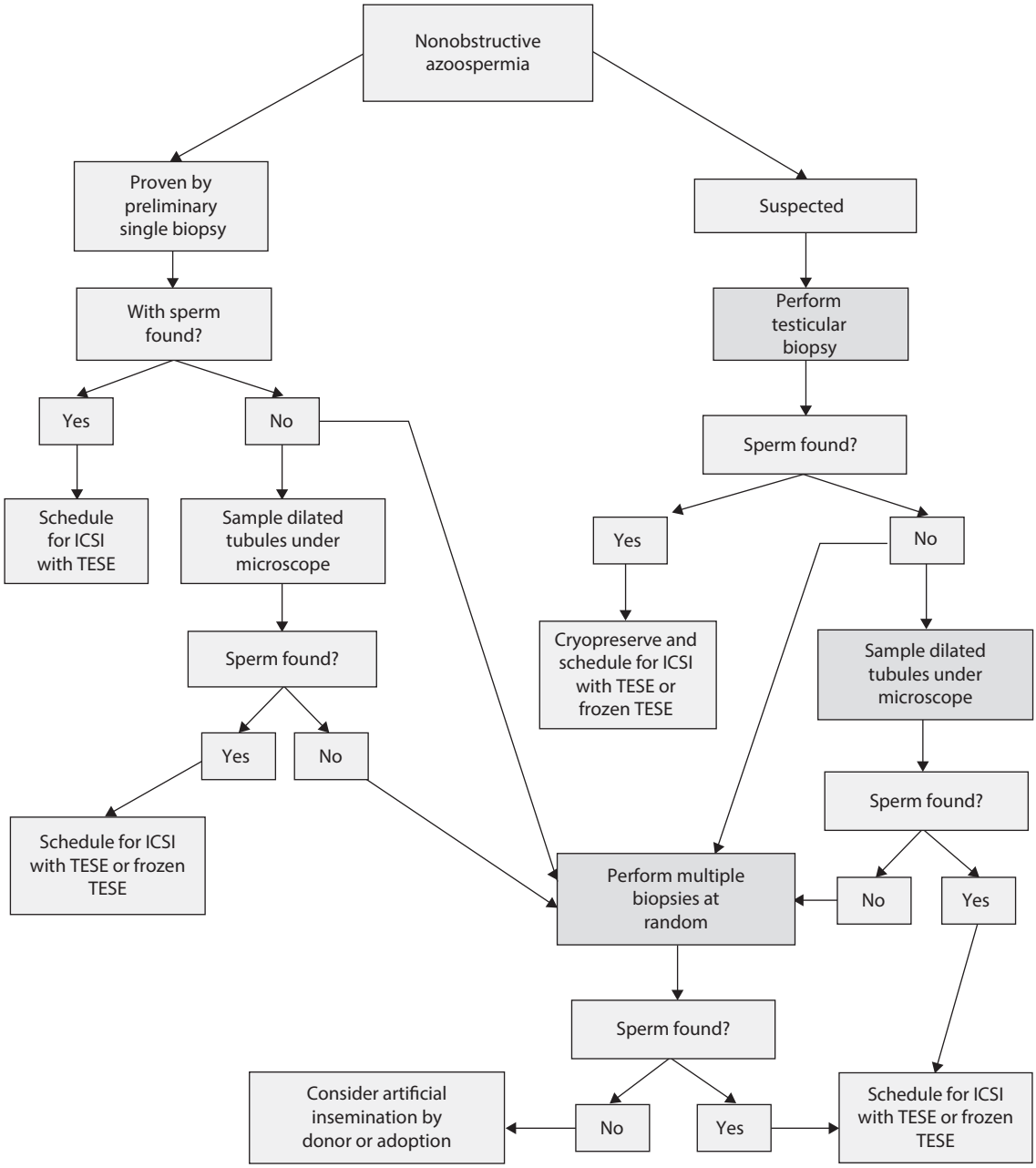

Figure 53.7 Treatment algorithm for patients with non-obstructive azoospermia. *Abbreviations*: ICSI, intracytoplasmic sperm injection; TESE, testicular sperm extraction.

population of NOA patients studied (42–44). There is still some controversy about the role of non-routine assessments such as inhibin-B for predicting successful sperm recovery (45–48). Recently, other non-routine markers (e.g., leptin) have been described as being useful in the prediction of sperm recovery after TESE (49). Since a single parameter has a low sensitivity for predicting TESE outcome, predictive models have been published combining several parameters (49,50). At present, it remains unclear whether such predictive models apply to all azoospermic men (51).

Apart from clinical parameters and hormonal tests, Doppler ultrasound of the testis has also been proposed as a method to predict successful recovery. But even such testicular vascularity assessment has a sensitivity not exceeding 50% (52,53).

The most invasive predictive strategy for TESE is "testicular mapping." According to an organized pattern, the testis is aspirated in different locations, followed by a cytological examination of these aspirates. Again, the sensitivity of this approach is below 50%. A mapping study by Bettella et al. reported that in 70 patients with a Sertoli cell-only pattern at testicular histology, mapping did not show any sperm; however, during a subsequent TESE procedure, sperm were recovered in 41% of these patients (54). To date, only histopathology has been shown to predict the probability of finding sperm with both acceptable sensitivity and specificity in subgroups of NOA men (55–57).

Although only applicable to a few patients, Yq deletion testing provides a robust prediction: no testicular sperm can be recovered in azoospermic men with AZFa and AZFb deletions (32,57).

The appropriate number of biopsies to be taken remains controversial. Although single testicular biopsy has been proposed as the best approach (58), it is currently recommended to take multiple samples from different sites of the testis, since a patchy distribution of spermatogenesis throughout the testis has been identified (42,59). In addition, it has been shown that TESE with multiple biopsies results in a higher chance of finding motile spermatozoa (60). Care should be taken to take small tissue pieces and to avoid cutting the arterioles as much as possible in order not to cause too much devascularization.

Concerning the best location to perform the biopsy, two small descriptive studies reported opposite results. Hauser et al. (60) found no differences in the sperm retrieval rate between three testicular sites, whereas Witt et al. concluded that the midline portion of the testis enabled the highest retrieval rate (61).

If a preliminary single biopsy has shown focal spermatogenesis with testicular spermatozoa present, the patient and his partner may be scheduled for ICSI with a TESE performed on the day of the oocyte retrieval. Vernaeve et al. reported finding sperm in up to 78% of patients in whom TESE had been previously successful (62).

When a preliminary single biopsy has not shown the presence of testicular spermatozoa, a testicular sperm retrieval procedure with multiple biopsies has to be proposed (Figure 53.8, protocol 6 in Appendix) (6,43). Because multiple biopsies may lead to extensive fibrosis and devascularization (63,64), multiple excisional biopsies may be taken under an operating microscope at ×40 and ×80 magnification (65). This microsurgical approach aims at sampling the more distended tubules in order to limit testicular damage. Micro-TESE (Figure 53.9) may theoretically be very useful in cases of Sertoli cell-only syndrome with focal spermatogenesis, but less useful in cases with maturation arrest where there is generally no difference in diameter of tubules with or without focal spermatogenesis. The technique is more time consuming than conventional TESE and may be influenced by the surgeon's case volume (66). Some authors recommend micro-TESE as a salvage technique when TESE is negative in NOA patients, reaching a sperm retrieval rate of 46.5%

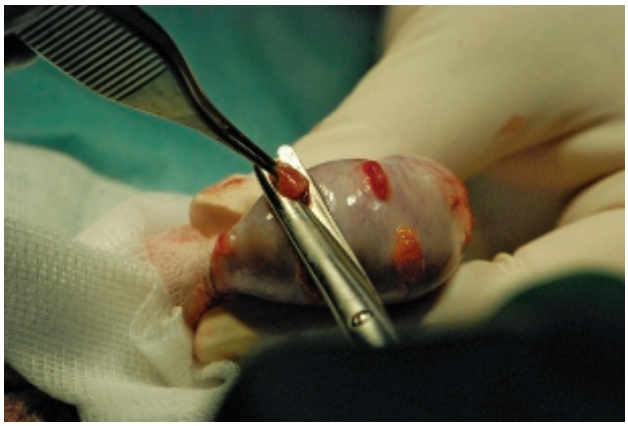

Figure 53.8 Multiple testicular sampling by open excisional testicular biopsy (testicular sperm extraction). Small tissue specimens are taken from the testicular mass while avoiding vascular injuries when incising the tunica albuginea.

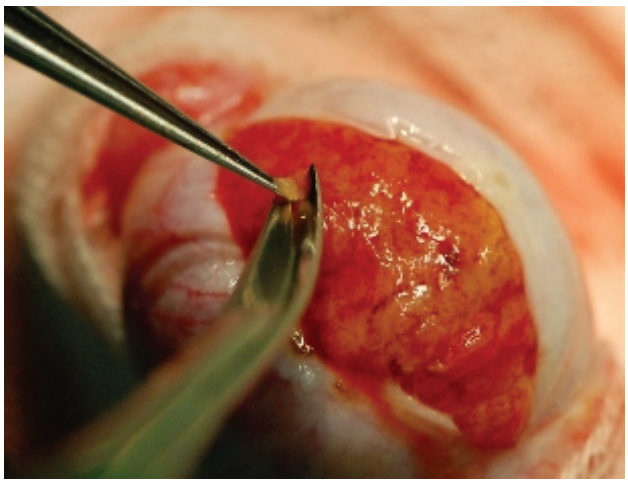

Figure 53.9 Testicular sampling by microscope-guided testicular sperm extraction (TESE) or micro-TESE. Under magnification, a dilated seminiferous tubule is excised using microscissors.

(67). A recent systematic review and meta-analysis comparing sperm recovery rate (SRR) in conventional TESE versus micro-TESE (mTESE) analyzed 15 studies with a total of 1890 patients. The authors concluded that mTESE was 1.5–times more likely to retrieve sperm than conventional TESE (95% CI 1.4–1.6) (68). Nonetheless, studies included in this meta-analysis were not randomized and showed high heterogeneity in both patient population and laboratory processing of samples, and thus definitive conclusions cannot be drawn. Another meta-analysis concluded that in patients with (incomplete) Sertoli cell-only syndrome, mTESE may have a benefit over TESE, but put a critical warning to the fact that their dataset only covered small pseudo-randomized studies (69). Not surprisingly, a recent large retrospective study reported retrieval rates after mTESE that were similar to those from large retrospective studies on classic random TESE (70,71).

When sperm are found, the samples may be frozen for later use with ICSI. If only a few spermatozoa are available or only a tiny amount of tissue is cryopreserved with only a few spermatozoa observed, we ask the patient to be on standby on the day of oocyte retrieval in case no spermatozoa can be observed after thawing. In the literature, data on the use of cryopreserved sperm from NOA patients is scarce. In the study by Verheyen et al., the frozen–thawed suspensions could not be used in 20 out of 97 cycles (20.6%), despite extensive search for motile sperm. However, a back-up fresh retrieval was successfully carried out in 14 cycles. Donor semen back-up should also be discussed prior to treatment (72). A recently published meta-analysis that evaluated the impact of fresh versus cryopreserved sperm in a large series of NOA men (574 ICSI cycles) showed no statistical difference in fertilization and clinical pregnancy rates (73).

A special subgroup of NOA is Klinefelter's syndrome patients, accounting for 11% of men with azoospermia. Again, in half of these patients, spermatozoa may be recovered for ICSI and pregnancies have been obtained after ICSI with testicular spermatozoa from 47,XXY non-mosaic Klinefelter's syndrome (74–76). Age represents an important parameter to predict sperm recovery in this group of patients, as shown by Okada et al., with a cutoff value of 35 years (77). Whether preimplantation genetic diagnosis should be performed because of the risk of aneuploidy in the embryos obtained in these patients remains controversial. Staessen and colleagues found that 46% of the embryos were chromosomally abnormal with a significant increase in sex and autosomal chromosome abnormalities, although without an increased specific risk for 47,XXY (78,79). This finding is in accordance with the hypothesis that Klinefelter's syndrome men in which testicular spermatozoa can be obtained produce these spermatozoa from 46,XY testicular stem cells (80).

Oncological patients are another subgroup at risk for NOA because of germ cell loss. Patients undergoing potentially sterilizing chemotherapy must bank their semen before starting any treatment (81). However, they may be azoospermic at the time of cancer diagnosis because of spermatogenic depression due to factors related to the malignancy. Yet these patients may be offered sperm recovery and banking before starting chemotherapy by vasal or epididymal sperm aspiration during orchiectomy (82) or TESE (onco-TESE) (83,84). Whenever sperm was not banked before starting chemotherapy, some patients with post-chemotherapy azoospermia may still benefit from TESE (85,86).

In order to improve retrieval rates in NOA, pre-surgical medical treatment has been proposed, attempting to improve spermatogenesis and eventually sperm recovery (87,88). Administration of estrogen receptor modulators such as clomiphene citrate or tamoxifen citrate and aromatase inhibitors primarily focuses on the enhancement of intratesticular testosterone levels and FSH production, but also increases plasmatic estrogen levels as well as testosterone production.

Treatment of infertile males with aromatase inhibitors like testolactone, anastrazole, and letrozole has been associated with increased sperm production and return of sperm to the ejaculate in men with NOA in small clinical trials and case reports (89–91). Some authors postulate that the use of aromatase inhibitors could enhance the sperm retrieval rate in selected group of patients with a low testosterone over estradiol (T/E2) ratio (<10), such as Klinefelter's syndrome patients (90). Unfortunately, no randomized controlled trials on either aromatase inhibitors or clomiphene citrate are available, hence the off-label use of this medical pre-treatment for NOA patients with low serum testosterone and abnormal T/E2 ratios should be carefully discussed before undergoing TESE.

The main complications of testicular retrieval techniques are hematoma, infection, fibrosis, and testicular atrophy (62,92,93).

Less invasive methods have been proposed in order to obtain testicular spermatozoa from patients with NOA (i.e., testicular aspiration). The main advantages of this technique are simplicity, low cost, being minimally invasive, and that it produces less postoperative pain compared to TESE under local anesthesia (30). However, multiple prospective studies have shown a lower recovery rate than with excisional biopsies (30,94–97). In patients with a history of cryptorchidism who have a higher risk of developing a testicular cancer from carcinoma *in situ* cells, an excisional biopsy must be performed in order to check for carcinoma *in situ* (98).

Because of the lack of randomized trials, controversy exists regarding which sperm retrieval technique should be preferred in NOA patients to guarantee the best chances of retrieving spermatozoa. While many studies (unfortunately poorly designed) focus on the surgical aspects of retrieving sperm, only a few studies address retrieval from testicular samples in the IVF laboratory. In many IVF laboratories, sperm retrieval is limited to microscopic observation of the wet preparation. However, the retrieval may be facilitated in the laboratory by using erythrocyte-lysing buffer (99,100) and enzymatic digestion (101). Some authors have reported that scheduling the testicular

recovery procedure one day before the ovum pickup (102) or the use of motility stimulants (e.g., pentoxifylline) may facilitate the retrieval of motile spermatozoa from the tissue (103,104).

A SUCCESSFUL TESTICULAR SPERM RECOVERY: WHAT IS NEXT?

In earlier reports, pregnancy rates after ICSI using testicular spermatozoa were comparable to those obtained after ICSI using epididymal spermatozoa patients with normal spermatogenesis (34,105). In a more recent series, results with testicular sperm were found to be inferior to epididymal sperm (38). Unfortunately, all these reports are based on retrospective case series and thus high-quality evidence in favor of the use of epididymal sperm in OA men is lacking.

In NOA men, ICSI with testicular sperm results in lower fertilization and embryo development compared with either the sperm of OA individuals or the ejaculated sperm of non-azoospermic men (106,107). The reasons for this finding are unclear, but are possibly associated with meiosis defects in NOA (108). Preimplantation genetic screening (PGS) has been proposed as a way to improve embryo selection in azoospermic men because of a higher frequency of aneuploid and mosaic embryos (109). PGS could be particularly important within the framework of a single-embryo transfer policy, where a higher chance of selecting a chromosomally abnormal embryo was reported when morphological criteria had solely been used (110). Whether PGS via comprehensive chromosome screening has any benefit for ICSI in NOA men remains to be studied (111).

When comparing reports on ICSI in NOA men, significant differences do exist between various reports, mainly because of differences in patient selection, the sample size of the study, and the definition of NOA (71). Typically, ICSI success rates in NOA patients are reported in different patient populations in which eventually testicular spermatozoa were invariably obtained (107,112). Only a few studies provide data on cumulative delivery or pregnancy rates after ICSI; however, again, these studies only include patients with successful sperm retrieval and thus overestimate pregnancy and live birth rates (113–115).

Currently, only one retrospective cohort study reports on a longitudinal follow-up of unselected NOA men undergoing TESE and eventually ICSI, concluding that while one out of four couples undergoing ICSI will have a live birth, eventually only one out of seven men undergoing TESE will father a child that is genetically their own (71).

Few data are available about the pregnancy outcomes and the neonatal data of children born after ICSI with surgically retrieved sperm in patients with azoospermia, and often such studies do not discriminate between either the source (epididymal vs. testicular) or the testicular function (obstructive vs. non-obstructive) (116,117).

So far, based on the few studies available, there is no indication that using either epididymal or testicular sperm from azoospermic men is associated with an increased risk of neonatal health problems, congenital malformation, or aneuploidy in comparison to children born after ICSI with ejaculated sperm (116,118).

Based on small sample sizes, these data have not shown any difference between pregnancies after the use of testicular sperm from NOA men compared to OA men (119,120).

Patients should thus be counseled that treating sterility because of OA is a successful approach, while ICSI for NOA has many limitations: firstly, there are limitations in the chances to recover testicular spermatozoa; and secondly, there are limitations in the outcomes after ICSI itself. ICSI with surgically retrieved sperm has been reported to be similar to ICSI with ejaculated sperm in terms of safety; however, there is an urgent need for longer follow-up with adequately powered and prospective cohort studies.

APPENDICES

Protocol 1: EEJ

Indication

Anejaculation refractory to PVS (see below)

Patient preparation

In spinal cord-injured men, a preliminary microbiological examination of the urine has to be performed. No rectal preparation (such as klysma). Fluid intake restricted to 500 mL in the 12 hours preceding the procedure.

The patient has to empty the bladder before EEJ. In spinal cord-injured men with lesions at T6 or higher, monitoring of blood pressure is mandatory. Nifedipine 10–20 mg may be given for preventing autonomic dysreflexia-related hypertension.

Patient wears a top only. He is placed in lithotomy position. Penal region cleansed with antiseptic solution (e.g., HAC, Zeneca: hospital antiseptic concentrate—contains chlorhexidine).

The EEJ procedure

The tip of a Nelaton bladder catheter is dipped into sterile liquid mineral oil as used in IVF. After instillation of 10 mL of sperm preparation medium into the urethra, the catheter is gently introduced into the bladder. The bladder is emptied and the urinary pH is measured. The bladder is then washed with 200 mL medium. After emptying, 50 mL of the medium is left in the bladder for collecting retrograde-ejaculated sperm. The patient is put into lateral decubitus. In spinal cord-injured men, an assistant should control leg spasm during the procedure.

Electrostimulation is performed using equipment with a built-in temperature sensor. After digital rectal examination and anoscopy, a standard probe is gently inserted into the rectum. Care is taken to orient the electrodes anteriorly. Electrostimulations are repeated, each stimulation lasting for two to four seconds. Baseline voltage should be 5 V and voltage can be increased or maintained according to the patient's reaction. In case of acute hypertension in patients with spinal cord lesions at T6 or higher, the procedure must be discontinued until blood pressure is again under control.

An assistant collects the antegrade fraction in a sterile container holding buffered sperm washing medium. The pendulous and bulbar urethra are continuously massaged by the assistant during the procedure. With the aid of a 1-mL syringe, ejaculated drops are flushed into the container. When no antegrade ejaculation is observed, indirect signs such as spasms of the lower abdominal muscles and legs and the appearance of goosebumps may indicate (retrograde) ejaculation. When ejaculation discontinues, the probe is removed and anoscopy is performed again to check for rectal lesions.

Then the patient is put again in lithotomy position. The bladder is re-catheterized and the bladder is emptied into a sterile container in order to collect any retrograde fraction. The bladder is flushed with 100 mL of medium until the flushing medium remains clear.

The collected fractions are transported to the andrology laboratory for identification of spermatozoa and further preparation. Centrifugation of the retrograde suspension may be necessary or open biopsy under local anesthetic should be performed.

Dressing after

Disposable underpants

Patient care post-operation

None

Requirements

- A runner
- Two assistants
- Seager Model 14 Electroejaculator (Dalzell Medical System, The Plains, VA, USA)
- Anoscope
- Manual manometer
- Nelaton catheter ch 14 (Cat.nr. 110)
- pH indicator strip (Merck, Germany)
- Mineral oil (Sigma-Aldrich, Darmstadt, Germany)
- Cleaning solution (3.5% HAC)
- Syringe 50 cc (BS-50 ES Terumo)
- Syringe Norm-Ject Cook 1 mL (K-ATS-1000)
- 100 mL modified Earle's balanced salt solution with 4-(2-hydroxyet4hyl)-1-piperazineethane sulfonic acid (HEPES), 0.4 Heparin Novo, and 2.25% human serum albumin
- Gauze squares 10 × 10

Protocol 2: PVS

Indication

Anejaculation

Patient preparation

As for EEJ

The PVS procedure

Patient empties his bladder before PVS and the urinary pH is measured. PVS is performed using high-amplitude equipment.

The antegrade fraction is collected into a sterile container holding buffered sperm washing medium. When no ejaculation occurs after five minutes, PVS is discontinued.

Then the patient is put again in lithotomy position. When no antegrade ejaculation is observed, but indirect signs are present (e.g., goosebumps and muscular spasms), the bladder is catheterized and emptied into a sterile container in order to collect any retrograde fraction (see above). The collected specimens are transported to the andrology laboratory for identification of spermatozoa and further preparation.

When PVS fails to induce ejaculation, EEJ has to be performed.

Patient care post-operation

None

Requirements

- Ferticare Personal Vibrostimulator (Multicept ApS, Denmark)
- Manual sphygmomanometer
- Ph indicator strip (Merck, Germany)
- Cleaning solution (3.5% HAC)
- Syringe Norm-Ject Cook 1 mL (K-ATS-1000)
- 50 mL modified Earle's balanced salt solution with HEPES, 0.4 Heparin Novo, and 2.25% human serum albumin.

Protocol 3: PESA

Indication

All cases of OA with normal spermatogenesis, such as congenital absence of the vas deferens and failed vasectomy reversal (CBAVD patients: read caveat in MESA section)

Patient preparation

The man is given hibitane soap to wash the area the night before and the morning of the operation. He is also asked to shave the area.

Meperidine hydrochloride 1 mg/kg intramuscularly and midazolam 2.5 mg intramuscularly may be given.

Patient has to empty the bladder before surgery.

Patient is fully draped with the operation site obscured to the patient. Patient wears a top only. Operation site cleansed with antiseptic solution (e.g.,, HAC, Zeneca—contains chlorhexidine). Penis is held up out of the way with a swab fixed underneath the drape. A drape with a small hole of 5 cm in diameter in the middle covers the operation site. The testes are gently pulled through to be in the field of the procedure. Local anesthetic—1–2 mL of 2% lidocaine (without epinephrine)—is injected in the spermatic cord in order to obtain locoregional anesthesia and into the scrotal skin.

The PESA procedure

A 19- or 21-gauge needle is used. Attached is a 10-mL syringe. The epididymis is held firmly between two fingers of one hand and the needle is inserted with the other

hand perpendicular to the epididymis. The needle is inserted into the epididymal mass and then gently withdrawn under slight suction. Care is taken not to move the needle in order to minimize contamination with blood and prevent epididymal damage. The embryologist/nurse brings a 1.5-mL Eppendorf micro-test tube filled with culture medium. The needle is placed in the micro-test tube and rinsed several times with the medium. The micro-test tube is then passed to the embryologist for identification of spermatozoa. Centrifugation of the suspension may be necessary. The procedure can be repeated if not enough sperm are retrieved. However, if after two aspirations there is no success, then an aspiration of the testis or open biopsy under local anesthetic should be performed.

Dressing after

Gauze squares and disposable underpants

Patient care post operation

The man is told that there may be some pain, but it should be minimal. Acetaminophen (paracetamol) can be taken. If more is required, then he should contact the clinic.

Requirements

- A runner
- Drape with central hole
- Non-iodine cleaning solution
- Syringe 10 cc (BS-10 ES Terumo)
- Micro-test tube 1.5 mL (Eppendorf 3810)
- (to be washed and sterilized first)
- Medium with modified Earle's balanced salt solution, HEPES, 0.4 Heparin Novo, and 2.25% human serum albumin
- Gauze squares 10 × 10(35813 Hartmann)

Protocol 4: FNA of testis for sperm retrieval

Indication

All cases of OA with normal spermatogenesis, such as congenital absence of the vas deferens and failed vasectomy reversal (CBAVD patients: read caveat in MESA section)

Patient preparation

As for PESA (see protocol 3)

The FNA procedure

A 21-guage 3/4-in butterfly needle is used, attached is a 20-mL syringe. A small amount of culture medium is drawn up into the tubing and the majority expelled until only about 1–2 mm is left in the butterfly tubing. There may be no air in the fluid. The butterfly needle is inserted perpendicular to the testis and a little away from the site of insertion of the needle used to inject the local anesthetic, as there is usually some blood at that site. The testis is held firmly in one hand and the butterfly needle is inserted with the other. Care is taken not to move the butterfly needle in order to minimize contamination with blood and prevent testicular damage. The patient may feel some pain only when the needle enters the tunica. The operator or assistant now "pumps" 5–10 times on the 20-mL syringe in order to generate suction to aspirate sperm. It is important to keep a slight negative pressure in order to make sure the aspirate is not pushed back into the testis. This is done by ensuring the plunger does not return all the way to the end. The butterfly needle tubing is then occluded near the needle and the butterfly needle subsequently removed with a smooth, sharp movement in order to minimize tissue trauma and contamination with blood. Occluding the tubing prevents aspirating blood from reaching the skin surface. With the tubing still occluded, the 20-mL syringe (must have rubber stop that may never be in contact with the medium) is removed and a 1-mL syringe with the plunger partially withdrawn is attached. Otherwise, the 20-mL syringe may be used.

The embryologist/nurse brings a dish with nine droplets of culture medium placed on it (one central droplet surrounded by eight droplets). The butterfly needle is placed in a droplet of culture medium and the butterfly needle tubing released, thereby removing the negative pressure. A small amount of the aspirate and the culture medium in the butterfly needle is then injected into each droplet in turn. Usually about three to five droplets will be used in this way. Fractionating the aspirate containing red blood cells will improve subsequent visualization under the microscope. The dish is then passed to the embryologist for identification of spermatozoa. The procedure can be repeated if not enough sperm are retrieved initially. However, if after three aspirations there is no success, then an open biopsy under local anesthetic should be performed.

Dressing after

As for PESA (see protocol 3)

Patient care post-operation

As for PESA (see protocol 3)

Requirements

- A runner
- Drape with central hole
- Non-iodine cleaning solution
- Syringe 20 cc (BS-20 ES Terumo)
- Surflo Winged Infusionset
- CE 0197 21-guage × 3/4" (SV-21BL Terumo)
- Flushed with medium
- Modified Earle's balanced salt solution with HEPES, 0.4 Heparin Novo, and 2.25% human serum albumin
- Syringe 1 cc (Air-Tite K-ATS-1000 Cook)
- Gauze squares 10 × 10 (35813 Hartmann)
- To transport sperm
- Tissue culture dishes (3200 Falcon Becton Dickinson)
- With droplets of medium (modified Earle's balanced salt solution with HEPES, 0.4 Heparin Novo, and 2.25% human serum albumin)

Protocol 5: open testicular biopsy under local anesthesia

Indication

Patients with OA with normal spermatogenesis who wish to have testicular sperm cryopreserved (CBAVD patients: read caveat in MESA section)

Patient preparation

As for PESA (see protocol 3)

Procedure

Approximately 5 mL lidocaine (2%) is injected into the skin and the underlying layers up to the tunica albuginea. The testis is fixed in the left hand and a 1–2-cm incision is then made into the scrotum and down through the tissue made edematous by the lignocaine to the tunica. The testis must remain fixed in order not to lose the alignment of the scrotal incision with the incision into the tunica. With the sharp point of the blade, the tunica is opened and the incision slightly extended. Under gentle pressure with the left hand, testicular tissue will protrude through the incision. By the use of a curved pair of Mayo scissors, a small sample is excised and placed into a Petri dish filled with sperm preparation medium (e.g., Earle's). Selective hemostasis with diathermy is performed since intratesticular bleeding may cause discomfort and fibrosis.

The testicular tissue is rinsed in the medium and then placed into another Petri dish filled with medium. After hemostasis, the tunica is closed with 3/0 Vicryl sutures. The skin is closed with interrupted 3/0 Vicryl sutures. A clean gauze swab covers the suture site and disposable underpants are given for support.

Patient care post-operation

As for PESA (see protocol 3).

The patient is told that the sutures will dissolve. There is increased risk of hematoma. The patient should report undue bruising or pain that is not alleviated with paracetamol.

Requirements

- An assistant and a runner
- Monopolar pencil with needle and cord (E 2502 Valleylab)
- Tubeholder (1×) (708130 Mölnlycke)
- To fix cords on drape (pencilcord off foot end)
- Needleholder Mayo-Hegar (20-642-16 Martin)
- Straight Mayo scissors (11-180-15 Martin)
- Adlerkreutz pincet (12-366-15 Martin)
- Allis forceps (30-134-15 Martin)
- Kryle forceps (13-341-14 Martin)
- Micro-Adson pincet (2×) (12-404-12 Martin)
- Micro-Adson pincet (2×) (12-406-12 Martin)
- Adson pincet (31-09770 Leibinger)
- Adson pincet (31-09772 Leibinger)
- Metzenbaum scissors (11-264-15 Martin)
- Metzenbaum scissors (11-939-14 Martin)
- Knifehandle with blades nr 15 (0505 Swann-Morton)
- Swabs 10 × 10 (35813 Hartmann)
- Vicryl 3/0 (JV 497 Ethicon Johnson/Johnson)
- Tissue culture dishes (2×) (3102 Falcon Becton Dickinson)
- With medium (modified Earle's balanced salt solution with HEPES, 0.4 Heparin Novo, and 2.25% human serum albumin)
- Local anesthesia
- Syringe 20 cc CE 0197 (BS-20 ES Terumo)
- Needle 18 gauge (NN 1838 S Terumo)
- Needle 26 gauge (NN 2613 R Terumo)
- Xylocaine 2% (Astra Pharmaceuticals)

Protocol 6: testicular biopsy under general anesthesia

Indication

All cases of NOA (primary testicular failure). When testicular biopsy is performed in such patients, a preliminary screening for deletions of the Yq region of the Y chromosome is preferable in the male partner, since deletions may be found in about 5%–10% of patients with unexplained primary testicular failure. Before undertaking the procedure, it is important to identify the best testis to explore. This is done by reading any previous histology reports and feeling the testis for size and consistency. If the testis is high or retracted, then the chance of retrieving spermatozoa is lower.

Patient preparation

As for PESA (see protocol 3)

Procedure

Biopsies taken at random

As for under local anesthetic (see protocol 4). The main difference is that a larger scrotal incision is made and the testis is delivered.

If no sperm are observed in the wet preparation, multiple small incisions can be made and biopsies taken accordingly. The incisions must avoid the arterial blood supply. The contralateral testis may be explored as well.

Biopsies taken with operating microscope (micro-TESE)

After scrototomy, the tunica albuginea is opened longitudinally with the sharp point of the blade, avoiding the arterial blood supply. Then the testicular pulpa containing the tubuli seminiferi is exposed to a 40–80× magnification using an operating microscope. Care is taken to keep the tubuli wet by a constant drip of saline. Distended tubules are spotted and sampled by micro-scissors, avoiding the arterial blood supply.

The tiny samples are placed into a Petri dish filled with sperm preparation medium (e.g., Earle's). The testicular samples are rinsed in the medium and then placed into another Petri dish filled with medium. After controlling hemostasis, the tunica is closed with a continuous 7/0 Ethilon suture. The skin is closed with interrupted 3/0

Vicryl sutures. A clean gauze swab covers the suture site and disposable underpants are given for support.

Patient care post-operation

See open biopsy under local anesthesia

Protocol 7: MESA

Indication

Patients with OA with normal spermatogenesis who wish to have epididymal sperm cryopreserved. The main drawback of MESA is that it is an invasive and expensive procedure requiring a basic knowledge of epididymal anatomy and of microsurgical techniques. However, the major benefit of this procedure is its diagnostic power: a full scrotal exploration can be performed and, whenever indicated, a vasoepididymostomy may be performed concomitantly. Furthermore, the number of spermatozoa retrieved is high, which facilitates cryopreservation.

Caveat

When MESA is performed in CBAVD patients, a preliminary screening for mutations of the cystic fibrosis (CF) gene is mandatory in both the male CBAVD patient and his partner, since mutations are found in 60%–70% of CBAVD patients without congenital renal malformations. If the female partner is found to be a carrier of a CF gene mutation, preimplantation genetic diagnosis should be proposed. Even where only the man is a carrier of a CF mutation, the couple has to be informed of the risk of having a boy with a genital CF phenotype with CBAVD.

Patient preparation

As for PESA (see protocol 3)

MESA procedure

MESA can be performed during any scrotal exploration taking place even long before the ICSI treatment is scheduled or in a satellite center (e.g., by a surgeon not involved in assisted reproduction).

Using an operating microscope, the epididymis is carefully dissected and after hemostasis. Using bipolar coagulation, a distended epididymal tubule is longitudinally opened by micro-scissors through a small opening in the serosa. The proximal corporal or distal head region of the epididymis is opened first. The epididymal fluid is aspirated by means of a disposable tip from an intravenous cannula mounted on a 1-mL syringe filled with 0.1 mL HEPES-buffered Earle's medium supplemented with 0.4% human serum albumin. The aspirated epididymal fluid is then transferred into a Falcon test tube, which is filled with 0.9 mL of this Earle's medium. When motile spermatozoa are recovered, as assessed by peri-operative microscopic examination of the aspirates, no further epididymal incision is made and a maximum of fluid is aspirated. If microscopic assessment does not show any motile sperm cells, a more proximal incision is made until motile sperm cells are found. In some instances, centrifugation (1800 × *g*, five minutes) of the epididymal aspirates is needed in order to observe spermatozoa under the microscope. In cases where no motile spermatozoa are recovered, a testicular biopsy is taken for sperm recovery (see below). The sperm suspension is further prepared and kept in the incubator until the moment of intracytoplasmic injection or cryopreservation.

Patient care post-operation

Same as for TESE under general anesthesia (see protocol 6)

Requirements

- An assistant and a runner
- Needleholder Mayo-Hegar (20-642-16 Martin)
- Straight Mayo scissors (11-180-15 Martin)
- Monopolar pencil and cord (E 2502 Valleylab)
- Bipolar pincet and cord (4055 Valleylab)
- Tubeholders (2×) (708130 Mölnlycke)
- to fix cords on drape (bipolar cord off head end, pencil-cord off foot end)
- Micro-scissors (OP 5503 V-Mueller)
- Micro-needleholder (GU 8170 V-Meuller)
- Jeweler's forceps (3×) (E 1947 Storz)
- (72 BD 330 Aesculaep)
- Curved blunt scissors (11-939-14 Martin)
- 1 cc syringe (4×) (Air-tite K-ATS-1000 Cook)
- with or 22 ga medicut (8888 100 107 Argyle)
- or Cook aspiration CT (K Sal 400 300 Cook)
- Micro-Adson pincet with teeth (2×) (12-406-12 Martin)
- Knife handle with blades nr 15 (0505 Swann-Morton)
- Knife handle with blades nr 11 (0503 Swann-Morton)
- NaCl 0.9% 500 mL (B1323 Baxter)
- with 2500 IU Heparin Novo
- (Heparin Novo Nordisk Pharma)
- Syringes 20 cc (2×) (SS 20 ES Terumo)
- with 22 ga Medicut tip (8888 100107 Argyle)
- Swabs 10 × 10 (35813 Hartmann)
- Tip cleaner
- (Surgikos 4315 Johnson-Johnson)
- Micro-sponges (NDC 8065-1000-02 Alcon)
- Sutures
- Ethilon 9/0 (W 1769 Ethicon)
- Vicryl 3/0 (JV 497 Ethicon)
- Microscope
- Surgical operating and diagnostic microscope Wild M 691 with 180° positioning for doctor and assistant and optical eyepiece opposite each other
- (M 691 Leica)
- Achromatic lens f = 200 mm (M 382162 Leica)

REFERENCES

1. Palermo G, Joris K, Devroey P et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992; 340: 17–8.
2. Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, Wisanto A, Devroey P. Higher fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 1993; 8: 1061–66.

3. Craft I, Benett V, Nicholson N. Fertilising ability of testicular spermatozoa (Letter). *Lancet* 1993; 342: 864.
4. Schoysman R, Vanderzwalmen P, Nijs M, Segal L, Segal-Bertin G, Geerts I, Roosendaal E, Schoysman D. Pregnancy after fertilisation with human testicular spermatozoa. *Lancet* 1993; 342: 1237.
5. Devroey P, Liu J, Nagy Z, Goosens A, Tournaye H, Camus M, Van Steirteghem AC, Silber S. Pregnancies alter testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod* 1995; 10: 1457–60.
6. Tournaye H, Camus M, Goosens A, Nagy Z, Silber S, Van Steirteghem AC, Devroey P. Recent concepts in the management of infertility because of non-obstructive azoospermia. *Hum Reprod* 1995; 10(Suppl 1): 115–9.
7. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patients. *J Urol* 1989; 142: 62.
8. Matsumiya K, Namiki M, Takahara S et al. Clinical study of azoospermia. *Int J Androl* 1994; 17: 140–2.
9. Rastrelli G, Corona G, Mannucci E, Maggi M. Factors affecting spermatogenesis upon gonadotropin-replacement therapy: A meta-analytic study. *Andrology* 2014; 2: 794–808.
10. Skakkebaek NE. Testicular dysgenesis syndrome. *Horm Res* 2003; 60(Suppl 3): 49.
11. Tournaye H, Camus M, Vandervorst M, Nagy Z, Joris H, Van Steirteghem A, Devroey P. Surgical sperm retrieval for intracytoplasmic sperm injection. *Int J Androl* 1997; 20(Suppl 3): 69–73.
12. Tournaye H. Gamete source and manipulation. *Current Practices and Controversies in Assisted Reproduction—Report of a WHO Meeting*. Vayena E, Rowe P J, Griffin P D (eds.). World Health Organization Geneva, pp. 83–101.
13. Van Steirteghem A, Tournaye H, Joris H, Filicori M (ed.), Flamigni C (ed.), Van Landuyt L, Verheyen G. In *Treatment of Infertility: The New Frontiers. Proceedings of the Conference*, January 22–24, 1998, Filicori M, Flamigni C (eds.). Communications Media for Education Inc., Boca Raton, Florida.
14. Ron-El R, Strassburger D, Friedler S et al. Extended sperm preparation: An alternative to testicular sperm extraction in non-obstructive azoospermia. *Hum Reprod* 1997; 12: 1222–6.
15. Swanton A, Itani A, McVeigh E, Child T. Azoospermia: Is a simple centrifugation indicated? A national survey of practice and the Oxford experience. *Fertil Steril* 2007; 88: 374–8.
16. Kafetsoulis A, Brackett N, Ibrahim E, Attia G, Lynne C. Current trends in the treatment of infertility in men with spinal cord injury. *Fertil Steril* 2006; 86: 781–9.
17. O'Kelly F, Manecksha RP, Cullen IM, McDermott TE, Flynn R, Grainger R. Electroejaculatory stimulation and its implications for male infertility in spinal cord injury: A short history through four decades of sperm retrieval (1975–2010). *J Urology* 2011; 77: 1349–52.
18. Hirsh A, Mills C, Tan SL et al. Pregnancy using spermatozoa aspirated from the vas deferens in a patient with ejaculatory failure due to spinal injury. *Hum Reprod* 1993; 8: 89–90.
19. Hovatta O, Reima I, Foudila T, Butzow T, Johansson K, von Smitten K. Vas deferens aspiration and intracytoplasmic sperm injection of frozen–thawed spermatozoa in a case of anejaculation in a diabetic man. *Hum Reprod* 1996; 11: 334–5.
20. Soeterik TF, Veenboer PW, Lock TM. Electroejaculation in psychogenic anejaculation. *Fertil Steril* 2014; 101: 1604–8.
21. Tur-Kaspa I, Segal S, Moffa F, Massobrio M, Meltzer S. Viagra for temporary erectile dysfunction during treatments with assisted reproductive technologies. *Hum Reprod* 1999; 14: 1783–4.
22. Engin-Ustun Y, Korkmaz C, Duru NK, Baser I. Comparison of three sperm retrieval techniques in spinal cord-injured men: Pregnancy outcome. *Gynecol Endocrinol* 2006; 22: 252–5.
23. Kamischke A, Nieschlag E. Update on medical treatment of ejaculatory disorders. *Int J Androl* 2002; 25: 333–44.
24. Chéhensse C, Bahrami S, Denys P, Clément P, Bernabé J, Giuliano F. The spinal control of ejaculation revisited: A systematic review and meta-analysis of anejaculation in spinal cord injured patients. *Hum Reprod Update* 2013; 19: 507–26.
25. Castle SM, Jenkins LC, Ibrahim E, Aballa TC, Lynne CM, Brackett NL. Safety and efficacy of a new device for inducing ejaculation in men with spinal cord injuries. *Spinal Cord* 2014; 52: S27–9.
26. Sonksen J, Fode M, Lochner-Ernst D, Ohl DA. Vibratory ejaculation in 140 spinal cord injured men and home insemination of their partners. *Spinal Cord* 2012; 50: 63–6.
27. Halstead LS, Vervoort S, Seager S. Rectal probe electrostimulation in the treatment of anejaculatory spinal cord injured men. *Paraplegia* 1987; 25: 120–9.
28. McGuire C, Manecksha RP, Sheils P, McDermott TED, Grainger R, Flynn R. Electroejaculatory stimulation for male infertility secondary to spinal cord injury: The Irish experience in National Rehabilitation Hospital. *J Urology* 2011; 77(1): 83–7.
29. Kathiresan AS, Ibrahim E, Aballa TC, Attia GR, Ory SJ, Hoffman DI, Maxson WS, Barrionuevo MJ, Lynne CM, Brackett NL. Comparison of *in vitro* fertilization/intracytoplasmic sperm injection outcomes in male factor infertility patients with and without spinal cord injuries. *Fertil Steril* 2011; 96(3): 562–6.
30. Tournaye H. Surgical sperm recovery for intracytoplasmic sperm injection: Which method is to be preferred? *Fertil Steril* 2011; 96(3): 562–6.
31. Shah R. Surgical sperm retrieval: Techniques and their indications. *Indian J Urol* 2011; 27: 102–9.
32. Gangrade B. Cryopreservation of testicular and epididymal sperm: Techniques and clinical outcomes of assisted conception. *Clinics* 2013; 68: 131–40.

33. Tournaye H, Merdad T, Silber S. No differences in outcome after intracytoplasmic sperm injection with fresh or with frozen-thawed epididymal sperm. *Hum Reprod* 1999; 14:101–6.
34. Nicopoullos J, Gilling-Smith C, Almeida P et al. Use of surgical sperm retrieval in azoospermic men: A meta-analysis. *Fertil Steril* 2004; 82: 691–701.
35. Ramos L, Kleingeld P, Meuleman E, van Kooy R, Kremer J, Braat D, Wetzels A. Assessment of DNA fragmentation of spermatozoa that were surgically retrieved from men with obstructive azoospermia. *Fertil Steril* 2002; 77: 233–7.
36. Yafi F, Zini A. Percutaneous epididymal sperm aspiration for men with obstructive azoospermia: Predictors of successful sperm retrieval. *Urology* 2013; 82: 341–4.
37. Gorgy A, Meniru GI, Bates S, Craft IL. Percutaneous epididymal sperm aspiration and testicular sperm aspiration for intracytoplasmic sperm injection under local anesthesia. *Assist Reprod Rev* 1998; 8: 79–93.
38. van Wely M, Barbey N, Meissner A, Repping S, Silber SJ. Live birth rates after MESA or TESE in men with obstructive azoospermia: Is there a difference? *Hum Reprod* 2015; 30: 761–6.
39. Tournaye H, Clasen K, Aytoz A, et al. Fine needle aspiration versus open biopsy for testicular sperm recovery: A controlled study in azoospermic patients with normal spermatogenesis. *Hum Reprod* 1998; 13: 901–4.
40. Morey AF, Deshon GE, Jr., Rozanski TA, Dresner ML. Technique of biopty gun testis needle biopsy. *Urology* 1993; 42:325–6.
41. Donoso P, Tournaye H, Devroey P. Which is the best sperm retrieval technique for non-obstructive azoospermia? A systematic review. *Hum Reprod Update* 2007; 13: 539–49.
42. Tournaye H, Liu J, Nagy Z et al. Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular sperm. *Hum Reprod* 1996; 11: 127–32.
43. Ezeh UIO, Moore HDM, Cooke ID. Correlation of testicular sperm extraction with morphological, biophysical and endocrine profiles in men with azoospermia due to primary gonadal failure. *Hum Reprod* 1998; 13: 3066–74.
44. Miitchell V, Robin G, Boitrelle F, Massart P, Marchetti C, Marcelli F, Rigot JM. Correlation between testicular sperm extraction outcomes and clinical, endocrine and testicular histology parameters in 120 azoospermic men with normal serum FSH levels. *Int J Androl* 2011; 34: 299–305.
45. Tunc L, Kirac M, Gurocak S, Yucel A, Kupeli B, Alkibay T, Bozkirli I. Can serum inhibin B and FSH levels, testicular histology and volume predict the outcome of testicular sperm extraction in patients with non-obstructive azoospermia? *Int Urol Nephrol* 2006; 38: 629–35.
46. Ballesca JL, Balasch J, Calafell JM et al. Serum inhibin B determination is predictive of successful testicular sperm extraction in men with non-obstructive azoospermia. *Hum Reprod* 2000; 15: 1734–8.
47. Vernaeve V, Tournaye H, Schiettecatte J et al. Serum inhibin B cannot predict testicular sperm retrieval in patients with non-obstructive azoospermia. *Hum Reprod* 2002; 17: 971–6.
48. Huang X, Bai Q, Yan LY, Zhang QF, Geng L, Qiao J. Combination of serum inhibin B and follicle-stimulating hormone levels cannot improve the diagnostic accuracy on testicular sperm extraction outcomes in Chinese non-obstructive azoospermic men. *Chin Med J (Engl)* 2012; 125: 2885–9.
49. Ma Y, Chen B, Wang H, Hu K, Huang Y. Prediction of sperm retrieval in men with non-obstructive azoospermia using artificial neural networks: Leptin is a good assistant diagnostic marker. *Hum Reprod* 2011; 26: 294–8.
50. Boitrelle F, Robin G, Marcelli F, Albert M, Leroy-Martin B, Dewailly D, Rigot J, Mitchell V. A predictive score for testicular sperm extraction quality and surgical intra-cytoplasmic sperm injection outcome in non-obstructive azoospermia: A retrospective study. *Hum Reprod* 2011; 26: 3215–21.
51. Tournaye H. How to predict fatherhood for men with non-obstructive azoospermia opting for TESE-ICSI? *Hum Reprod* 2011; 26: 3213–4.
52. Har-Toov J, Eytan O, Hauser R, Yavetz H, Elad D, Jaffa AJ. A new power Doppler ultrasound guiding technique for improved testicular sperm extraction. *Fertil Steril* 2004; 81: 430–4.
53. Herwig R, Tosun K, Pinggera GM, Soelder E, Moeller KT, Pallwein L, Frauscher E, Bartsch G, Wildt L, Illmensee K. Tissue perfusion essential for spermatogenesis and outcome of testicular sperm extraction (TESE) for assisted reproduction. *J Assist Reprod Genet* 2004; 21: 175–80.
54. Bettella A, Ferlin A, Menegazzo M, Ferigo M, Tavolini M, Bassi PF, Foresta C. Testicular fine needle aspiration as a diagnostic tool in non-obstructive azoospermia. *Asian J Androl* 2005; 7: 289–94.
55. Tournaye H, Verheyen G, Nagy P et al. Are there any predictive factors for successful testicular sperm recovery? *Hum Reprod* 1997; 12: 80–6.
56. Ezeh UI, Taub NA, Moore HD, Cooke ID. Establishment of predictive variables associated with testicular sperm retrieval in men with non-obstructive azoospermia. *Hum Reprod* 1999; 14: 1005–12.
57. Bonarriba CR, Burgués JP, Vidaña V, Ruiz X, Pizá P. Predictive factors of successful sperm retrieval in azoospermia. *Actas Urol Esp* 2013; 37: 266–72.
58. Silber S, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. Distribution of spermatogenesis in the testicles of azoospermic men: The presence or abscence of spermatids in the testes of men with germinal failure. *Hum Reprod* 1997; 12: 2422–8.

59. Gil-Salom M, Minguez Y, Rubio C, de los Santos MJ, Remohi J, Pellicer A. Efficacy of intracytoplasmic sperm injection using testicular spermatozoa. *Hum Reprod* 1995; 10: 3166–70.
60. Hauser R, Botchan A, Amit A, Ben Yosef D, Gamzu R, Paz G, Lessing JB, Yogev L, Yavetz H. Multiple testicular sampling in non-obstructive azoospermia—Is it necessary? *Hum Reprod* 1998; 13: 3081–5.
61. Witt MA, Richard JR, Smith SE, Rhee EH, Tucker MJ. The benefit of additional biopsy sites when performing testicular sperm extraction in non-obstructive azoospermia. *Fertil Steril* 1997; 67: S79–80.
62. Vernaeve V, Verheyen G, Goossens A et al. How successful is repeat testicular sperm extraction in patients with azoospermia? *Hum Reprod* 2006; 21: 1551–4.
63. Schlegel P, Su LM. Physiological consequences of testicular sperm extraction. *Hum Reprod* 1997; 12: 1688–92.
64. Ron-El R, Strauss S, Friedler S, Strassburger D, Komarovsky D, Raziel A. Serial sonography and colour flow Doppler imaging following testicular and epididymal sperm extraction. *Hum Reprod* 1998; 13: 3390–3.
65. Schlegel PN, Li PS. Microdissection TESE: Sperm retrieval in non-obstructive azoospermia. *Hum Reprod Update* 1998; 4: 439.
66. Ishikawa T, Nose R, Yamaguchi K et al. Learning curves of microdissection testicular sperm extraction for nonobstructive azoospermia. *Fertil Steril* 2010; 94: 1008–11.
67. Kalsi JS, Shah P, Thum Y, Muneer A, Ralph DJ, Minhas S. Salvaje micro-dissection testicular sperm extraction; outcome in men with non-obstructive azoospermia with previous failed sperm retrievals. *BJU Int* 2014; 116: 460–5.
68. Bernie A, Mata D, Ramasamy R, Schlegel P. Comparison of microdissection sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azooaspermia 2015; 104: 1099–103.
69. Deruyver Y, Vanderschueren D, Van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: A systematic review. *Andrology* 2014; 2: 20–4.
70. Berookhim BM, Palermo GD, Zaninovic N, Rosenwaks Z, Schlegel PN. Microdissection testicular sperm extraction in men with Sertoli cell-only testicular histology. *Fertil Steril* 2014; 102: 1282–6.
71. Vloeberghs V, Verheyen G, Haentjens P, Goossens A, Polyzos NP, Tournaye H. How successful is TESE-ICSI in couples with non-obstructive azoospermia? *Hum Reprod* 2015; 30: 1790–6.
72. Verheyen G, Vernaeve V, Van Landuyt L et al. Should diagnostic testicular sperm retrieval followed by cryopreservation for later ICSI be the procedure of choice for all patients with non-obstructive azoospermia? *Hum Reprod* 2004; 19: 2822–30.
73. Ohlander S, Hotaling J, Kirshenbaum E et al. Impact of fresh versus cryopreserved testicular sperm upon intracytoplasmic sperm injection pregnancy outcomes in men with azoospermia due to spermatogenic dysfunction: A meta-analysis. *Feril Steril* 2014; 101: 344–9.
74. Tournaye H, Staessen C, Liebaers I et al. Testicular sperm recovery in 47, XXY Klinefelter patients. *Hum Reprod* 1996; 11: 1644–9.
75. Dávila Garza SA, Patrizio P. Reproductive outcomes in patients with male infertility because of Klinefelter's syndrome, Kartagener's syndrome, round-head sperm, dysplasia fibrous sheath, and 'stump' tail sperm: An updated literature review. *Curr Opin Obstet Gynecol* 2013; 25:229–46.
76. Tournaye H, Camus M, Vandervorst M et al. Sperm retrieval for ICSI. *Int J Andrology* 1997; 20(Suppl 3): 69–73.
77. Okada H, Goda K, Yamamoto Y, Sofikitis N, Miyagawa I, Mio Y, Koshida M, Horie S. Age as a limiting factor for successful sperm retrieval in patients with nonmosaic Klinefelter's syndrome. *Fertil Steril* 2005; 84: 1662–4.
78. Staessen C, Coonen E, Van Assche E et al. Preimplantation diagnosis for X and Y normality in embryos from three Klinefelter patients. *Hum Reprod* 1996; 11:1650–3.
79. Staessen C, Tournaye H, Van Assche E et al. Preimplantation diagnosis in 47, XXY Klinefelter patients. *Hum Reprod Update* 2003; 9: 319–30.
80. Sciurano RB, Luna Hisano CV, Rahn MI et al. Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. *Hum Reprod* 2009; 24: 2353–60.
81. Tournaye H, Dohle GR, Barratt CL. Fertility preservation in men with cancer. *Lancet* 2014; 384: 1295–301.
82. Rosenlund B, Sjoblom P, Tornblom M et al. *In-vitro* fertilization and intracytoplasmic sperm injection in the treatment of infertility after testicular cancer. *Hum Reprod* 1998; 13: 414–8.
83. Baniel J, Sella A. Sperm extraction at orchiectomy for testis cancer. *Fertil Steril* 2001; 75: 260–2.
84. Schrader M, Muller M, Straub B et al. Testicular sperm extraction in azoospermic patients with gonadal germ cell tumors prior to chemotherapy—A new therapy option. *Urology* 2003; 61: 421–5.
85. Damani MN, Masters V, Meng MV et al. Postchemotherapy ejaculatory azoospermia: Fatherhood with sperm from testis tissue with intracytoplasmic sperm injection. *J Clin Oncol* 2002; 15: 930–6.
86. Hsiao W, Stahl PJ, Osterberg EC, Nejat E, Palermo GD, Rosenwaks Z, Schlegel PN. Successful treatment of postchemotherapy azoospermia with microsurgical testicular sperm extraction: The Weill Cornell experience. *J Clin Oncol* 2011; 29: 1607–11.

87. Hussein A, Ozgok Y, Ross L, Niederberger C. Clomiphene administration for cases of nonobstructive azoospermia: A multicenter study. *J Androl* 2005; 26: 787–91.
88. Schlegel P. Aromatase inhibitors for male infertility. *Fertil Steril* 2012; 98: 1359–62.
89. Reifsnyder J, Ramasamy R, Husseini J, Schlegel P. Role of optimizing testosterone before microdissection testicular sperm extraction in men with nonobstructive azoospermia. *J Urol* 2012; 188: 532–7.
90. Mehta A, Bolyakov A, Roosma J, Schlegel P, Paduch D. Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor. *Fertil Steril* 2013; 100: 970–4.
91. Kyrou D, Kosmas I, Popovic-Todorovic B, Donoso P, Devroey P, Fatemi H. Ejaculatory sperm production in non-obstructive azoospermic patients with a history of negative testicular biopsy after the administration of an aromatase inhibitor: Report of two cases. *Eur J Obstet Gynecol Reprod Biol* 2014; 173: 120–1.
92. Friedler S, Raziel A, Schachter M, Strassburger D, Bern O, Ron-El R. Outcome of first and repeated testicular sperm extraction in patients with non-obstructive azoospermia. *Hum Reprod* 2002; 17: 2356–61.
93. Kamal A, Fahmy I, Mansour R, Abou-Setta A, Serour G, Aboulghar M. Outcome of repeated testicular sperm extraction and ICSI in patients with non-obstructive azoospermia. *MEFSJ* 2004; 9: 42–6.
94. Hauser R, Yogev L, Paz G, Yavetz H, Azem F, Lessing JB, Botchan A. Comparison of efficacy of two techniques for testicular sperm retrieval in nonobstructive azoospermia: Multifocal testicular sperm extraction versus multifocal testicular sperm aspiration. *J Androl* 2006; 27: 28–33.
95. Rosenlund B, Kvist U, Ploen L, Lundh Rozell B, Sjoblom P, Hillensjo T. A comparison between open and percutaneous needle biopsies in men with azoospermia. *Hum Reprod* 1998; 13: 1266–71.
96. Friedler S, Raziel A, Strassburger D et al. Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum Reprod* 1997; 12: 1488–93.
97. Ezeh UIO, Moore HDM, Cooke ID. A prospective study of multiple needle biopsies versus a single open biopsy for testicular sperm extraction in men with non-obstructive azoospermia. *Hum Reprod* 1998; 13: 3075–80.
98. Novero V, Goossens A, Tournaye H, Silber S, Van Steirteghem A, Devroey P. Seminoma discovered in two males undergoing successful testicular sperm extraction for intracytoplasmic sperm injection. *Fertil Steril* 1996; 65: 1015–54.
99. Nagy P, Verheyen G, Tournaye H, Devroey P, Van Steirteghem A. An improved treatment procedure for testicular biopsy offers more efficient sperm recovery: Case series. *Fertil Steril* 1997; 68: 376–9.
100. Popal W, Nagy ZP. Laboratory processing and intracytoplasmic sperm injection using epididymal and testicular spermatozoa: What can be done to improve outcomes? *Clinics (Sao Paulo)* 2013; 68(Suppl 1): 125–30.
101. Crabbé E, Verheyen G, Tournaye H, Van Steirteghem A. The use of enzymatic procedures to recover testicular sperm. *Hum Reprod* 1997; 12: 1682–7.
102. Angelopoulos T, Adler A, Krey L, Licciardi F, Noyes N, McCullough A. Enhancement or initiation of testicular sperm motility by *in vitro* culture of testicular tissue. *Fertil Steril* 1999; 71: 240–3.
103. Tasdemir I, Tasdemir M, Tavukcuoglu S. Effect of pentoxifylline on immotile testicular spermatozoa. *J Assisted Reprod Genet* 1998; 15: 90–2.
104. Griveau JF, Lobel B, Laurent MC, Michardière L, Le Lannou D. Interest of pentoxifylline in ICSI with frozen-thawed testicular spermatozoa from patients with non-obstructive azoospermia. *Reprod Biomed Online* 2006; 12(1): 14–8.
105. Nagy Z, Liu J, Janssenwillen C et al. Comparison of fertilization, embryo development and pregnancy rates after intracytoplasmic sperm injection using ejaculated, fresh and frozen–thawed epididymal and testicular spermatozoa. *Fertil Steril* 1995; 63: 808–5.
106. Vernaeve V, Tournaye H, Osmanagaoglu K et al. Intracytoplasmic sperm injection with testicular spermatozoa is less successful in men with nonobstructive azoospermia than in men with obstructive azoospermia. *Fertil Steril* 2003; 79: 529–33.
107. Esteves SC, Agarwal A. Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: Case series and systematic review. *Clinics (Sao Paulo)* 2013; 68(Suppl 1): 141–50.
108. Levron J, Aviram-Goldring A, Madgar I et al. Sperm chromosome abnormalities in men with severe male factor infertility who are undergoing *in-vitro* fertilization with intracytoplasmic sperm injection. *Fertil Steril* 2001; 76: 479–84.
109. Platteau P, Staessen C, Michiels A et al. Comparison of the aneuploidy frequency in embryos derived from testicular sperm extraction in obstructive and non-obstructive azoospermic men. *Hum Reprod* 2004; 19: 1570–4.
110. Donoso P, Platteau P, Papanikolaou EG et al. Does PGD for aneuploidy screening change the selection of embryos derived from testicular sperm extraction in obstructive and non-obstructive azoospermic men? *Hum Reprod* 2006; 21: 2390–5.
111. Chen M, Wei S, Hu J, Quan S. Can comprehensive chromosome screening technology improve IVF/ICSI outcomes? A meta-analysis. *PLoS One* 2015; 10: e0140779.
112. Schlegel PN. Nonobstructive azoospermia: A revolutionary surgical approach and results. *Semin Reprod Med* 2009; 27: 165–70.

113. Osmanagaoglu K, Vernaeve V, Kolibianakis E, Tournaye H, Camus M, Van Steirteghem A, Devroey P. Cumulative delivery rates after ICSI treatment cycles with freshly retrieved testicular sperm: A 7-year follow-up study. *Hum Reprod* 2003; 18: 1836–40.
114. Giorgetti C, Chinchole JM, Hans E, Charles O, Franquebalme JP, Glowaczower E, Salzmann J, Terriou P, Roulier R. Crude cumulative delivery rate following ICSI using intentionally frozen-thawed testicular spermatozoa in 51 men with non-obstructive azoospermia. *Reprod Biomed Online* 2005; 11: 319–24.
115. Dafopoulos K, Griesinger G, Schultze-Mosgau A, Orief Y, Schöpper B, Nikolettos N, Diedrich K, Al-Hasani S. Cumulative pregnancy rate after ICSI with cryopreserved testicular tissue in non-obstructive azoospermia. *Reprod Biomed Online* 2005; 10: 461–6.
116. Fedder J, Loft A, Parner ET, Rasmussen S, Pinborg A. Neonatal outcome and congenital malformations in children born after ICSI with testicular or epididymal sperm: A controlled national cohort study. *Hum Reprod* 2013; 28(1): 230–40.
117. Woldringh GH, Besselink DE, Tillema AH, Hendriks JC, Kremer JA. Karyotyping, congenital anomalies and follow-up of children after intracytoplasmic sperm injection with non-ejaculated sperm: A systematic review. *Hum Reprod Update* 2010; 16: 12–9.
118. Woldringh GH, Horvers M, Janssen AJ, Reuser JJ, de Groot SA, Steiner K, D'Hauwers KW, Wetzels AM, Kremer JA. Follow-up of children born after ICSI with epididymal spermatozoa. *Hum Reprod* 2011; 26(7): 1759–67.
119. Vernaeve V, Bonduelle M, Tournaye H et al. Pregnancy outcome and neonatal data on children born after ICSI with testicular sperm in obstructive and non-obstructive azoospermia. *Hum Reprod* 2003; 18: 2093–7.
120. Belva F, De Schrijver F, Tournaye H et al. Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. *Hum Reprod* 2011; 26: 1752–8.

Processing and cryopreservation of testicular sperm

54

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HANDLING AND CRYOPRESERVATION OF TESTICULAR SPERM

For the infertile couple, each patient must be addressed individually, yet managed in the context of the couple. Coordination of care is paramount amongst the male and female treatment teams. For the couple requiring *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), facilitating fresh sperm retrieval to coincide with the female's cycle and oocyte retrieval requires significant logistical efforts. Additionally, in the setting of male factor infertility, there is no certainty that sperm extraction will be successful. Failed retrieval may result in an unutilized cycle of ovarian hyperstimulation. The emotional and financial implications of such results can be devastating.

Cryopreservation of testicular sperm offers the means combating these challenges. In 1995, Craft and Tsirigotis first demonstrated successful recovery of spermatozoa following a freeze–thaw cycle of testicular sperm (1). Romero et al. later demonstrated successful fertilization with cryopreserved testicular sperm, and then subsequently with ICSI, utilizing cryopreserved testicular sperm from azoospermic men (2). Although Romero et al. only reported on two cases of fertilization with ICSI, neither of which ultimately resulted in pregnancy, their work demonstrated a significant advancement in reproductive medicine.

However, the true clinical acceptance and widespread utilization of cryopreservation can be attributed to the work of Oates and colleagues (3). In 1997, they published a case series of 10 couples with non-obstructive azoospermic (NOA) males that utilized frozen–thawed testicular sperm for ICSI with a 48% fertilization rate. While they reported only an 11% pregnancy rate, continued evolution of cryopreservation techniques has led to improved success. A recent meta-analysis by Ohlander and colleagues identified no statistical difference in fertilization or pregnancy rates when using fresh versus cryopreserved testicular sperm from men with NOA for ICSI (4).

Much of the applicability of sperm cryopreservation relates to the dilemmas posed by the male with spermatogenic dysfunction. First, the chance of successful microsurgical sperm retrieval in a patient with NOA ranges from 40% to 60% (5). As previously noted, the emotional and financial implications of a failed retrieval and wasted ovarian cycle cannot be understated. Unfortunately, despite significant efforts, we still do not have means of predicting a successful sperm retrieval, and a scenario of adoption versus donor sperm remains a realistic family planning decision. Cryopreservation allows for the couple to make an informed decision, on their own timeline, based upon the outcome of attempted retrieval. Second, cryopreservation limits the necessity of repeat sperm retrieval procedures for each cycle should the couple desire multiple children. It has been shown that significant testicular injury can be incurred with each extraction (6). This injury is compounded by the fact that both androgen production and spermatogenesis are often already impaired in the patient with NOA. Taken together, these confounders illustrate why cryopreservation of testicular sperm is so crucially important to patients with NOA.

Unfortunately, limitations to cryopreservation persist. As many as 50% of viable sperm may be lost with each freeze–thaw cycle even in experienced hands (7); in patients with severe oligospermia or azoospermia, this can result in an unacceptable rate of loss when viable testicular spermatozoa are rare. We must continue to scientifically advance efforts on the evolution of reproductive medicine and, more specifically, cryopreservation techniques focused on single-digit numbers of preserved cells.

This chapter will serve as an update on the retrieval, processing, and cryopreservation of testicular sperm. We will outline handling techniques that are crucial to increasing the likelihood of successful sperm preservation after freeze–thaw.

TECHNIQUE FOR TESTICULAR SPERM EXTRACTION

Testicular sperm extraction (TESE) and diagnostic testis biopsies are performed at our institution using an open window technique (8). The procedure is either performed under intravenous conscious sedation with local anesthesia or under general anesthesia. A transverse scrotal incision is made with dissection carried down to the tunica vaginalis. A 2–3-cm window incision is made through the tunica vaginalis, which is retracted using an eyelid retractor (Figure 54.1). A similarly small incision is made through the tunica albuginea, and seminiferous tubules are then extruded through the incision. These tubules are removed with iris scissors and placed into human tubule fluid medium (Figure 54.2). A small portion of the sample is sent to pathology for formal histologic evaluation. A second small portion is mechanically disrupted with two jeweler's forceps and then examined with phase contrast microscopy. If mature spermatozoa are identified, additional tubules are harvested via the same incision until the sample is complete. If mature spermatozoa are not seen, additional biopsies are

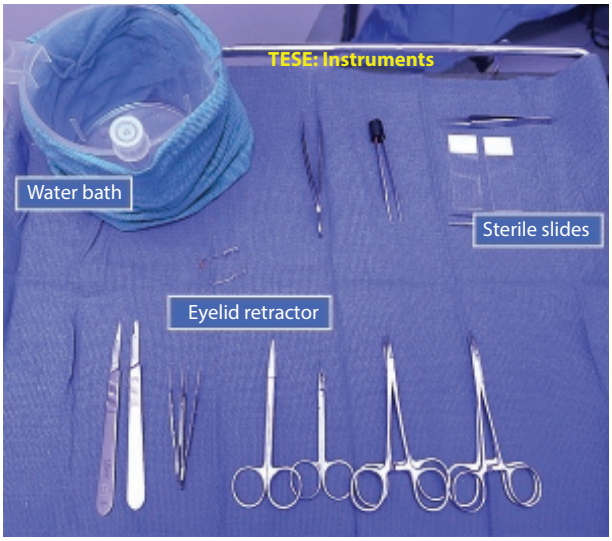

Figure 54.1 Standard instruments and vessels required for TESE. *Abbreviation*: TESE, testicular sperm extraction.

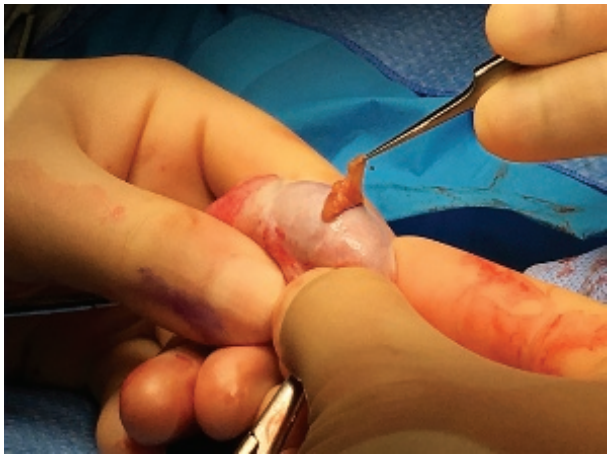

Figure 54.2 Testicular tissue is gently grasped by the jeweler's forceps prior to separation from the testis with iris scissors.

performed on the ipsilateral testis. If still no mature spermatozoa are seen, the contralateral testis is then evaluated by performing a formal microdissection TESE (microTESE). We have found that this stepwise approach helps many men avoid the more invasive microdissection procedure. For the microTESE, the tunica albuginea is opened widely to allow microscopic examination of all tubules using up to 25× magnification, which facilitates identification of healthier-appearing tubules that may be more likely to harbor spermatogenesis. Microscopy also allows identification of vessels to minimize injury while providing complete evaluation of the testis. The sampled tubules are examined for mature spermatozoa. If spermatozoa are found, the specimen is processed and cryopreserved.

PROCESSING OF SPECIMENS

Several methods are available to process surgically harvested testis tissue and prepare the derived spermatozoa for cryopreservation. Irrespective of the method used, it is important to optimize the technique to achieve the highest likelihood of detecting sperm while simultaneously minimizing tissue loss to prevent testicular atrophy/hypogonadism and protecting the spermatozoa from unnecessary mechanical, enzymatic, oxidative, and thermal stress in order to preserve their fertilization potential (6). This is particularly true in the subset of NOA men who possess rare sperm.

The initial step in processing testis biopsy tissues involves mechanical disruption to disperse spermatozoa present within the seminiferous tubules. Harvested tissue should be rinsed with sperm-wash media to rinse away common contaminants, such as erythrocytes and fibroblasts, and minced in a Petri dish using sterile iris scissors. Early studies comparing mincing to other mechanical processing methods, such as rough shredding, vortexing, and crushing with an electric potter, found mincing to achieve the highest yield of total motile spermatozoa and percentage of normal morphology (9). Finer homogenization can be performed by repeat aspiration and passage of the testis tissue through a fine-gauge hypodermic or angiocatheter needle (3). Mechanical dispersion and passage through a 24-gauge angiocatheter needle has been associated with a 470% improvement in the sperm retrieval rates compared to mincing alone (10). A small aliquot of the resultant specimen can be used for an intraoperative wet preparation analysis to confirm the presence of sperm and the remainder of the processed specimen stored in polypropylene tubes (Figure 54.3). If mechanical processing and microscopic examination using 400× magnification of a wet preparation fails to identify spermatozoa, biopsy samples can be purified of erythrocytes using density gradient centrifugation, the supernatant discarded, and remaining pellet re-suspended in culture media and re-examined (11,12). Although mechanical processing is rapid, it introduces the risk of cellular injury through shearing force.

Non-mechanical processing methods include ones such as enzymatic digestion, which can be performed in

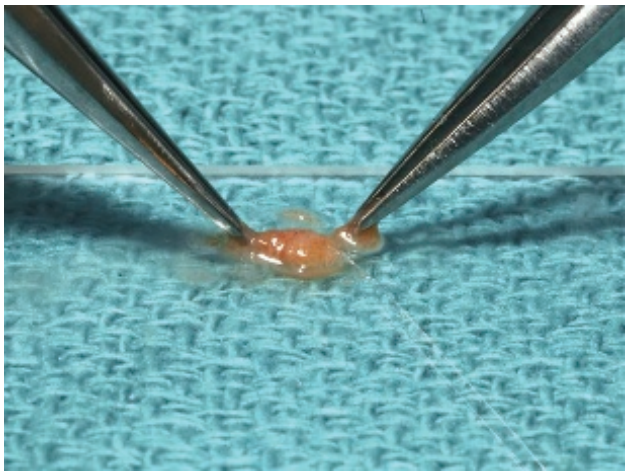

Figure 54.3 Jeweler's forceps mechanically teasing the testicular tissue as part of the "wet" preparation.

combination with mechanical disruption or as an additional rescue procedure if no sperm are seen (12–14). Enzymatic preparation is performed by incubating the testicular tissue with type IV collagenase and DNase for up to two hours at 37°C and mixing the solution every 30 minutes to increase the amount of tissue exposure to the collagenase. The digested tissue is then centrifuged and washed with sperm media (15). To address the potential for reintroduction of cell contaminants back into digested specimens following centrifugation, Wober et al. (12) recently described their technique of enzymatic digestion and density gradient centrifugation to further improve testicular sperm purity. Although the enzymatic and mechanical preparations have never been compared in a randomized trial, a retrospective multicenter comparison of mechanical versus enzymatic preparations of 839 ICSI cycles using testicular sperm revealed a statistically significantly higher percentage of cycles with motile sperm following mechanical preparation ($p = 0.03$) (16). Despite the potential for collagenase to damage the sperm cell membrane, a 24-hour vitality study showed no difference between enzyme-exposed sperm and untreated sperm obtained from men undergoing orchiectomy as part of androgen deprivation therapy or from residual fragments of testis tissue following an ICSI treatment (17).

Additional consideration must be given to prepubescent boys who undergo testis biopsy for fertility preservation prior to undergoing chemotherapy or radiation. A seven-year longitudinal study of the onset of sperm production (spermarche) identified spermarche as an early pubertal event occurring at a median age of 13.4 years (range 11.7–15.3 years) in 40 boys (18). Therefore, it is reasonable to perform a testicular biopsy for sperm extraction in boys over the age of 10 years (19). If no mature spermatozoa are found or the patient is less than 10 years of age, the biopsy sample should be cleansed of erythrocytes, sectioned into 1–4-mm^3 fragments, and placed into cryovials containing 5% dimethyl sulfoxide (DMSO) in order to preserve the architecture of the seminiferous tubules and to maintain immature spermatogonial cell-to-cell contact with its adjacent cells (20). Maintaining this cell-to-cell contact is important for post-thaw maturation of spermatogonia cells (21).

Challenges arise from the processing and cryopreservation of testicular specimens that harbor only a single or few sperm (<100 sperm). In addition to the labor-intensive approach necessary for the identification of these sperm, suboptimal post-thaw recovery has been shown when standard techniques are applied to specimens with poor sperm density (22). Moreover, the size of the sperm storage vessel and freezing volume can make the scarce sperm difficult to re-identify post-thaw. As a result, significant effort has been made to develop cryopreservation carriers and techniques suitable for tissue samples with scant or single sperm. Biologic carriers that have been used include alginic acid capsules (23,24) and empty zona pellucidae (25–27). Similarly, non-biologic vessels that have been used include mini-straws (28), 5-mm copper loops (29), calcium alginate beads (30), hyaluronan microcapsules (31,32), microdroplets (33), Cryolock (Irvine Scientific, Santa Ana, CA) (34), and agarose gel microspheres (35).

A 2009 systematic review of the different carriers and techniques for cryopreservation of individual or small numbers of human sperm found a 79.5% (range 59%–100%) recovery rate, a 46.5% (range 8%–85%) cryosurvival rate, and a 42.5% (range 18%–67%) fertilization rate for all carriers (36). Technical and biological challenges that exist with these systems include the necessity for the possession of and proficiency with expensive micromanipulators in order to handle single sperm, the potential for trans-species viral transmission with the use of non-human, emptied zona pellucidae, and the potential for transmission of foreign algae DNA residue from alginate beads to the oocyte during sperm injection (23,34). Nevertheless, development and application of novel technologies, such as a microfluidic platforms to isolate spermatozoa from testis tissue, are likely to make meaningful steps forward in testicular sperm processing (37).

CRYOPRESERVATION

As previously mentioned in this chapter, cryopreservation offers the unique advantages of sperm acquisition prior to use for assisted reproductive techniques, the ability to quarantine sperm until infectious and genetic testing have been performed, and the potential for indefinite storage (38). Since the first pregnancy using cryopreserved sperm was achieved in 1953 by Bunge and Sherman (39), advances in our understanding of cryophysiology have enabled modification to the cryopreservation technique to improve post-thaw sperm survival.

What distinguishes cryopreservation from cryoablation is the prevention of dehydration due to intracellular ice crystal formation, stabilization of intracellular ionic concentrations, and preservation of plasma membrane integrity. Slow cooling rates permit phase transition to occur and transform one state of matter into another by heat transfer (e.g., water transformation into its solid state of ice). The impact of slow cooling on spermatozoa was originally described by Sawada et al. (40) in 1967 and later corroborated by Leibo (41) with demonstration of extracellular ice formation, plasma membrane damage, and cell death. Slow thaw rates can also create additional destructive forces by allowing maximal growth of ice crystals (42). Furthermore, reactive oxygen species formation during the freeze–thaw cycle pose a threat to the diverse cellular compartments and genetic integrity of the sperm. Regulation of cooling and warming rates of the freeze–thaw cycle and the use of cryoprotectants and semen extenders therefore represent means of preventing phase transition's lethal cellular injury and ultimately improve sperm cryosurvival. In addition to optimizing osmotic pressures and preserving membrane integrity, semen extenders provide an alternative energy source for sperm metabolism and reduce the breakdown of intracellular sperm phospholipid (43). The addition of antibiotics to semen extenders can also prevent bacterial contamination.

Current testicular sperm cryopreservation techniques include slow freeze (SF), rapid freeze (RF), and ultra-rapid freeze (URF) protocols. In the three protocols, testicular spermatozoa are mixed with commonly used cryoprotectants such as egg yolk, which supplement lipids necessary for membrane fluidity and stability of the acrosin/proacrosin enzyme system, and intracellular glycerol, which penetrates the cell to replace lost intracellular water and lowers the intracellular freezing point (38,44,45). Stepwise cooling then occurs for SF protocols at a rate of −1°C/minute until a temperature of 5°C is reached; more rapid cooling takes place next at a rate of −10°C/minute until a temperature of −80°C is reached, following which the sample is plunged in liquid nitrogen to be cryopreserved at a temperature of −196°C (46). Conventional SF protocols take approximately one hour to complete and can be automated for more precise temperature regulation using programmable controlled-rate freezers (47). While these programmable freezers reduce the physical constraints on laboratory personnel, they are expensive and time consuming to oversee.

RF protocols utilize a cryoprotectant medium, but the spermatozoa are loaded directly into 0.25-mL straws and incubated at 4°C for 10 minutes. The straws are rapidly frozen by positioning the straws 15–20 cm above the liquid nitrogen to expose the straws to −80°C for 15 minutes and are subsequently immersed into liquid nitrogen (−196°C). Comparisons of SF and RF protocols have demonstrated superior post-thaw motility and cryosurvival with RF, but no differences in post-thaw sperm morphology and sperm DNA integrity (47). Alternatively, a more time-efficient URF protocol is available that induces an initial freezing rate of −10°C/minute by exposing cryostraws to liquid nitrogen vapor 10 cm above the liquid nitrogen surface for 10 minutes before immersing the straws in liquid nitrogen (48). A recent comparison of the URF and SF protocols demonstrated a statistically significant reduction in post-thaw sperm motility after one month of cryopreservation with both protocols; however, the difference between the protocols was not significant ($p > 0.05$) (49).

The impact of various storage and thawing temperatures has similarly been evaluated to determine the optimal cryopreservation temperatures. Comparisons of storage at −70°C to the conventional storage temperature at −196°C demonstrated superiority of −196°C in post-thaw sperm motility at seven days and three months after initial freeze (50). Thaw protocols regulate the ascent of specimen temperature in order to prevent rapid and dramatic changes in cell volume and cell injury associated with shifts of water into the cell and exchange with glycerol (38). Three thaw protocols include thaw at room temperature for 15 minutes, combination of thaw at room temperature for 10 minutes and 10 minutes in a 37°C water bath, or thaw in a 37°C water bath for 10–20 minutes. Comparison of thawing protocols of using the combined 10–minute room temperature thaw and 37°C water bath versus placing the sample in a 37°C water bath for 20 minutes have shown a higher percentage of fast linear movement and viability with less acrosomal damage associated with the 37°C water bath (51).

FERTILITY PRESERVATION

Significant improvements in childhood cancer treatment and survival rates have led to increased attention to survivorship issues, such as future fertility. The five-year overall survival rate of cancer in children has improved from 58% in the 1970s to 83% in the time period from 2005 to 2011 (52). Nevertheless, therapies such as radiation and chemotherapy, while improving survival rates, may destroy the patient's germ cells and impact their fertility potential. Cryopreservation of sperm prior to gonadotoxic treatment is an effective method to help ensure the patient could still father biologic children if he is rendered sterile by life-saving cancer treatments. Unfortunately, while more common among adults, sperm banking is not universally offered or performed in pediatric oncology centers for adolescents. These sensitive discussions can be positive by both emphasizing the future and providing assurance that curative treatment is the aim (53). The American Society of Clinical Oncology recommends that sperm cryopreservation should be widely available and considered the standard of care (54). If the young patient is unable to provide a semen sample, ejaculation can be induced by penile vibratory stimulation or electroejaculation, or a method of surgical sperm extraction can be discussed (55).

Particularly challenging is the problem posed by the prepubertal patient about to undergo gonadotoxic treatment. At the time of this writing, all options remain experimental, but cryopreservation of prepubertal testicular tissue has emerged as a viable strategy for preserving fertility potential in this patient population (56). Although the cryopreservation protocol is not well established, using DMSO as a permeating cryoprotectant to control slow-freezing has proven to be a promising approach to preserving human immature testicular tissue biopsies (20,57). Investigators evaluated vitrification as an alternative approach in both animal and human models, as this technique avoids ice crystal formation and potential freeze injuries (58,59), and this shows great promise in the emerging field of immature testicular tissue cryopreservation.

A potential strategy for using the immature cryopreserved testicular tissue involves spermatogonial stem cells. Spermatogonial stem cells are capable of self-renewal and differentiation into mature spermatozoa for the sole purpose of transmission of the genome to the next generation. Germ cell transplantation was developed in rodent models and successfully performed by Brinster and Avarbock in 1994 (60). Microinjection of spermatogonial stem cell suspensions into the seminiferous tubules of infertile mice stimulated spermatogenesis. Cryopreservation of spermatogonial stem cells before the start of any cancer therapy followed by autologous intratesticular transplantation of these cells after cure offers potential for preserving fertility (60,61). Offering human spermatogonial stem cell auto-transplantation as an option for fertility preservation to patients becomes more tangible every day. There

are institutions that recognize the realistic potential of this being a valid option for patients in the near future (61), so much so that they have begun to offer cryopreservation of testicular tissue in the hope that within the next 10 years science will have solved all of the intricacies of stem cell transfer to revolutionize the ability to preserve fertility.

CONCLUSION

Cryopreservation of testicular spermatozoa is a critical component in the treatment of patients with male infertility. Coupled with IVF–ICSI, cryopreservation allows for minimizing morbidity and unnecessary procedures, while facilitating pregnancy in patients with NOA. Future directions for inquiry include safe and effective means for cryopreservation of specimens with limited numbers of spermatozoa and minimization of cell death with the freeze–thaw cycle. Additionally, with increasing awareness among practitioners of the fertility issues that can arise after gonadotoxic cancer treatments in young patients, it is crucial that an evidence-based approach to the management of these patients is developed. As these and other advances in cryopreservation are made, these new processes will continue to directly benefit patients with severe male factor infertility.

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REFERENCES

1. Craft I, Tsirigotis M. Simplified recovery, preparation and cryopreservation of testicular spermatozoa. *Hum Reprod* 1995; 10(7): 1623–6.
2. Romero J, Remohi J, Minguez Y, Rubio C, Pellicer A, Gil-Salom M. Fertilization after intracytoplasmic sperm injection with cryopreserved testicular spermatozoa. *Fertil Steril* 1996; 65(4): 877–9.
3. Oates RD, Mulhall J, Burgess C, Cunningham D, Carson R. Fertilization and pregnancy using intentionally cryopreserved testicular tissue as the sperm source for intracytoplasmic sperm injection in 10 men with non-obstructive azoospermia. *Hum Reprod* 1997; 12(4): 734–9.
4. Ohlander S, Hotaling J, Kirshenbaum E, Niederberger C, Eisenberg ML. Impact of fresh versus cryopreserved testicular sperm upon intracytoplasmic sperm injection pregnancy outcomes in men with azoospermia due to spermatogenic dysfunction: A meta-analysis. *Fertil Steril* 2014; 101(2): 344–9.
5. Schlegel PN. Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999; 14(1): 131–5.
6. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. *Hum Reprod* 1997; 12(8): 1688–92.
7. Pegg DE. The history and principles of cryopreservation. *Semin Reprod Med* 2002; 20(1): 5–13.
8. Coburn M, Wheeler T, Lipshultz LI. Testicular biopsy. Its use and limitations. *Urol Clin North Am* 1987; 14(3): 551–61.
9. Verheyen G, De Croo I, Tournaye H, Pletincx I, Devroey P, van Steirteghem AC. Comparison of four mechanical methods to retrieve spermatozoa from testicular tissue. *Hum Reprod* 1995; 10(11): 2956–9.
10. Ostad M, Liotta D, Ye Z, Schlegel PN. Testicular sperm extraction for nonobstructive azoospermia: Results of a multibiopsy approach with optimized tissue dispersion. *Urology* 1998; 52(4): 692–6.
11. Popal W, Nagy ZP. Laboratory processing and intracytoplasmic sperm injection using epididymal and testicular spermatozoa: What can be done to improve outcomes? *Clinics (Sao Paulo)* 2013; 68(Suppl 1): 125–30.
12. Wober M, Ebner T, Steiner SL et al. A new method to process testicular sperm: Combining enzymatic digestion, accumulation of spermatozoa, and stimulation of motility. *Arch Gynecol Obstet* 2015; 291(3): 689–94.
13. Ramasamy R, Reifsnyder JE, Bryson C et al. Role of tissue digestion and extensive sperm search after microdissection testicular sperm extraction. *Fertil Steril* 2011; 96(2): 299–302.
14. Aydos K, Demirel LC, Baltaci V, Unlu C. Enzymatic digestion plus mechanical searching improves testicular sperm retrieval in non-obstructive azoospermia cases. *Eur J Obstet Gynecol Reprod Biol* 2005; 120(1): 80–6.
15. World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen [press release]*. Geneva, Switzerland: World Health Organisation Press, 2010.
16. Baukloh V. Retrospective multicentre study on mechanical and enzymatic preparation of fresh and cryopreserved testicular biopsies. *Hum Reprod* 2002; 17(7): 1788–94.
17. Crabbe E, Verheyen G, Tournaye H, Van Steirteghem A. The use of enzymatic procedures to recover testicular germ cells. *Hum Reprod* 1997; 12(8): 1682–7.
18. Nielsen CT, Skakkebaek NE, Richardson DW et al. Onset of the release of spermatozoa (spermarche) in boys in relation to age, testicular growth, pubic hair, and height. *J Clin Endocrinol Metab* 1986; 62(3): 532–5.
19. Sadri-Ardekani H, Atala A. Testicular tissue cryopreservation and spermatogonial stem cell transplantation to restore fertility: From bench to bedside. *Stem Cell Res Ther* 2014; 5(3): 68.
20. Keros V, Hultenby K, Borgstrom B, Fridstrom M, Jahnukainen K, Hovatta O. Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment. *Hum Reprod* 2007; 22(5): 1384–95.

21. Ogawa T, Ohmura M, Ohbo K. The niche for spermatogonial stem cells in the mammalian testis. *Int J Hematol* 2005; 82(5): 381–8.
22. Degl'Innocenti S, Filimberti E, Magini A et al. Semen cryopreservation for men banking for oligospermia, cancers, and other pathologies: Prediction of post-thaw outcome using basal semen quality. *Fertil Steril* 2013; 100(6): 1555–63.e1–3.
23. Just A, Gruber I, Wober M, Lahodny J, Obruca A, Strohmer H. Novel method for the cryopreservation of testicular sperm and ejaculated spermatozoa from patients with severe oligospermia: A pilot study. *Fertil Steri.* 2004; 82(2): 445–7.
24. Herrler A, Eisner S, Bach V, Weissenborn U, Beier HM. Cryopreservation of spermatozoa in alginic acid capsules. *Fertil Steril* 2006; 85(1): 208–13.
25. Cohen J, Garrisi GJ, Congedo-Ferrara TA, Kieck KA, Schimmel TW, Scott RT. Cryopreservation of single human spermatozoa. *Hum Reprod* 1997; 12(5): 994–1001.
26. Walmsley R, Cohen J, Ferrara-Congedo T, Reing A, Garrisi J. The first births and ongoing pregnancies associated with sperm cryopreservation within evacuated egg zonae. *Hum Reprod* 1998; 13(Suppl 4): 61–70.
27. Verheyen G, Vernaeve V, Van Landuyt L, Tournaye H, Devroey P, Van Steirteghem A. Should diagnostic testicular sperm retrieval followed by cryopreservation for later ICSI be the procedure of choice for all patients with non-obstructive azoospermia? *Hum Reprod* 2004; 19(12): 2822–30.
28. Isachenko V, Isachenko E, Montag M et al. Clean technique for cryoprotectant-free vitrification of human spermatozoa. *Reprod Biomed Online* 2005; 10(3): 350–4.
29. Schuster TG, Keller LM, Dunn RL, Ohl DA, Smith GD. Ultra-rapid freezing of very low numbers of sperm using cryoloops. *Hum Reprod* 2003; 18(4): 788–95.
30. Desai NN, Blackmon H, Goldfarb J. Single sperm cryopreservation on cryoloops: An alternative to hamster zona for freezing individual spermatozoa. *Reprod Biomed Online* 2004; 9(1): 47–53.
31. Chen Y, Li L, Qian Y et al. Small-volume vitrification for human spermatozoa in the absence of cryoprotectants by using Cryotop. *Andrologia* 2015; 47(6): 694–9.
32. Tomita K, Sakai S, Khanmohammadi M et al. Cryopreservation of a small number of human sperm using enzymatically fabricated, hollow hyaluronan microcapsules handled by conventional ICSI procedures. *J Assist Reprod Genet* 2016; 33(4): 501–11.
33. Coetzee K, Ozgur K, Berkkanoglu M, Bulut H, Isikli A. Reliable single sperm cryopreservation in Cell Sleepers for azoospermia management. *Andrologia* 2016; 48(2): 203–10.
34. Stein A, Shufaro Y, Hadar S, Fisch B, Pinkas H. Successful use of the Cryolock device for cryopreservation of scarce human ejaculate and testicular spermatozoa. *Andrology* 2015; 3(2): 220–4.
35. Araki Y, Yao T, Asayama Y, Matsuhisa A, Araki Y. Single human sperm cryopreservation method using hollow-core agarose capsules. *Fertil Steril* 2015; 104(4): 1004–9.
36. AbdelHafez F, Bedaiwy M, El-Nashar SA, Sabanegh E, Desai N. Techniques for cryopreservation of individual or small numbers of human spermatozoa: A systematic review. *Hum Reprod Update* 2009; 15(2): 153–64.
37. Plouffe BD, Murthy SK. Perspective on microfluidic cell separation: A solved problem? *Anal Chem* 2014; 86(23): 11481–8.
38. Rothman S. What is sperm banking? When and how is it (or should it be) used in humans? Animals? In: *Handbook of Andrology*, 2nd edition. Robaire B, Chan P (eds). Lawrence, KS: Allen Press, Inc., 2010, pp. 41–4.
39. Bunge RG, Sherman JK. Fertilizing capacity of frozen human spermatozoa. *Nature* 1953; 172(4382): 767–8.
40. Sawada Y, Ackerman D, Behrman SJ. Motility and respiration of human spermatozoa after cooling to various low temperatures. *Fertil Steril* 1967; 18(6): 775–81.
41. Leibo SP. Fundamental cryobiology of mouse ova and embryos. *Ciba Found Symp* 1977; (52): 69–96.
42. Gage AA, Baust JM, Baust JG. Experimental cryosurgery investigations *in vivo*. *Cryobiology* 2009; 59(3): 229–43.
43. Anger JT, Gilbert BR, Goldstein M. Cryopreservation of sperm: Indications, methods and results. *J Urol* 2003; 170(4 Pt 1): 1079–84.
44. Jeyendran RS, Gunawardana VK, Barisic D, Wentz AC. TEST-yolk media and sperm quality. *Hum Reprod Update* 1995; 1(1): 73–9.
45. Nallella KP, Sharma RK, Allamaneni SS, Aziz N, Agarwal A. Cryopreservation of human spermatozoa: Comparison of two cryopreservation methods and three cryoprotectants. *Fertil Steril* 2004; 82(4): 913–8.
46. Di Santo M, Tarozzi N, Nadalini M, Borini A. Human sperm cryopreservation: Update on techniques, effect on DNA integrity, and implications for ART. *Adv Urol* 2012; 2012: 854837.
47. Vutyavanich T, Piromlertamorn W, Nunta S. Rapid freezing versus slow programmable freezing of human spermatozoa. *Fertil Steril* 2010; 93(6): 1921–8.
48. Stanic P, Tandara M, Sonicki Z, Simunic V, Radakovic B, Suchanek E. Comparison of protective media and freezing techniques for cryopreservation of human semen. *Eur J Obstet Gynecol Reprod Biol* 2000; 91(1): 65–70.

49. Tongdee P, Sukprasert M, Satirapod C, Wongkularb A, Choktanasiri W. Comparison of cryopreserved human sperm between ultra rapid freezing and slow programmable freezing: Effect on motility, morphology and DNA integrity. *J Med Assoc Thai* 2015; 98(Suppl 4): S33-42.
50. Trummer H, Tucker K, Young C, Kaula N, Meacham RB. Effect of storage temperature on sperm cryopreservation. *Fertil Steril* 1998; 70(6): 1162–4.
51. Martinez-Soto JC, Garcia-Vazquez FA, Gumbao D, Landeras J, Gadea J. Assessment of two thawing processes of cryopreserved human sperm in pellets. *Cryobiology* 2011; 63(3): 131–6.
52. *Cancer Facts and Figures*. American Cancer Society, https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2016.html. Date Accessed July 4, 2016.
53. Schover LR, Brey K, Lichtin A, Lipshultz LI, Jeha S. Knowledge and experience regarding cancer, infertility, and sperm banking in younger male survivors. *J Clin Oncol* 2002; 20(7): 1880–9.
54. Lee SJ, Schover LR, Partridge AH et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol* 2006; 24(18): 2917–31.
55. Schmiegelow ML, Sommer P, Carlsen E, Sonksen JO, Schmiegelow K, Muller JR. Penile vibratory stimulation and electroejaculation before anticancer therapy in two pubertal boys. *J Pediatr Hematol Oncol* 1998; 20(5): 429–30.
56. Wyns C, Curaba M, Vanabelle B, Van Langendonckt A, Donnez J. Options for fertility preservation in prepubertal boys. *Hum Reprod Update* 2010; 16(3): 312–28.
57. Wyns C, Van Langendonckt A, Wese FX, Donnez J, Curaba M. Long-term spermatogonial survival in cryopreserved and xenografted immature human testicular tissue. *Hum Reprod* 2008; 23(11): 2402–14.
58. Curaba M, Poels J, van Langendonckt A, Donnez J, Wyns C. Can prepubertal human testicular tissue be cryopreserved by vitrification? *Fertil Steril* 2011; 95(6): 2123 e9–12.
59. Gouk SS, Loh YF, Kumar SD, Watson PF, Kuleshova LL. Cryopreservation of mouse testicular tissue: Prospect for harvesting spermatogonial stem cells for fertility preservation. *Fertil Steril* 2011; 95(7): 2399–403.
60. Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. *Proc Natl Acad Sci USA* 1994; 91(24): 11303–7.
61. Schlatt S, Ehmcke J, Jahnukainen K. Testicular stem cells for fertility preservation: Preclinical studies on male germ cell transplantation and testicular grafting. *Pediatr Blood Cancer* 2009; 53(2): 274–80.

Embryo transfer technique

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RAGAA MANSOUR

INTRODUCTION

Despite major advances in assisted reproduction technology (ART), the implantation rates remain too low to allow the widespread use of single-embryo transfer. Successful implantation requires a viable embryo, a receptive endometrium, and an optimal embryo transfer (ET) technique.

Various *in vitro* fertilization (IVF) steps proceed successfully up to the ET stage in about 80% of cases (1). However, only a small percentage of them achieve pregnancy. There are numerous technical aspects that affect the ET results. The variables in IVF are so numerous that it is difficult to fix all other factors while studying only one other factor (2).

Unfortunately, most clinicians consider the ET technique to be a simple procedure. To them, it is only a simple task of inserting the ET catheter into the uterine cavity and delivering the embryos. However, it is not as simple as it appears, and it is easier said than done (3).

The ET technique has a great impact on the IVF results. It has been proven that the pregnancy rates differ significantly among different individuals performing ET within the same IVF program (4,5). However, when the ET technique is standardized, the probability of pregnancy is not dependent on the physician performing the ET (6).

We need to standardize the protocol for the ET technique. In a survey of 80 IVF practitioners, standardization of the ET technique was considered the most important factor influencing the success rate in IVF (7). Furthermore, it was estimated that poor ET technique may account for as much as 30% of all IVF failures (8). Individualizing ET training for timing and performance demonstrated that the ET technique is easy to learn (9). In a large study, it was demonstrated that fellows can be trained to perform ET without compromising results (10).

Extra attention and time should be given to the procedure of ET so that it will be performed meticulously (11).

ET is routinely performed using the transcervical route, which is basically a blind technique associated with multiple negative factors.

POTENTIAL NEGATIVE FACTORS ASSOCIATED WITH ET

Uterine contractions

Initiation of uterine contractions may lead to an immediate or delayed expulsion of the embryos and has been considered a big concern in ART. In a study on cows, "artificial embryos" in the form of resin spheres impregnated with radioactive gold were traced after ET (12). After 1.5 hours, a large proportion of the spheres were expelled from the uterus.

In human IVF, Fanchin et al. (13) noted that more uterine contractions at the time of ET was associated with a lower pregnancy rate. In a study by Woolcott and Stanger, it was observed that the embryos could move as easily toward the cervical canal as toward the fallopian tubes (14). As a result, it is possible that the embryos may be expelled from the uterus partially or totally after the transfer (15–17). In a study using radio-opaque dye and mimicking ET, it was found that the dye remained primarily in the uterine cavity in only 58% of cases (18). In another study using Methylene Blue, the dye was extruded in 42% of the cases after dummy ET (16).

Failure to pass the internal cervical os

It is crucial for a successful ET that the catheter pass through the cervical canal and internal os to enter the uterine cavity. The ET catheter, especially soft ones, can be unnoticeably curved inside the cervical canal.

An important cause of failure of the catheter to pass the internal cervical os is the lack of alignment between the catheter (straight) and the utero-cervical angle (curved or acutely angulated). The acute degree of anteversion/retroversion or utero-cervical angulation and cervical stenosis are the most common reasons for difficult transfer (19,20).

In some rare cases, it may be impossible to pass the catheter inside the uterine cavity. Scaring of the lower uterine segment and cervix, distorted anatomy with fibroids, previous surgery, or congenital anomalies may lead to very difficult ET (20–22).

Cervical mucus

Cervical mucus can seriously impair proper embryo replacement. Plugging the tip of the catheter can cause embryo retention, especially with such a small volume of culture media to inject with the embryos (23).

The mucus can also drag the embryos outside during withdrawal of the catheter. It may also interfere with implantation if the mucus is pushed or injected in the uterine cavity. In an experimental dummy ET using Methylene Blue, it was demonstrated that the dye was extruded at the external os at a significantly higher rate when the cervical mucus was not removed (16).

In a large study by Nabi et al. (24), the authors reported that the embryos were much more likely to be retained when the catheter contained mucus or blood. Moreover, cervical mucus may be a source of bacterial contamination with subsequent lower pregnancy rates (25,26). It can also be contaminated with vaginal progesterone, which has been demonstrated to significantly impair mouse embryo development (27). In a prospective randomized trial by Eskandar et al. (28), removal of cervical mucus prior to ET significantly improved the pregnancy rates. In another

randomized controlled trial (RCT) by Visschers et al., this positive effect of mucus removal was not significant (29).

OPTIMIZING THE ET TECHNIQUE

Evaluation of the uterine cavity

To ensure proper embryo replacement, it is important to evaluate the uterine cavity before starting the IVF cycle by following the steps outlined in the sections below.

Dummy ET (mock transfer)

Performing a mock ET before the IVF cycle has been shown to significantly improve the pregnancy rate (30). This procedure is recommended to be performed before the start of the IVF cycle (23,30) or immediately before the actual ET. At our center, we usually do both. Performing mock ET at the time of oocyte retrieval, 3 to 5 days before ET does not have a deleterious effect on the endometrium (31). During the mock ET, we can measure the length of the uterine cavity and evaluate its direction and the degree of cervico-uterine angulation. It is also a good test for choosing the most suitable kind of catheter and for discovering any unanticipated difficulty such as pinpoint external os, cervical polyps or fibroids, stenosed cervix from previous surgery, or congenital anomalies. If cervical stenosis is diagnosed, it is advisable to perform cervical dilatation before starting the IVF cycle (32,33). The use of cervical laminaria one month before the IVF cycle is an adequate means of cervical dilation (34). When cervical dilatation is done at the time of ovum pickup, however, the pregnancy rate was very low (35).

Ultrasonographic evaluation

The ultrasound (US) is a precise method for measuring the length of the uterine cavity and the cervical canal. It is very important in evaluating the cervico-uterine angle (20,36). Ultrasonography is also essential in diagnosing the presence of fibroids and their encroachment on the uterine cavity or cervical canal.

The accuracy of measuring the uterine length using a catheter was determined with US (37). The authors reported a discrepancy of $\geq$1.5 cm in approximately 19% of the patients and $\geq$1 cm in 30% of the patients.

Hysteroscopy

Hysteroscopy is considered as the definitive diagnostic tool for evaluating any uterine cavity abnormalities suspected on hysterosalpingography or US (38).

Avoiding the initiation of uterine contractions

The precautions outlined in the following sections have to be taken to avoid the initiation of uterine contractions.

Avoid touching the uterine fundus

It was demonstrated that touching the uterine fundus with the catheter stimulated uterine contractions (13,39). In an experiment by Lesny et al. (39) using ultrasonically visible material, the authors demonstrated that touching the fundus with the catheter initiated strong random uterine contractions, relocating the contrast material. That is probably why early sources in IVF described the optimal location for embryo placement as between 0.5 and 1.0 cm from the fundus (40,41). Not touching the fundus was ranked high as a prognostic factor for IVF success in a recent survey (42).

It is a routine procedure for some IVF specialists to place the catheter approximately 0.5 cm (43,44) or 1–1.5 cm (45) below the fundus to avoid touching.

Depositing the embryos in the mid-fundal area of the uterus improved the pregnancy rates (46–50). It was also found by another study that the position of the catheter 2 cm from the fundus was superior to 1 cm from the fundus (51).

Therefore, individual measurement of the cervical canal and uterine cavity length is extremely important. However, the use of a fixed-distance technique greatly reduced the variation in pregnancy rates among physicians (52), probably due to reducing the rate of touching the fundus.

Soft catheters

The value of soft ET catheters has been recognized since the beginning of IVF. The ideal catheter should be soft enough to avoid any trauma to the endometrium and malleable enough to find its way through the cervical canal into the uterine cavity (20). A soft ET catheter means a combination of physical flexibility, malleability, and smoothness of the tip (8). Soft catheters usually have an outer rigid sheath that should be stopped short of the internal cervical os to benefit from the advantages of soft catheters. If the outer sheath is introduced, it will convert a "soft" catheter into a "stiff" catheter (23). The stimulus of the ET catheter passing through the internal cervical os could possibly cause the release of prostaglandins and lead to uterine contractions (53).

Different kinds of catheters were studied, and soft catheters were associated with improved pregnancy rates (8,30,54,55), but in other studies there was no significant difference (43,45,56–60). Two large prospective randomized studies have shown significantly higher ongoing clinical pregnancy rates using soft catheters as compared to rigid ones (61,62). Changing from rigid catheters to soft ones has been associated with improved pregnancy rates (47,63). It is recommended to use soft catheters for ET of embryos with assisted hatching (54). A recent meta-analysis has shown that the clinical pregnancy rates were significantly better when using soft ET catheters as compared with rigid ones (64). Different soft catheters are more or less the same and the pregnancy rates associated with them are not significantly different (65).

Gentle manipulations

Atraumatic delivery of embryos into the endometrial cavity is the prime goal of ET (23). As a rule, the ET procedure should be a simple and painless procedure, and gentle manipulations should be observed, even when introducing

the vaginal speculum. Holding the cervix with a vulsellum should be avoided except in rare cases (42). In a clinical study of humans, serial blood samples were collected in time intervals of 20 seconds during the ET procedure to measure the serum oxytocin concentrations (66). When the tenaculum was used, there was a temporary elevation of oxytocin level, which remained elevated until the end of ET. In another study of humans, the use of tissue forceps to hold the cervix was found to trigger uterine contractions (67).

Applying 1–2 mL of local anesthetic (1% procaine) to the anterior lip of the cervix through a fine needle before applying the tenaculum was very acceptable to the patients and did not affect the outcome (45). Technically difficult ETs were associated with reduced pregnancy rates in a number of studies (30,54,68–71). Difficult manipulation initiates uterine contractions with possible embryo expulsion (13). Despite all the precautions to ensure atraumatic ET, sometimes it can be difficult with repeated sequential attempts, without an adverse effect on the pregnancy rate or outcome (72).

Uterine relaxing substances

Serum progesterone levels on the day of ET inversely correlate with the frequency of uterine contractions (13). Starting progesterone on the day of oocyte pickup to relax uterine contractility at the time of ET was suggested (73,74), although it did not improve the pregnancy rates as compared to starting it on the day of ET (75).

Sedation with 10 mg diazepam 30–60 minutes before ET did not make a difference to the pregnancy rates (43).

Similarly, tocolytic agents or prostaglandin synthetase inhibitors did not have a positive effect (43). In some patients who experience severe stress and anxiety during ET, propofol anesthesia can be used, and this was found to have no effect on the pregnancy rates (76). Non-steroidal anti-inflammatory drugs to inhibit prostaglandin production (77) were given (10 mg piroxicam), and there were significant improvements in the implantation and pregnancy rates (78). In a prospective randomized study, an oxytocin antagonist (atosiban) was given intravenously before ET and the results showed significant improvements in the implantation and pregnancy rates (79), but a recent RCT failed to show a beneficial effect (80).

Removal of cervical mucus

Removing the cervical mucus before ET is advisable in order to avoid the adverse effects mentioned previously. It can be removed by repeated gentle aspiration using a 1-cm^3 syringe with its tip placed at the external cervical os or using a soft catheter.

The endocervix can be cleaned of mucus using a sterile cotton swab or small brush and then small amounts of culture media (45,68).

Vigorous cervical washing was reported in a retrospective study to improve the pregnancy rates (81); however, a prospective randomized study showed no significant difference (82). Another large multicenter study showed no significant difference (83). In a large retrospective study including 470 ET procedures, it was found that the presence of macroscopic or microscopic blood and mucus did not affect the clinical pregnancy or implantation rates (84). In a Cochrane review, no evidence of benefit was found when the cervical mucus was removed before ET (85).

Make sure the ET catheter passes the internal cervical os

The ultimate goal at the end of an IVF cycle is to safely deposit the embryos inside the uterine cavity. We have to be sure that the catheter has passed the internal os and is not kinked or curved inside the cervical canal. Performing the ET under US guidance makes it easy to ensure proper passage of the catheter. For clinicians performing ET without US guidance, soft catheters can sometimes be misleading. A simple test of rotating the catheter 360° can discover kinking of the catheter if it recoils. It is advisable to perform a dummy ET to choose the most suitable kind of catheter before loading the embryos in order to avoid harshly navigating the cervix during the actual ET.

One of the most common causes of failure to pass the ET catheter is the pronounced curvature or angulation of the cervico-uterine angle. Proper curving of the catheter to follow the cervico-uterine curvature should be done before loading the embryos in the catheter. This is why it is important to perform a dummy ET and revise the US picture of the uterus before loading the embryos. It has been reported that molding the ET catheter according to the US cervico-uterine angle improved the clinical pregnancy rate and diminished the incidence of difficult ET (36). Straitening the utero-cervical angle can be achieved with a full bladder before ET (70,86). This effect can be achieved indirectly by performing ET under US (86,87). However, in a Cochrane review, no evidence of benefit with a full bladder was found (85).

Sometimes, simple maneuver of the vaginal speculum can change the direction of the cervix and facilitate introduction of the ET catheter.

If the soft catheter cannot pass the cervical canal, a more rigid one should be used. Rigid catheters should be malleable in order to achieve the required curve to overcome the acute cervico-uterine angulations.

Using a malleable stylet to place the outer sheath correctly through the cervical canal before introducing the soft catheter did not have a negative impact on the implantation and pregnancy rates (88).

In difficult cases, it may be necessary to hold the cervix with a vulsellum to stabilize the uterus during the introduction of the ET catheter. Holding the cervix with a vulsellum is painful and it should be done under local or general anesthesia (45,76).

In rare cases, it is very difficult or even impossible to pass the catheter. More rigid, stiffer catheters have to be used (30,70). Another system that can be used in difficult cases is the coaxial catheter (21). Cannulation of a resistant internal os can be done with the outer sheath, followed by introducing the soft inner catheter (89).

Cervical dilatation can be resorted to in cases of cervical stenosis. A short interval between cervical dilation and ET can result in a very low pregnancy rate (35,68). Performing cervical dilatation before the IVF cycle resulted in easier ET and improved pregnancy rates (32,33).

Another helpful approach in cases of cervical stenosis is to place a laminaria before the start of the IVF cycle (34), or to place the hygroscopic rods in the cervix prior to ovarian stimulation (90). Cannulation of the cervix under fluoroscopic guidance then dilatation was successfully tried in some tortuous and stenotic cervical canals (22).

In the early days of IVF, the rate of difficult ET was high. In a report of 867 ET procedures, 1.3% were impossible, 3.2% were very difficult (manipulation of more than five minutes or cervical dilatation), and 5.6% were difficult (91). Twenty-five years after this report, the current experience of most IVF centers makes the rate of impossible and difficult ET procedures extremely low. In these rare difficult cases, transmyometrial surgical ET can be used (35,92).

Surgical ET has been used successfully, achieving results comparable to the transcervical route (93). The surgical technique is straightforward and requires no greater experience than that necessary for US-guided oocyte collection (93). The surgical ET set is composed of a metal needle (like an oocyte pickup needle) with a stylet and an ET catheter that fits into the needle after withdrawal of the stylet.

Prevention of embryo expulsion

Recently, a technique using the vaginal speculum to prevent embryo expulsion after ET was described (94). After introducing the ET catheter into the uterine cavity, the screw of the vaginal speculum is loosened so that its two valves press on the portio vaginalis of the cervix, occluding the cervical canal. After waiting for one minute, the embryos are ejected and the catheter is withdrawn slowly. The speculum is kept in place gently pressing on the cervix for about seven more minutes and then removed. The results of this study demonstrated significant improvements in the implantation and pregnancy rates.

ET USING US

Using US guidance for ET was reported by a number of investigators as simple, reassuring, and significantly improved the pregnancy rates (8,55,95–101). However, other investigators found no significant difference in the pregnancy rates when ET was performed under US guidance as compared to clinical touch ET (45,102,103). A RCT by Kosmas et al. (104) showed that US-guided ET does not offer any benefit in terms of clinical outcome.

The results of a meta-analysis of eight prospective randomized trials showed that the pregnancy and implantation rates were significantly better when US was used (105).

Another recent meta-analysis has reached a similar conclusion (106). A Cochrane review of 17 RCTs showed that US-guided ET increased the ongoing pregnancy rates compared with clinical touch (107). For easier identification of the catheter, some kinds have an ultrasonically visible tip (59). Transrectal US can be used in obese women during ET (108).

LOADING THE EMBRYOS IN THE ET CATHETER

Putting the embryos in the ET catheter should start after completing the dummy ET and making sure that the dummy catheter passed the internal os. After the dummy ET, the suitable kind of ET catheter will be selected and flushed with tissue culture medium. Then the ET catheter is filled with ET culture medium and up to 10–15 μL will be aspirated first. The embryos are then aspirated in another 10–15 μL of medium and moved in the catheter to stop away from the tip.

Using a continuous fluid column without air bubbles is recommended (23,96). The volume of fluid used for ET should be as small as possible to prevent flowing out of the embryos into the cervical canal or the fallopian tubes. A large volume (60 μL) of transfer medium and a large air bubble in the catheter result in the expulsion of the embryos (16). A continuous fluid column of 30 μL without air bubbles is recommended (23).

Two prospective randomized trials were performed to investigate the effect of the presence of air bubbles in the ET catheter on the IVF outcome (109,110). The authors concluded that the presence of air bubbles had no negative effect. However, there is no definitive reason to support the enclosure of air spaces before and after the medium containing the embryos in the ET catheter. It was suggested that the air bubbles mark the position of the embryos inside the catheter (109) and protect against loss (111) or entangling with mucus (109).

The protein concentration in the transfer medium does not affect the result, nor does increasing the viscosity (112). Hyaluronan-enriched transfer medium has been recently used in a large randomized trial and showed significant improvements in implantation and pregnancy rates (113). However, another RCT showed no beneficial effect (114). In patients with repeated IVF failures, the use of hyaluronan improved the pregnancy rate (115). Recently, a Cochrane review was performed to determine whether ET medium enriched with adherence compounds has an impact on live birth rate in ART compared to regular ET medium (116). The review included 15 studies using hyaluronic acid and the results showed no evidence of a treatment effect on live birth rate. However, the clinical pregnancy rate and multiple pregnancy rate were significantly higher in hyaluronic acid groups.

INFUSION OF HUMAN CHORIONIC GONADOTROPIN

It was recently published that intrauterine infusion with 40 μL of tissue culture medium containing 500 IU of human chorionic gonadotropin (hCG) significantly improved the clinical pregnancy rates and implantation rates (117). This positive effect was confirmed by three other RCTs (118–120). However, two other RCTs did not show a beneficial effect (121,122). The conflicting results in the literature could be due to the variable degrees of purity

of hCG, and the use of recombinant hCG could avoid such problem. It could also be due to variable days of ET ranging from days 2 to 5. Moreover, hCG is a fragile molecule and it should be prepared immediately before its use and not kept overnight in the incubator. Another factor is preventing its expulsion after injection by using the vaginal speculum to press on the portio vaginalis of the cervix.

RETAINED EMBRYOS AFTER ET

The ET catheter must be checked for retained embryos after ET. This problem occurs more frequently after a difficult ET (24) or when the catheter was filled with mucus or blood (123). This decreases the implantation rates (68,124). It is advisable to retransfer the retained embryos immediately (24,72,125,126).

The volume of tissue culture medium for the ET is another cause of retained embryos. It is advisable to aspirate approximately 10–15 μL of culture medium first before aspirating the embryos to ensure the presence of enough medium to push out the embryos (20). It is also important to keep the pressure on the plunger of the syringe after ejecting the embryos until complete withdrawal of the catheter (5) to avoid re-aspirating the embryos.

It is also advisable to withdraw the catheter slowly after ejecting the embryos to avoid creating negative pressure and withdrawal of the embryos following the catheter. Leong et al. (127) reported that withdrawal of the catheter by about 1 cm and brisk injection avoided retrograde flow of the transfer media along the catheter by "capillary action." An automated device that generates a standardized injection speed has been described (128). It is a peristaltic pump system that ensures the pumping of a small volume of medium containing the embryos, but it needs further research in order to investigate its clinical value.

BED REST AFTER ET

Bed rest after ET was originally practiced in most IVF centers for several hours due to the fear of mechanical expulsion of the embryos (129–132). Different investigators have reported that there is no need for bed rest after ET (45,70,133–135). The position of the embryos was ultrasonically traced immediately after ET in a standing position (136). The authors reported that standing shortly after ET did not play a significant role in the final position of embryos. It has also been reported in a prospective controlled trial of 406 patients that immediate ambulation following the ET has no adverse effect on the pregnancy rate (137). The same conclusion was reached in a Cochrane review (138). It was even found in one RCT that bed rest after ET negatively affected the pregnancy rate (139).

The so-called endometrial cavity is only a potential space and not a real cavity. The ET catheter only separates the opposed endometrial surfaces, and once the catheter is removed, the endometrial surfaces re-oppose. Then, the embryos and fluid injected into the potential space are relocated by the endometrial and myometrial peristalsis, as well as the surface tension between the fluid–solid interfaces (136,137,140,141). It is believed that the embryos generally implant near where they were deposited (142). Dummy ET was performed using small microspheres immediately before hysterectomy. The uterine cavity was then inspected and the microspheres were found within 1 cm of the site of deposition (141). In another study by Baba et al., it was found that 26 of 32 gestational sacs as seen by US were in the area where the air bubble was seen immediately after ET (143).

DURATION OF ET

The time interval from loading the embryos in the catheter to depositing them in the uterine cavity should be kept to a minimum in order to prevent prolonged exposure of the embryos to ambient temperature, light, or other factors. A long time of more than 60 seconds (144) or 120 seconds (145) has been shown to lower the pregnancy and implantation rates. On the other hand, another study showed no adverse effect of the duration of the procedure on the results, even with transfers lasting up to 7.5 minutes (146).

ET TECHNIQUE AS A CAUSE OF ECTOPIC PREGNANCY

The risk of ectopic pregnancy following IVF was estimated to be 5% in a multicenter study of 1163 pregnancies (147). In a recent review, the prevalence of ectopic pregnancy secondary to ART ranged between 2.1% and 8.6% of all pregnancies (148). This figure is much higher than in natural conception.

The distance from the fundus to the tip of the ET catheter was studied in relation to the ectopic rate (149). The authors reported a decrease in the ectopic rate associated with an increased distance between the fundus and the tip of the catheter. The mid-fundal technique resulted in a lower percentage of ectopic pregnancy and did not negatively affect the pregnancy rate (150).

Ectopic pregnancy was 3.9-times more frequently associated with difficult ET than with an easy procedure (151).

Another factor in the etiology of ectopic pregnancy in IVF is the size of the uterus. It was reported that the ectopic pregnancy rate was significantly higher in women with uterine cavity lengths of less than 7 cm (25). The ET technique can also be a cause of ectopic pregnancy due to forcing the embryo(s) through tubal ostia by hydrostatic pressure or by using a large volume of ET medium (152,153). The speed of the transfer was also implicated in inducing ectopic pregnancy. It was recommended to be performed slowly over 10 seconds (154).

Finally, it was found that the uterine contractions in the early luteal phase are generally cervico-fundal in origin (151) and may be the cause of some ectopic pregnancies in IVF (23). As a result of uterine peristalsis, the embryos could move as easily toward the cervical canal as toward the fallopian tubes (14).

CONCLUDING REMARKS

ET is the final and most critical step in ART. Individual training and monitoring of doctors are essential to standardize the technique. Several factors have been studied to develop guidelines for optimizing the ET technique

(2,20,155). The evidence based factors include: (a) soft catheters; (b) ultrasonic guidance; (c) dummy ET; (d) curving the ET catheter according to the cervico-uterine angulations; (e) not touching the fundus; and (f) small volumes of medium to deposit the embryos. Other factors that are based on clinical experts' recommendations include: (a) gentle manipulation; (b) removing cervical mucus; (c) slow withdrawal of the catheter to avoid negative pressure; and (d) minimizing the time of the procedure.

DESCRIPTION OF AN ET PROCEDURE

- The patient is instructed to be fasting as the need for general anesthesia may arise.
- The patient/couple is informed of the fertilization rate, the number of embryos selected for the transfer, and if there are extra embryos for cryopreservation. The patient should be assured of the simplicity of the procedure.
- The file of the patient is revised to see the US picture of the uterus and the comments on the previous dummy ET, particularly the length of the uterine cavity and the degree and direction of the utero-cervical angulation.
- The patient is put in the lithotomy position and the cervix is visualized using Cusco's speculum.
- The cervix and vaginal vaults are wiped with sterile gauze and tissue culture medium to remove excess cervical mucus and vaginal secretions.
- The cervical mucus at the external os is aspirated gently and repeatedly using a 1-cm^3 syringe.
- A dummy ET is performed using a sterile soft ET catheter. Only the soft inner catheter is introduced to pass the internal os and the rigid outer sheath is stopped short of the internal os. To make sure that the soft catheter is not kinked inside the cervical canal, the catheter is rotated 360°, then left alone resting on the hand. If it recoils, this indicates that the catheter is coiled. The catheter can be withdrawn to be reintroduced again after modifying the position of the cervix by manipulating the vaginal speculum (the degree of opening and how far it is introduced). If the soft catheter cannot be introduced, a more rigid and malleable one can be tried. The rigid, malleable catheter can be molded to follow the curvature of the cervico-uterine angle (reported previously in US picture). Sometimes the catheter needs to be moved gently in different directions until its tip passes the internal os. The catheter curvature may need to be increased in order to overcome the acute angulation of the cervico-uterine angle. In almost all cases, it is possible to introduce the correctly curved rigid catheter.
- If the above maneuvers fail, the procedure is stopped and the patient is put under general anesthesia. General anesthesia is given in the form of propofol 2 mg/kg as an induction dose and anesthesia is maintained by inhalation of isoflurane 1.5% and oxygen 100% through a face mask. The previous trial is repeated with or without a vulsellum to support the cervix. As a last resort, a rigid introducer may be used. In extremely rare cases, the rigid (or even metal) introducer cannot pass the cervical canal and enter the internal os. If this happens, you can either resort to transmyometrial surgical ET or you can cryopreserve the embryos and postpone the ET for later after doing cervical dilatation or hysteroscopic cannulation of the cervix.
- After introduction of the dummy ET catheter, 40 μL ET medium containing 500 IU hCG is injected intrauterine.
- The embryologist will start loading the embryos in a new catheter similar to the one that was introduced in the dummy ET.
- The ET catheter is flushed with tissue culture medium, and then filled with ET medium. About 10–15 μL of transfer medium is aspirated first in the ET catheter, then the embryos are aspirated in another 10–15 μL of medium to withdraw the embryos away from the tip of the catheter.
- The loaded ET catheter is introduced gently through the cervical canal to pass the internal os, then advanced slowly up to the mid-uterine cavity and stopped 1–2 cm short of the uterine fundus.
- The screw of the vaginal speculum is loosened so that the two valves of the speculum gently press on the portio vaginalis of the cervix. At this moment, some patients experience suprapubic heaviness or discomfort. After one minute, when this complaint disappears, the embryos are ejected and pressure is kept on the plunger of the syringe while slowly withdrawing the catheter out (the inner catheter and outer sheath simultaneously). The ET catheter is returned to the laboratory to check for the presence of any retrained embryos. If found, re-transfer is done immediately. The speculum is kept in place for an average of about seven minutes, then removed.

REFERENCES

1. Human Fertilisation and Embryology Authority. *Fifth Annual Report*. London, UK: Human Fertilisation and Embryology Authority.
2. Mains L, Van Voorhis BJ. Optimizing the technique of embryo transfer. *Fertil Steril* 2010; 94(3): 785–90.
3. Naaktgeboren N, Dieben S, Heijnsbroek I. Embryo transfer easier said than done. *Fertil Steril* 1998; 70: S352.
4. Karande VC, Morris R, Chapman C, Rinehart J, Gleicher N. Impact of the "physician factor" on pregnancy rates in a large assisted reproductive technology program: Do too many cooks spoil the broth? *Fertil Steril* 1999; 71(6): 1001–9.
5. Hearns-Stokes RM, Miller BT, Scott L, Creuss D, Chakraborty PK, Segars JH. Pregnancy rates after embryo transfer depend on the provider at embryo transfer. *Fertil Steril* 2000; 74(1): 80–6.
6. van Weering HG, Schats R, McDonnell J, Hompes PG. Ongoing pregnancy rates in *in vitro* fertilization are not dependent on the physician performing the embryo transfer. *Fertil Steril* 2005; 83(2): 316–20.
7. Salha OH, Lamb VK, Balen AH. A postal survey of embryo transfer practice in the UK. *Hum Reprod* 2001; 16(4): 686–90.

8. Cohen J. *Embryo replacement technology. 31 Annual Post Graduate Course*. San Francisco, CA: ASRM, 1998.
9. López MJ, García D, Rodríguez A, Colodrón M, Vassena R, Vernaeve V. Individualized embryo transfer training: Timing and performance. *Hum Reprod* 2014; 29(7): 1432–7.
10. Eaton JL, Zhang X, Barnes RB. Embryo transfer by reproductive endocrinology fellows vs attending physicians: Are live birth rates comparable? *Am J Obstet Gynecol* 2014; 211(5): 494.e1–5.
11. Meldrum DR, Chetkowski R, Steingold KA, de Ziegler D, Cedars MI, Hamilton M. Evolution of a highly successful *in vitro* fertilization-embryo transfer program. *Fertil Steril* 1987; 48(1): 86–93.
12. Harper MJK, Bennett JP, Rowson LEA. Movement of the ovum in the reproductive tract. *Nature* 1961; 190: 788.
13. Fanchin R, Righini C, Olivennes F, Taylor S, de Ziegler D, Frydman R. Uterine contractions at the time of embryo transfer alter pregnancy rates after *in-vitro* fertilization. *Hum Reprod* 1998; 13(7): 1968–74.
14. Woolcott R, Stanger J. Potentially important variables identified by transvaginal ultrasound-guided embryo transfer. *Hum Reprod* 1997; 12(5): 963–6.
15. Poindexter AN 3rd, Thompson DJ, Gibbons WE, Findley WE, Dodson MG, Young RL. Residual embryos in failed embryo transfer. *Fertil Steril* 1986; 46(2): 262–7.
16. Mansour RT, Aboulghar MA, Serour GI, Amin YM. Dummy embryo transfer using methylene blue dye. *Hum Reprod* 1994; 9(7): 1257–9.
17. Schulman JD. Delayed expulsion of transfer fluid after IVF/ET. *Lancet* 1986; 1: 44.
18. Knutzen V, Stratton CJ, Sher G, McNamee PI, Huang TT, Soto-Albors C. Mock embryo transfer in early luteal phase, the cycle before *in vitro* fertilization and embryo transfer: A descriptive study. *Fertil Steril* 1992; 57(1): 156–62.
19. Garzo VG. Embryo transfer technique. *Clin Obstet Gynecol* 2006; 49: 117–22.
20. Mansour RT, Aboulghar MA. Optimizing the embryo transfer technique. *Hum Reprod* 2002; 17(5): 1149–53.
21. Patton PE, Stoelk EM. Difficult embryo transfer managed with a coaxial catheter system. *Fertil Steril* 1993; 60(1): 182–3.
22. Zreik TG, Dickey KW, Keefe DL, Glickman MG, Olive DL. Fluoroscopically guided cervical dilatation in patients with infertility. *J Am Assoc Gynecol Laparosc* 1996; 3(Suppl 4): S56.
23. Schoolcraft WB, Surrey ES, Gardner DK. Embryo transfer: Techniques and variables affecting success. *Fertil Steril* 2001; 76(5): 863–70.
24. Nabi A, Awonuga A, Birch H, Barlow S, Stewart B. Multiple attempts at embryo transfer: Does this affect *in-vitro* fertilization treatment outcome? *Hum Reprod* 1997; 12(6): 1188–90.
25. Egbase PE, al-Sharhan M, al-Othman S, al-Mutawa M, Udo EE, Grudzinskas JG. Incidence of microbial growth from the tip of the embryo transfer catheter after embryo transfer in relation to clinical pregnancy rate following *in-vitro* fertilization and embryo transfer. *Hum Reprod* 1996; 11(8): 1687–9.
26. Moore DE, Soules MR, Klein NA, Fujimoto VY, Agnew KJ, Eschenbach DA. Bacteria in the transfer catheter tip influence the live-birth rate after *in vitro* fertilization. *Fertil Steril* 2000; 74(6): 1118–24.
27. Ying LY, Ying Y, Mayer J, Imudia AN, Plosker SM. Embryo transfer catheter contamination with intravaginal progesterone preparations in a simulated embryo transfer model impairs mouse embryo development: Are there implications for human embryo transfer technique? *Reprod Sci* 2014; 21(8): 1000–5.
28. Eskandar MA, Abou-setta AM, El-Amin M, Almushait MA, Sobande AA. Removal of cervical mucus prior to embryo transfer improves pregnancy rates in women undergoing assisted reproduction. *Reprod Biomed Online* 2007; 14: 308–13.
29. Visschers BA, Bots RS, Peeters MF, Mol BW, van Dessel JH. Removal of cervical mucus: Effect on pregnancy rates in IVF/ICSI. *Reprod Biomed Online* 2007; 15: 310–5.
30. Mansour R, Aboulghar M, Serour G. Dummy embryo transfer: A technique that minimizes the problems of embryo transfer and improves the pregnancy rate in human *in vitro* fertilization. *Fertil Steril* 1990; 54(4): 678–81.
31. Katariya KO, Bates GW, Robinson RD, Arthur NJ, Propst AM. Does the timing of mock embryo transfer affect *in vitro* fertilization implantation and pregnancy rates? *Fertil Steril* 2007; 88: 1462–4.
32. Abusheikha N, Lass A, Akagbosu F, Brinsden P. How useful is cervical dilatation in patients with cervical stenosis who are participating in an *in vitro* fertilization–embryo transfer program? The Bourn Hall experience. *Fertil Steril* 1999; 72(4): 610–2.
33. Prapas N, Prapas Y, Panagiotidis Y, Prapa S, Vanderzwalmen P, Makedos G. Cervical dilatation has a positive impact on the outcome of IVF in randomly assigned cases having two previous difficult embryo transfers. *Hum Reprod* 2004; 19(8): 1791–5.
34. Glatstein IZ, Pang SC, McShane PM. Successful pregnancies with the use of laminaria tents before embryo transfer for refractory cervical stenosis. *Fertil Steril* 1997; 67(6): 1172–4.
35. Groutz A, Lessing JB, Wolf Y, Azem F, Yovel I, Amit A. Comparison of transmyometrial and transcervical embryo transfer in patients with previously failed *in vitro* fertilization–embryo transfer cycles and/or cervical stenosis. *Fertil Steril* 1997; 67(6): 1073–6.
36. Sallam HN, Agameya AF, Rahman AF, Ezzeldin F, Sallam AN. Ultrasound measurement of the uterocervical angle before embryo transfer: A prospective controlled study. *Hum Reprod* 2002; 17(7): 1767–72.

37. Shamonki MI, Spandorfer SD, Rosenwaks Z. Ultrasound-guided embryo transfer and the accuracy of trial embryo transfer. *Hum Reprod* 2005; 20(3): 709–16.
38. Pundir J, El Toukhy T. Uterine cavity assessment prior to IVF. *Womens Health (Lond)* 2010; 6(6): 841–8.
39. Lesny P, Killick SR, Tetlow RL, Robinson J, Maguiness SD. Embryo transfer—Can we learn anything new from the observation of junctional zone contractions? *Hum Reprod* 1998; 13(6): 1540–6.
40. Kerin JF, Jeffrey R, Warnes GM, Cox LW, Broom TJ. A simple technique for human embryo transfer into the uterus. *Lancet* 1981; 2(8249): 726–7.
41. Leeton J, Trounson A, Jessup D, Wood C. The technique for embryo transfer. *Fertil Steril* 1982; 14: 2417.
42. Kovacs GT. What factors are important for successful embryo transfer after *in-vitro* fertilization? *Hum Reprod* 1999; 14(3): 590–2.
43. Diedrich K, van der Ven H, al-Hasani S, Krebs D. Establishment of pregnancy related to embryo transfer techniques after *in-vitro* fertilization. *Hum Reprod* 1989; 4(Suppl 8): 111–4.
44. Egbase PE, Al-Sharhan M, Grudzinskas JG. Influence of position and length of uterus on implantation and clinical pregnancy rates in IVF and embryo transfer treatment cycles. *Hum Reprod* 2000; 15(9): 1943–6.
45. al-Shawaf T, Dave R, Harper J, Linehan D, Riley P, Craft I. Transfer of embryos into the uterus: How much do technical factors affect pregnancy rates? *J Assist Reprod Genet* 1993; 10(1): 31–6.
46. Waterstone J, Curson R, Parsons J. Embryo transfer to low uterine cavity. *Lancet* 1991; 337(8754): 1413.
47. Rosenlund B, Sjöblom P, Hillensjö T. Pregnancy outcome related to the site of embryo deposition in the uterus. *J Assist Reprod Genet* 1996; 13(6): 511–3.
48. Naaktgeboren N, Broers FC, Heijnsbroek I. Hard to believe hardly discussed, nevertheless very important for the IVF/ICSI result: Embryo transfer technique can double or halve the pregnancy rate. *Hum Reprod* 1997; 12: 149.
49. Frankfurter D, Trimarchi JB, Silva CP, Keefe DL. Middle to lower uterine segment embryo transfer improves implantation and pregnancy rates compared with fundal embryo transfer. *Fertil Steril* 2004; 81(5): 1273–7.
50. Oliveira JB, Martins AM, Baruffi RL, Mauri AL, Petersen CG, Felipe V, Contart P, Pontes A, Franco Júnior JG. Increased implantation and pregnancy rates obtained by placing the tip of the transfer catheter in the central area of the endometrial cavity. *Reprod Biomed Online* 2004; 9(4): 435–41.
51. Coroleu B, Barri PN, Carreras O, Martínez F, Parriego M, Hereter L, Parera N, Veiga A, Balasch J. The influence of the depth of embryo replacement into the uterine cavity on implantation rates after IVF: A controlled, ultrasound-guided study. *Hum Reprod* 2002; 17(2): 341–6.
52. van de Pas MM, Weima S, Looman CW, Broekmans FJ. The use of fixed distance embryo transfer after IVF/ICSI equalizes the success rates among physicians. *Hum Reprod* 2003; 18(4): 774–80.
53. Fraser IS. Prostaglandins, prostaglandin inhibitors and their roles in gynaecological disorders. *Baillieres Clin Obstet Gynaecol* 1992; 6(4): 829–57.
54. Choe JK, Nazari A, Check JH, Summers-Chase D, Swenson K. Marked improvement in clinical pregnancy rates following *in vitro* fertilization-embryo transfer seen when transfer technique and catheter were changed. *Clin Exp Obstet Gynecol* 2001; 28(4): 223–4.
55. Wood EG, Batzer FR, Go KJ, Gutmann JN, Corson SL. Ultrasound-guided soft catheter embryo transfers will improve pregnancy rates in *in-vitro* fertilization. *Hum Reprod* 2000; 15(1): 107–12.
56. Wisanto A, Janssens R, Deschacht J, Camus M, Devroey P, Van Steirteghem AC. Performance of different embryo transfer catheters in a human *in vitro* fertilization program. *Fertil Steril* 1989; 52(1): 79–84.
57. Ghazzawi IM, Al-Hasani S, Karaki R, Souso S. Transfer technique and catheter choice influence the incidence of transcervical embryo expulsion and the outcome of IVF. *Hum Reprod* 1999; 14(3): 677–82.
58. De Placido G, Wilding M, Stina I, Mollo A, Alviggi E, Tolino A, Colacurci N, De ML, Marino M, Dale B. The effect of ease of transfer and type of catheter used on pregnancy and implantation rates in an IVF program. *J Assist Reprod Genet* 2002; 19(1): 14–8.
59. Karande V, Hazlett D, Vietzke M, Gleicher N. A prospective randomized comparison of the Wallace catheter and the Cook Echo-Tip catheter for ultrasound-guided embryo transfer. *Fertil Steril* 2002; 77(4): 826–30.
60. Burke LM, Davenport AT, Russell GB, Deaton JL. Predictors of success after embryo transfer: Experience from a single provider. *Am J Obstet Gynecol* 2000; 182(5): 1001–4.
61. van Weering HG, Schats R, McDonnell J, Vink JM, Vermeiden JP, Hompes PG. The impact of the embryo transfer catheter on the pregnancy rate in IVF. *Hum Reprod* 2002; 17(3): 666–70.
62. McDonald JA, Norman RJ. A randomized controlled trial of a soft double lumen embryo transfer catheter versus a firm single lumen catheter: Significant improvements in pregnancy rates. *Hum Reprod* 2002; 17(6): 1502–6.
63. Penzias A, Harris D, Barrett C et al. Outcome oriented research in an IVF program: Transfer catheter type affects IVF outcome [abstr]. In: *Proceedings of the 53rd Annual Meeting of the American Society for Reproductive Medicine*, 1997, S163.
64. Abou-Setta AM, Al-Inany HG, Mansour RT, Serour GI, Aboulghar MA. Soft versus firm embryo transfer catheters for assisted reproduction: A systematic review and meta-analysis. *Hum Reprod* 2005; 20(11): 3114–21.

65. Saldeen P, Abou-Etta A, Bergh T, Sundstrom P, Holte J. A prospective randomized controlled trial comparing two embryo transfer catheters in an ART program. *Fertil Steril* 2008; 90: 599–603.
66. Dorn C, Reinsberg J, Schlebusch H, Prietl G, van der Ven H, Krebs D. Serum oxytocin concentration during embryo transfer procedure. *Eur J Obstet Gynecol Reprod Biol* 1999; 87(1): 77–80.
67. Lesny P, Killick SR, Robinson J, Raven G, Maguiness SD. Junctional zone contractions and embryo transfer: Is it safe to use a tenaculum? *Hum Reprod* 1999; 14(9): 2367–70.
68. Visser DS, Fourie FL, Kruger HF. Multiple attempts at embryo transfer: Effect on pregnancy outcome in an *in vitro* fertilization and embryo transfer program. *J Assist Reprod Genet* 1993; 10(1): 37–43.
69. Englert Y, Puissant F, Camus M, Van Hoeck J, Leroy F. Clinical study on embryo transfer after human *in vitro* fertilization. *J In Vitro Fert Embryo Transf* 1986; 3(4): 243–6.
70. Sharif K, Afnan M, Lenton W. Mock embryo transfer with a full bladder immediately before the real transfer for *in-vitro* fertilization treatment: The Birmingham experience of 113 cases. *Hum Reprod* 1995; 10(7): 1715–8.
71. Tomás C, Tikkinen K, Tuomivaara L, Tapanainen JS, Martikainen H. The degree of difficulty of embryo transfer is an independent factor for predicting pregnancy. *Hum Reprod* 2002; 17(10): 2632–5.
72. Tur-Kaspa I, Yuval Y, Bider D, Levron J, Shulman A, Dor J. Difficult or repeated sequential embryo transfers do not adversely affect *in-vitro* fertilization pregnancy rates or outcome. *Hum Reprod* 1998; 13(9): 2452–5.
73. Fanchin R, Righini C, de Ziegler D, Olivennes F, Ledée N, Frydman R. Effects of vaginal progesterone administration on uterine contractility at the time of embryo transfer. *Fertil Steril* 2001; 75(6): 1136–40.
74. Ayoubi JM, Fanchin R, Kaddouz D, Frydman R, de Ziegler D. Uterorelaxing effects of vaginal progesterone: Comparison of two methodologies for assessing uterine contraction frequency on ultrasound scans. *Fertil Steril* 2001; 76(4): 736–40.
75. Baruffi R, Mauri AL, Petersen CG, Felipe V, Franco JG, Jr. Effects of vaginal progesterone administration starting on the day of oocyte retrieval on pregnancy rates. *J Assist Reprod Genet* 2003; 20(12): 517–20.
76. Al-Inany HG, Waseef M, Aboulghar, MA, Mansour RT, Serour GI, Abou-Setta AM. Embryo transfer under propofol anesthesia: The impact on implantation and pregnancy rate. *Middle East Fertil Soc J* 2003; 8: 269.
77. Dawood MY. Nonsteroidal antiinflammatory drugs and reproduction. *Am J Obstet Gynecol* 1993; 169(5): 1255–65.
78. Moon HS, Park SH, Lee JO, Kim KS, Joo BS. Treatment with piroxicam before embryo transfer increases the pregnancy rate after *in vitro* fertilization and embryo transfer. *Fertil Steril* 2004; 82(4): 816–20.
79. Moraloglu O, Tonguc E, Var T, Zeyrek T, Batioglu S. Treatment with oxytocin antagonists before embryo transfer may increase implantation rates after IVF. *Reprod Biomed Online* 2010; 21(3): 338–43.
80. Ng EH, Li RH, Chen L, Lan VT, Tuong HM, Quan S. A randomized double blind comparison of atosiban in patients undergoing IVF treatment. *Hum Reprod* 2014; 29(12): 2687–94.
81. McNamee P, Huang T, Carwile A. Significant increase in pregnancy rates achieved by vigorous irrigation of endocervical mucus prior to embryos transfer with a Wallace catheter in an IVF-ET program [abstr]. *Fertil Steril* 1998; 70(Suppl 1): S228.
82. Sallam HN, Farrag A, Ezzeldin F, Agameya A, Sallam AN. The importance of flushing the cervical canal with culture medium prior to embryo transfer. *Fertil Steril* 2000; 74(3 Suppl 11): S64–5.
83. Glass KB, Green CA, Fluker MR, Schoolcraft WB, McNamee PI, Meldrum D. Multicenter randomized trial of cervical irrigation at the time of embryo transfer [abstr]. *Fertil Steril* 2000; 74(Suppl 1): S31.
84. Moragianni VA, Cohen JD, Smith SE, Schinfeld JS, Somkuti SG, Lee A, Barmat LI. Effect of macroscopic or microscopic blood and mucus on the success rates of embryo transfers. *Fertil Steril* 2010; 93(2): 570–3.
85. Derks RS, Farquhar C, Mol BW, Buckingham K, Heineman MJ. Techniques for preparation prior to embryo transfer. *Cochrane Database Syst Rev* 2009; 4: CD007682.
86. Lewin A, Schenker JG, Avrech O, Shapira S, Safran A, Friedler S. The role of uterine straightening by passive bladder distension before embryo transfer in IVF cycles. *J Assist Reprod Genet* 1997; 14(1): 32–4.
87. Sundström P, Wramsby H, Persson PH, Liedholm P. Filled bladder simplifies human embryo transfer. *Br J Obstet Gynaecol* 1984; 91(5): 506–7.
88. Nielsen IK, Lindhard A, Loft A, Ziebe S, Andersen AN. A Wallace malleable stylet for difficult embryo transfer in an *in vitro* fertilization program: A case–control study. *Acta Obstet Gynecol Scand* 2002; 81(2): 133–7.
89. Silberstein T, Weitzen S, Frankfurter D, Trimarchi JR, Keefe DL, Plosker SM. Cannulation of a resistant internal os with the malleable outer sheath of a coaxial soft embryo transfer catheter does not affect *in vitro* fertilization–embryo transfer outcome. *Fertil Steril* 2004; 82(5): 1402–6.
90. Serhal P, Ranieri DM, Khadum I, Wakim RA. Cervical dilatation with hygroscopic rods prior to ovarian stimulation facilitates embryo transfer. *Hum Reprod* 2003; 18(12): 2618–20.
91. Wood C, McMaster R, Rennie G, Trounson A, Leeton J. Factors influencing pregnancy rates following *in vitro* fertilization and embryo transfer. *Fertil Steril* 1985; 43(2): 245–50.
92. Kato O, Takatsuka R, Asch RH. Transvaginal–transmyometrial embryo transfer: The Towako method; experiences of 104 cases. *Fertil Steril* 1993; 59(1): 51–3.

93. Sharif K, Afnan M, Lenton W, Bilalis D, Hunjan M, Khalaf Y. Transmyometrial embryo transfer after difficult immediate mock transcervical transfer. *Fertil Steril* 1996; 65(5): 1071–4.
94. Mansour R. Minimizing embryo expulsion after embryo transfer: A randomized controlled study. *Hum Reprod* 2005; 20(1): 170–4.
95. Lindheim SR, Cohen MA, Sauer MV. Ultrasound guided embryo transfer significantly improves pregnancy rates in women undergoing oocyte donation. *Int J Gynaecol Obstet* 1999; 66(3): 281–4.
96. Coroleu B, Carreras O, Veiga A, Martell A, Martinez F, Belil I, Hereter L, Barri PN. Embryo transfer under ultrasound guidance improves pregnancy rates after *in-vitro* fertilization. *Hum Reprod* 2000; 15(3): 616–20.
97. Prapas Y, Prapas N, Hatziparasidou A, Vanderzwalmen P, Nijs M, Prapa S, Vlassis G. Ultrasound-guided embryo transfer maximizes the IVF results on day 3 and day 4 embryo transfer but has no impact on day 5. *Hum Reprod* 2001; 16(9): 1904–8.
98. Tang OS, Ng EH, So WW, Ho PC. Ultrasound-guided embryo transfer: A prospective randomized controlled trial. *Hum Reprod* 2001; 16(11): 2310–5.
99. Anderson RE, Nugent NL, Gregg AT, Nunn SL, Behr BR. Transvaginal ultrasound-guided embryo transfer improves outcome in patients with previous failed *in vitro* fertilization cycles. *Fertil Steril* 2002; 77(4): 769–75.
100. Matorras R, Urquijo E, Mendoza R, Corcóstegui B, Expósito A, Rodríguez-Escudero FJ. Ultrasound-guided embryo transfer improves pregnancy rates and increases the frequency of easy transfers. *Hum Reprod* 2002; 17(7): 1762–6.
101. Li R, Lu L, Hao G, Zhong K, Cai Z, Wang W. Abdominal ultrasound-guided embryo transfer improves clinical pregnancy rates after *in vitro* fertilization: Experiences from 330 clinical investigations. *J Assist Reprod Genet* 2005; 22(1): 3–8.
102. Kan AK, Abdalla HI, Gafar AH, Nappi L, Ogunyemi BO, Thomas A, Ola-ojo OO. Embryo transfer: Ultrasound-guided versus clinical touch. *Hum Reprod* 1999; 14(5): 1259–61.
103. García-Velasco JA, Isaza V, Martinez-Salazar J, Landazábal A, Requena A, Remohí J, Simón C. Transabdominal ultrasound-guided embryo transfer does not increase pregnancy rates in oocyte recipients. *Fertil Steril* 2002; 78(3): 534–9.
104. Kosmas IP, Janssens R, De Munch L et al. Ultrasound-guided embryo transfer does not offer any benefit in clinical outcome: A randomized controlled trial. *Hum Reprod* 2007; 22: 1327–34.
105. Buckett WM. A meta-analysis of ultrasound-guided versus clinical touch embryo transfer. *Fertil Steril* 2003; 80(4): 1037–41.
106. bou-Setta AM, Mansour RT, Al-Inany HG, Aboulghar MM, Aboulghar MA, Serour GI. Among women undergoing embryo transfer, is the probability of pregnancy and live birth improved with ultrasound guidance over clinical touch alone? A systemic review and meta-analysis of prospective randomized trials. *Fertil Steril* 2007; 88: 333–41.
107. Brown J, Buckingham K, Abou-Setta AM, Buckett W. Ultrasound versus "clinical touch" for catheter guidance during embryo transfer in women. *Cochrane Database Syst Rev* 2010; 1: CD006107.
108. Sohan K, Woodward B, Ramsewak SS. Successful use of transrectal ultrasound for embryo transfer in obese women. *J Obstet Gynaecol* 2004; 24(7): 839–40.
109. Krampl E, Zegermacher G, Eichler C, Obruca A, Strohmer H, Feichtinger W. Air in the uterine cavity after embryo transfer. *Fertil Steril* 1995; 63(2): 366–70.
110. Moreno V, Balasch J, Vidal E, Calafell JM, Cívico S, Vanrell JA. Air in the transfer catheter does not affect the success of embryo transfer. *Fertil Steril* 2004; 81(5): 1366–70.
111. Ebner T, Yaman C, Moser M, Sommergruber M, Pölz W, Tews G. The ineffective loading process of the embryo transfer catheter alters implantation and pregnancy rates. *Fertil Steril* 2001; 76(3): 630–2.
112. Menezo Y, Arnal F, Humeau C, Ducret L, Nicollet B. Increased viscosity in transfer medium does not improve the pregnancy rates after embryo replacement. *Fertil Steril* 1989; 52(4): 680–2.
113. Urman B, Yakin K, Ata B, Isiklar A, Balaban B. Effect of hyaluronan-enriched transfer medium on implantation and pregnancy rates after day 3 and day 5 transfers: A prospective randomized study. *Fertil Steril* 2008; 90: 604–12.
114. Karimian L, Rezazadeh VM, Baghestani AR, Moeini A. A prospective randomized comparison of two commercial embryo transfer medium in IVF/ ICSI cycles. *Hum Reprod* 2004; 19(Suppl 1): i52.
115. Friedler S, Schacter M, Strassburger D, Esther K, Ron El R, Raziel A. A randomized clinical trial comparing recombinant hyaluronan/recombinant albumin versus human tubal fluid for cleavage stage embryo transfer in patients with multiple IVF–embryo transfer failure. *Hum Reprod* 2007; 22: 2444–8.
116. Bontekoe S, Blake D, Heineman MJ, Williams EC, Johnson N. Adherence compounds in embryo transfer media for assisted reproductive technologies. *Cochrane Database Syst Rev* 2010; 7: CD007421.
117. Mansour R, Tawab N, Kamal O, El-Faissal Y, Serour A, Aboulghar M, Serour G. Intrauterine injection of human chorionic gonadotropin before embryo transfer significantly improves the implantation and pregnancy rates in *in vitro* fertilization/intracytoplasmic sperm injection: A prospective randomized study. *Fertil Steril* 2011; 96(6): 1370–1374.
118. Santibañez A, García J, Pashkova O, Colín O, Castellanos G, Sánchez AP, De la Jara JF. Effect of intrauterine injection of human chorionic gonadotropin before embryo transfer on clinical pregnancy rates from *in vitro* fertilisation cycles: A prospective study. *Reprod Biol Endocrinol* 2014; 12: 9.

119. Zarei A, Parsanezhad ME, Younesi M, Alborzi S, Zolghadri J, Samsami A, Amooee S, Aramesh S. Intrauterine administration of recombinant human chorionic gonadotropin before embryo transfer on outcome of *in vitro* fertilization/ intracytoplasmic sperm injection: A randomized clinical trial. *Iran J Reprod Med* 2014; 12(1): 1–6.
120. Aaleyasin A, Aghahosseini M, Rashidi M, Safdarian L, Sarvi F, Najmi Z, Mobasseri A, Amoozgar B. *In vitro* fertilization outcome following embryo transfer with or without preinstillation of human chorionic gonadotropin into the uterine cavity: A randomized controlled trial. *Gynecol Obstet Invest* 2015; 79(3): 201–5.
121. Hong KH, Forman EJ, Werner MD, Upham KM, Gumeny CL, Winslow AD, Kim TJ, Scott RT, Jr. Endometrial infusion of human chorionic gonadotropin at the time of blastocyst embryo transfer does not impact clinical outcomes: A randomized, double-blind, placebo-controlled trial. *Fertil Steril* 2014; 102(6): 1591–5.
122. Wirleitner B, Schuff M, Vanderzwalmen P, Stecher A, Okhowat J, Hradecký L, Kohoutek T, Králícková M, Spitzer D, Zech NH. Intrauterine administration of human chorionic gonadotropin does not improve pregnancy and life birth rates independently of blastocyst quality: A randomised prospective study. *Reprod Biol Endocrinol* 2015; 13: 70.
123. Awonuga A, Nabi A, Govindbhai J, Birch H, Stewart B. Contamination of embryo transfer catheter and treatment outcome in *in vitro* fertilization. *J Assist Reprod Genet* 1998; 15(4): 198–201.
124. Alvero R, Hearns-Stokes RM, Catherino WH, Leondires MP, Segars JH. The presence of blood in the transfer catheter negatively influences outcome at embryo transfer. *Hum Reprod* 2003; 18(9): 1848–52.
125. Goudas VT, Hammitt DG, Damario MA, Session DR, Singh AP, Dumesic DA. Blood on the embryo transfer catheter is associated with decreased rates of embryo implantation and clinical pregnancy with the use of *in vitro* fertilization–embryo transfer. *Fertil Steril* 1998; 70(5): 878–82.
126. Leeton HC, Seifer DB, Shelden RM. Impact of retained embryos on the outcome of assisted reproductive technologies. *Fertil Steril* 2004; 82(2): 334–7.
127. Leong M, Leung C, Tucker M, Wong C, Chan H. Ultrasound-assisted embryo transfer. *J In Vitro Fert Embryo Transf* 1986; 3(6): 383–5.
128. Groeneveld E, de Leeuw B, Vergouw CG, Visser OW, Lambers MJ, Heymans MW, Langeveld LH, Schats R, Hompes PG. Standardization of catheter load speed during embryo transfer: Comparison of manual and pump-regulated embryo transfer. *Reprod Biomed Online* 2012; 24(2): 163–9.
129. Garcia J. Conceptus transfer. In: *In Vitro Fertilization*. Jones G, Jones G, Hodgen G, Rosen-Waks Z (eds). Baltimore, MD: Williams and Wilkins, 1986, p. 215.
130. Edwards RG, Steptoe PC, Purdy JM. Establishing full-term human pregnancies using cleaving embryos grown *in vitro*. *Br J Obstet Gynaecol* 1980; 87(9): 737–56.
131. Lopata A, Johnston IW, Hoult IJ, Speirs AI. Pregnancy following intrauterine implantation of an embryo obtained by *in vitro* fertilization of a preovulatory egg. *Fertil Steril* 1980; 33(2): 117–20.
132. Damewood MD, Meng SG. Personal and the organization of the IVF team. In: *The Johns Hopkins Handbook of In vitro Fertilization and Assisted Reproductive Technologies*. Damewood MD (ed.). Boston, MA: Little, Brown and Company, 1990, p. 39.
133. Botta G, Grudzinskas G. Is a prolonged bed rest following embryo transfer useful? *Hum Reprod* 1997; 12(11): 2489–92.
134. Sherif K, Afnan M, Lashen H, Elgendy M, Morgan C, Sinclair L. Is bed rest following embryo transfer necessary? *Fertil Steril* 1998; 69(3): 478–81.
135. Purcell KJ, Schembri M, Telles TL, Fujimoto VY, Cedars MI. Bed rest after embryo transfer: A randomized controlled trial. *Fertil Steril* 2007; 87: 322–6.
136. Woolcott R, Stanger J. Ultrasound tracking of the movement of embryo-associated air bubbles on standing after transfer. *Hum Reprod* 1998; 13(8): 2107–9.
137. Bar-Hava I, Kerner R, Yoeli R, Ashkenazi J, Shalev Y, Orvieto R. Immediate ambulation after embryo transfer: A prospective study. *Fertil Steril* 2005; 83(3): 594–7.
138. Abou-Setta AM, Peters LR, D'Angelo A, Sallam HN, Hart RJ, Al-Inany HG. Post-embryo transfer interventions for assisted reproduction technology cycles. *Cochrane Database Syst Rev* 2014; 8: CD006567.
139. Gaikwad S, Garrido N, Cobo A, Pellicer A, Remohi J. Bed rest after embryo transfer negatively affects *in vitro* fertilization: A randomized controlled clinical trial. *Fertil Steril* 2013; 100(3): 729–35.
140. Birnholz JC. Ultrasonic visualization of endometrial movements. *Fertil Steril* 1984; 41(1): 157–8.
141. de Vries K, Lyons EA, Ballard G, Levi CS, Lindsay DJ. Contractions of the inner third of the myometrium. *Am J Obstet Gynecol* 1990; 162(3): 679–82.
142. Lindholm P, Sundstrom P, Wramsby H. A model for experimental studies on human egg transfer. *Arch Androl* 1980; 5: 92.
143. Baba K, Ishihara O, Hayashi N, Saitoh M, Taya J, Kinoshita K. Where does the embryo implant after embryo transfer in humans? *Fertil Steril* 2000; 73(1): 123–5.
144. Cetin MT, Kumtepe Y, Kiran H, Seydaoglu G. Factors affecting pregnancy in IVF: Age and duration of embryo transfer. *Reprod Biomed Online* 2010; 20(3): 380–6.
145. Matorras R, Mendoza R, Expósito A, Rodriguez-Escudero FJ. Influence of the time interval between embryo catheter loading and discharging on the success of IVF. *Hum Reprod* 2004; 19(9): 2027–30.

146. Ciray HN, Tosun S, Hacifazlioglu O, Mesut A, Bahceci M. Prolonged duration of transfer does not affect outcome in cycles with good embryo quality. *Fertil Steril* 2007; 87(5): 1218–21.
147. Cohen J, Mayaux MJ, Guihard-Moscato ML, Schwartz D. *In-vitro* fertilization and embryo transfer: A collaborative study of 1163 pregnancies on the incidence and risk factors of ectopic pregnancies. *Hum Reprod* 1986; 1(4): 255–8.
148. Refaat B, Dalton E, Ledger WL. Ectopic pregnancy secondary to *in vitro* fertilization–embryo transfer: Pathogenic mechanisms and management strategies. *Reprod Biol Endocrinol* 2015; 13(1): 30.
149. Pope CS, Cook EK, Arny M, Novak A, Grow DR. Influence of embryo transfer depth on *in vitro* fertilization and embryo transfer outcomes. *Fertil Steril* 2004; 81(1): 51–8.
150. Nazari A, Askari HA, Check JH, O'Shaughnessy A. Embryo transfer technique as a cause of ectopic pregnancy in *in vitro* fertilization. *Fertil Steril* 1993; 60(5): 919–21.
151. Lesny P, Killick SR, Tetlow RL, Robinson J, Maguiness SD. Uterine junctional zone contractions during assisted reproduction cycles. *Hum Reprod Update* 1998; 4(4): 440–5.
152. Azem F, Yaron Y, Botchan A et al. Ectopic pregnancy after *in vitro* fertilization–embryo transfer (IVF-ET): The possible role of the ET technique. *J Assist Reprod Genet* 1993; 10: 302–4.
153. Keegan DA, Morelli SS, Noyes N, Flisser ED, Berkeley AS, Grifo JA. Low ectopic pregnancy rates after *in vitro* fertilization: Do practice habits matter? *Fertil Steril* 2007; 88: 734–6.
154. Eytan O, Elad D, Jaffa AJ. Evaluation of the embryo transfer protocol by a laboratory model of the uterus. *Fertil Steril* 2007; 88: 485–93.
155. Healy MW, Hill MJ, Levens ED. Optimal oocyte retrieval and embryo transfer techniques: Where we are and how we got here. *Semin Reprod Med* 2015; 33(2): 83–91.

Cycle regimes for frozen–thawed embryo transfer

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Ovarian stimulation commonly results in the generation of more embryos than are necessary for the fresh embryo transfer. Therefore, cryopreservation and subsequent replacement of frozen–thawed embryos is an integral part of assisted reproductive technology (ART) programs. Frozen embryo replacement (FER) cycles contribute to around 25% of all ART births (1). FER clinical pregnancy rates (CPRs) vary widely. This is at least in part because clinics have varying protocols as to the quality of embryos suitable for cryopreservation, the day of development at which the embryo is frozen, and the technique (slow freezing or vitrification) used. Blastocyst vitrification is now being used more extensively since its widespread introduction over a decade ago (2). As much of the evidence used to guide practice currently is derived from studies using slow freezing, practice will change with increasing evidence from vitrification studies.

Multiple pregnancy remains the most significant risk of ART. Studies have shown that elective single-embryo transfer (eSET) dramatically reduces the rate of multiple pregnancy (3). Importantly, there is increasing evidence that eSET followed by subsequent FER, if pregnancy does not occur, can lead to a cumulative live birth rate (LBR) per oocyte retrieval equivalent to double-embryo transfer. This enables countries with eSET policies and clinics aiming to reduce their multiple pregnancy rate to achieve similar LBRs to those performing multiple-embryo transfer (3–6). Furthermore, there is evidence that ART with blastocyst culture, eSET, and cryopreservation of surplus embryos is acceptable to patients (7,8).

FER PROTOCOLS

It is vital that a frozen–thawed embryo is replaced during the window of endometrial receptivity and that there is synchronization between embryo and endometrial development. A number of different protocols have been developed to achieve this: replacement during a natural ovulatory cycle; hormone (estrogen and progesterone) replacement cycles (with or without prior or synchronous pituitary downregulation); and ovulation induction cycles.

Endometrial receptivity may be negatively affected by ovarian stimulation (9,10). Although most clinicians would advise the use of fresh embryo transfer over FER cycles, a number of studies, including a meta-analysis of three randomized controlled trials (RCTs), have suggested that there may be an advantage to freezing all suitable embryos and replacing them in a natural or medicated cycle (11). A further large U.K. multicenter RCT comparing fresh embryo transfer to freezing all embryos followed by thawed FER is currently ongoing (trial number ISRCTN61225414).

Natural FER cycles

In natural FER cycles, embryo transfer is usually timed using a combination of ultrasound monitoring to confirm follicular development and urinary or serum detection of the luteinizing hormone (LH) surge. The major advantage to replacement in a natural FER cycle is that no medication is required and the time taken to complete the cycle is short. However, there will be a significant proportion of women for whom this approach is not suitable, such as women with anovulatory polycystic ovary syndrome.

Some clinics advocate the use of human chorionic gonadotropin (hCG) to trigger ovulation and to aid in the timing of embryo replacement. A small RCT has shown that the use of an hCG trigger (compared to ultrasound and LH monitoring) decreased the number of monitoring visits required with no difference in pregnancy rate (12). However, if urinary LH testing is undertaken, multiple visits for ultrasound monitoring should be unnecessary. A further RCT contradicted these findings and was terminated after interim analysis because of a significantly increased ongoing pregnancy rate in the group having LH-timed embryo transfer compared to those randomized to an hCG trigger (13). The question of luteal-phase progesterone supplementation in natural cycle FER has been addressed in two RCTs with mixed results. One study found a significant increase in LBR, though not CPR, in women receiving vaginal progesterone (14). However, a further RCT (using an hCG trigger) found no improvement in CPR when luteal intramuscular progesterone was given (15).

Hormone-replacement cycles

One benefit of medicated FER cycles may be increased flexibility as to the timing of embryo transfer that may suit both the patient and the clinic (e.g., the avoidance of weekend thawing and transfers). A number of different protocols exist. First, ovarian down-regulation can be achieved by the use of a gonadotropin-releasing hormone (GnRH) agonist for two to three weeks, after which estrogen and then progesterone is used. A simpler regime commencing estrogen on day 2 of the cycle (which prevents follicular recruitment) with the addition of progesterone later, with or without the use of a GnRH antagonist, is also commonly followed.

Four RCTs have evaluated the use of a GnRH analogue and subsequent hormone replacement compared to using estrogen and progesterone alone. A meta-analysis showed no difference in pregnancy rate, cycle cancelation, endometrial

thickness, or miscarriage rate (16). Notably, the only study to report LBR found a statistically significant increase in the group that underwent downregulation (17). In that study, ovarian activity was not monitored and the authors concluded that in medicated cycles, when embryo replacement was determined by endometrial thickness alone, ovarian activity should either be monitored or suppressed.

HORMONE PREPARATIONS IN FER CYCLES

A number of preparations of both estrogen and progesterone have been used in FER cycles. Commonly, estrogen is administered in tablet form or transdermal patches. One RCT comparing the use of estradiol patches with oral estradiol in FER cycles found no difference in CPR (18). Progesterone may be given as a tablet, pessary (rectal or transvaginal), or by intramuscular injection. Two RCTs have compared progesterone preparations in non-down-regulated medicated cycles. No difference in pregnancy rates were identified using vaginal pessaries versus intramuscular progesterone (19), or when comparing vaginal micronized tables and vaginal progesterone gel (20).

Regimes differ in the length of time progesterone is given prior to transfer. One RCT transferring cleavage-stage embryos found significantly higher implantation and pregnancy rates with three days versus four days of progesterone (21). Another compared three and five days of progesterone administration prior to transfer of cleavage-stage embryos. The CPRs did not differ, but early pregnancy loss was significantly higher in the group receiving three days of progesterone (22). In blastocyst transfer, no difference in CPR was found comparing five days to six days of progesterone administration before transfer (23). A further study investigating five or seven days of progesterone prior to day-5 FER is ongoing (trial number NCT02032797 registered at clinicaltrials.gov). Although there are no studies as to the optimal duration of continued progesterone support in pregnancy following FER, most clinics advise patients to continue progesterone treatment for 8–12 weeks, by which time placental progesterone production is adequate.

It has been hypothesized that hCG may have a beneficial effect on the secretory endometrium, stimulating cytokines and proteins that are important to implantation. However, in an RCT of hCG supplementation versus no treatment in non-down-regulated, hormonally induced cycles, no significant difference in the CPR was identified (24). Two RCTs investigated whether glucocorticoids improve implantation or CPR in FER; however, neither study showed a benefit (25,26). One RCT has looked at the use of sildenafil citrate in artificial FER cycles. Although the endometrial thickness and presence of a triple line were significantly higher in the treatment group, this did not translate to higher implantation or CPRs (27).

Efficacy of natural cycle versus hormone replacement in frozen–thawed embryo transfers

Whilst a number of retrospective studies comparing estrogen and progesterone (with no prior down-regulation) with natural cycles showed a higher pregnancy rate in the natural cycle group (28,29), a number of non-RCTs and one RCT in abstract form (30) showed no difference. Furthermore, a recent RCT investigating the efficacy of natural FER cycles compared to down-regulated hormone-replacement cycles showed no difference in implantation, CPR, or LBR (31).

Stimulation regimes for FER

An alternative approach to endometrial preparation for FER cycles is to use low-dose ovarian stimulation. One RCT of 199 women compared 150 IU follicle-stimulating hormone on days 6, 8, and 10 of the menstrual cycle to estrogen and progesterone endometrial preparation. No differences were identified in implantation or pregnancy rate, cancellation rate, or endometrial thickness (32). Mild ovarian stimulation with low-dose human menopausal gonadotropin was also compared to natural cycle in another RCT of 410 women. There were no differences in implantation rate, endometrial thickness, or LBR between the two groups (33). Clomiphene citrate has also been used for stimulation, but the only RCT using this intervention showed no benefit over estrogen and progesterone used with or without a GnRH analogue (34). Ovulation induction cycles require increased monitoring, are relatively expensive, and do not have the advantage of flexibility with regard to the timing of embryo replacement, thus few centers use this regime.

THE USE OF ULTRASOUND-GUIDED EMBRYO TRANSFER IN FER CYCLES

Ultrasound-guided transfer increases the pregnancy rate when compared to the "clinical touch" technique (35). A recent systematic review (although not differentiating between fresh and frozen cycles) has confirmed the benefit of ultrasound guidance (36).

ENDOMETRIAL THICKNESS AND QUALITY IN FER CYCLES

Several studies have failed to identify differences in endometrial thickness and morphology between conception and non-conception cycles in both natural and medicated cycles (37,38). However, a large retrospective study of medicated (non-down-regulated) FER cycles found that implantation and pregnancy rates were significantly lower when the endometrial thickness was less than 7 mm or greater than 14 mm (39). Although in fresh *in vitro* fertilization (IVF) cycles a triple line is associated with an increased CPR, in FER cycles, no such association has been identified (38,40). However, a non-homogenous hyperechogenic endometrial echo three days after FER was shown to be associated with a reduced pregnancy rate (41). Several studies have evaluated endometrial blood flow parameters in FER cycles. One group has reported a decreased mean uterine artery pulsatility index in conception compared to non-conception cycles (38). An observational study of 165 medicated FER cycles found that the presence of subendometrial–endometrial blood flow on

two-dimensional power Doppler was associated with a significant improvement in implantation, CPR, and LBR (42). No differences have been identified in three-dimensional subendometrial blood flow parameters in natural or clomiphene-induced cycles (40).

THE DEVELOPMENTAL STAGE OF THE EMBRYO AT THE TIME OF FREEZING

Embryo cryopreservation has been successfully achieved at the zygote, cleavage, and blastocyst stages.

Pronuclear versus cleavage-stage freezing

If cryopreservation takes place at the two pronuclear (2PN) zygote stage all embryos are frozen without selection. The temperature-sensitive spindles are not present at 2PN and the pronuclear membrane protects the nuclear material, leading to survival rates of 70%–80% (43–45).

Once thawed, zygotes are usually cultured overnight prior to transfer, since day-1 transfer may be suboptimal (46). Two pronuclear-stage cryopreservation is used extensively in countries where the law does not allow culture of multiple embryos beyond the 2PN stage. Additionally, 2PN cryopreservation is often undertaken for medical reasons, such as in women at very high risk of ovarian hyperstimulation syndrome or for fertility preservation prior to chemotherapy. Although clinics apply different protocols, if a good number of zygotes have been cryopreserved, a number of embryos are often thawed and cultured to days 3 or 5 prior to transfer in order to aid embryo selection.

In the day-2 embryo, the number of blastomeres appears to be related to implantation potential, as those with four cells have a significantly higher implantation rate than those with two cells. On day 3, the data are inconsistent. Studies have shown significantly fewer embryos surviving intact on day 3 compared to day 2 (47,48). However, retrospective studies comparing day-2 and -3 frozen–thawed embryo transfers have shown similar (48) or improved (49) pregnancy rates with day-3 transfer. Comparing 2PN and cleavage-stage cumulative pregnancy rates, RCTs have shown conflicting results, one with similar pregnancy rates (50) and another with increased CPR with 2PN freezing (43).

Cleavage-stage versus blastocyst freezing

In fresh IVF treatment, it has been shown that blastocyst transfer increases the LBR compared to cleavage-stage transfer. One consequence of blastocyst transfer is that the number of embryos available for cryopreservation is reduced (51). Historically, blastocysts were cryopreserved using slow freezing, which was generally less successful than zygote or cleavage-stage freezing. However, vitrification has radically changed the potential of blastocyst cryopreservation. Post-thaw survival of vitrified blastocysts is in the range of 80%–100% (52,53). A recent meta-analysis found higher cumulative pregnancy rates (fresh followed by FER) with blastocyst compared to cleavage-stage transfer when vitrification was used. However, when slow freezing was used, there was evidence of a benefit for cleavage transfer (51). CPRs, even from SET of frozen–thawed embryos, may be as high as 35%–40% (54,55). Of course the majority of ART patients will not have supernumerary high-quality blastocysts suitable for vitrification, and therefore these success rates will only be seen in a percentage of the overall ART population.

EFFECT OF EMBRYO QUALITY AT THE TIME OF FREEZING

Clinics often have very different protocols for determining the quality of embryos they choose to cryopreserve. The policy adopted by an individual clinic regarding the embryo quality threshold for cryopreservation will play a large part in the success rates of the FER cycles. For example, if only top-quality blastocysts are cryopreserved, the pregnancy rate per FER cycle will be significantly higher than a clinic with a policy of freezing all surplus cleavage-stage embryos. However, the latter approach will lead to more FER cycles, albeit with lower success rates per ET, but perhaps the same overall cumulative LBR.

Pronuclear-stage embryo quality

Features of 2PN embryos have been suggested as viability markers that may assist in the decision as to which zygotes to freeze (56–59). However, the effectiveness of these criteria has been questioned, and most centers, if freezing at 2PN, will cryopreserve all zygotes (60). One study has shown that cryopreserved and fresh 2PN embryos have the same implantation potential (61); however, other authors have shown a decreased implantation potential even when the thawed zygote remained intact (59). Particular morphological features including nucleolar precursor body patterns and the presence of a cytoplasmic halo have been found to correlate most highly with implantation rates in both fresh and frozen cycles (62).

Cleavage-stage embryo quality

Evaluation of cleavage-stage embryo quality is more reliable than 2PN as there is significant variability between embryos. At the cleavage stage, the number and regularity of blastomeres along with the speed of cleavage can be observed. In addition, the appearance of the membrane and cytoplasm and the level of cellular fragmentation can be assessed. The loss of blastomeres after thawing has been shown to decrease the implantation potential of cleavage-stage embryos, whilst minimal fragmentation (0%–30%) was found to have no effect (63).

Blastocyst quality

Embryos reach the blastocyst stage on day 5 or day 6 of development. Studies have shown conflicting results as to whether the rate of blastocyst formation affects the treatment outcome. A meta-analysis has shown a significant increase in the CPR and LBR when day 5 rather than day 6 frozen–thawed blastocysts are transferred. However, this difference was no longer seen where the day 5 and day 6 embryos had the same morphological quality (64). Morphological grading of blastocysts is also related to

outcome. In a large series of nearly 1500 frozen–thawed embryo transfers, the chance of a clinical pregnancy and live birth was significantly reduced after the transfer of lower-morphological quality blastocysts. This was found to be the case in each of the age groups studied. Age was also found to be independent of morphological quality as a predictor of pregnancy (65).

ZONA PELLUCIDA BREACHING

It is thought that the process of cryopreservation may cause hardening of the zona pellucida (66), and therefore assisted hatching may be beneficial in FER cycles. However, evidence on this is mixed, and a recent meta-analysis of eight RCTs found no difference in CPR with the use of assisted hatching (67).

REFREEZING OF THAWED EMBRYOS

There are a number of reports of successful live births after the transfer of embryos that have been frozen and thawed more than once (68,69). Perinatal outcomes appear to be reassuring (70). Although the routine use of multiple freeze thaws is not recommended because of the potential stress of cryopreservation, it may be of value in particular circumstances. If, for example, embryos have been frozen at 2PN for fertility preservation, the decision may be made to thaw a number of zygotes to try and reach the blastocyst stage. If at the end of this process there are surplus good-quality blastocysts, these could be vitrified for future use.

SAFETY AND FOLLOW-UP OF CHILDREN BORN AFTER FER CYCLES

The safety of embryo cryopreservation has been questioned. Concerns have been raised regarding its effects on embryonic gene expression and metabolism, as well as the potential negative effects of cryoprotectants (71). However, a recent register-based study showed no significant difference in the physical health outcomes at three years of age between children born from fresh compared to frozen cycles (72). In addition, no difference in obstetric outcome or congenital malformation has been found (73), and one systematic review of 11 observational studies has actually found better obstetric and perinatal outcomes with frozen compared to fresh IVF cycles (74). However, a number of cohort studies have reported increased birthweights in singletons born after FER. This finding was supported by a recent meta-analysis that found a significant increase in both macrosomic and large-for-gestational-age babies when slow freezing FER cycles were compared to fresh cycles (75). Given that vitrification is a relatively new technique, fewer data are available. It is essential that continuing long-term follow-up studies of all cryopreservation techniques are carried out.

REFERENCES

1. Nygren KG, Sullivan E, Zegers-Hochschild F et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) world report: Assisted reproductive technology 2003. *Fertil Steril* 2011; 95: 2209–22; 22.e1–17.
2. Rienzi L, Gracia C, Maggiulli R et al. Oocyte, Embryo and blastocyst cryopreservation in ART: Systematic review and meta-analysis comparing slow freezing versus vitrification to produce evidence for the development of global guidance. *Hum Reprod Update* 2017; 23(2): 139–55.
3. Thurin A, Hausken J, Hillensjo T et al. Elective single-embryo transfer versus double-embryo transfer in *in vitro* fertilization. *N Engl J Med* 2004; 351: 2392–402.
4. Tiitinen A, Halttunen M, Harkki P et al. Elective single embryo transfer: The value of cryopreservation. *Hum Reprod* 2001; 16: 1140–4.
5. Pandian Z, Marjoribanks J, Ozturk O et al. Number of embryos for transfer following *in-vitro* fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev* 2013; 2: CD003416.
6. McLernon DJ, Harrild K, Bergh C et al. Clinical effectiveness of elective single versus double embryo transfer: Meta-analysis of individual patient data from randomised trials. *BMJ* 2010; 341: c6945.
7. Csokmay JM, Hill MJ, Chason RJ, et al. Experience with a patient-friendly, mandatory, single-blastocyst transfer policy: The power of one. *Fertil Steril* 2011; 96: 580–4.
8. Martini S, Van Voorhis BJ, Stegmann BJ et al. *In vitro* fertilization patients support a single blastocyst transfer policy. *Fertil Steril* 2011; 96: 993–7.
9. Haouzi D, Assou S, Dechanet C et al. Controlled ovarian hyperstimulation for *in vitro* fertilization alters endometrial receptivity in humans: Protocol effects. *Biol Reprod* 2010; 82: 679–86.
10. Haouzi D, Assou S, Mahmoud K et al. Gene expression profile of human endometrial receptivity: Comparison between natural and stimulated cycles for the same patients. *Hum Reprod* 2009; 24: 1436–45.
11. Roque M, Lattes K, Serra S et al. Fresh embryo transfer versus frozen embryo transfer in *in vitro* fertilization cycles: A systematic review and meta-analysis. *Fertil Steril* 2013; 99:156–162.
12. Weissman A, Horowitz E, Ravhon A et al. Spontaneous ovulation versus HCG triggering for timing natural-cycle frozen–thawed embryo transfer: A randomized study. *Reprod Biomed Online* 2011; 23: 484–9.
13. Fatemi HM, Kyrou D, Bourgain C et al. Cryopreserved-thawed human embryo transfer: Spontaneous natural cycle is superior to human chorionic gonadotropin-induced natural cycle. *Fertil Steril* 2010; 94: 2054–8.
14. Bjuresten K, Landgren B, Hovatta O et al. Luteal phase progesterone increases live birth rate after frozen embryo transfer. *Fertil Steril* 2011; 95: 534–7.
15. Maryam E, Mozhgan R, Elham R. Effect of progesterone supplementation on natural frozen–thawed embryo transfer cycles: A randomized controlled trial. *Int J Fertil Steril* 2013; 7: 13–20.

16. Ghobara T, Vandekerckhove P. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* 2008; 1: CD003414.
17. El-Toukhy T, Taylor A, Khalaf Y et al. Pituitary suppression in ultrasound-monitored frozen embryo replacement cycles. A randomised study. *Hum Reprod* 2004; 19: 874–9.
18. Davar R, Janati S, Mohseni F et al. A comparison of the effects pf transdermal estradiol and estradiol valerate on endometrial receptivity in frozen-thawed embryo transfer cycles: A randomized clinical trial. *J Reprod Infertil* 2016; 17: 97–103.
19. Lightman A, Kol S, Itskovitz-Eldor J. A prospective randomized study comparing intramuscular with intravaginal natural progesterone in programmed thaw cycles. *Hum Reprod* 1999; 14: 2596–9.
20. Lan VT, Tuan PH, Canh LT et al. Progesterone supplementation during cryopreserved embryo transfer cycles: Efficacy and convenience of two vaginal formulations. *Reprod Biomed Online* 2008; 17: 318–23.
21. Sharma S, Majumdar A. Determining the optimal duration of progesterone supplementation prior to transfer of cryopreserved embryos and its impact on implantation and pregnancy rates: A pilot study. *Int J Reprod Med* 2016; 2016: 7128485.
22. Van der Vijver A, Polyzos NP, Van Landuyt L et al. What is the optimal duration of progesterone administration before transferring a vitrified-warmed cleavage stage embryo? A randomized controlled trial. *Hum Reprod* 2016; 31: 1097–104.
23. Ding J, Rana N, Dmowski WP. Length of progesterone treatment before transfer and implantation rates of frozen–thawed blastocysts. *Fertil Steril* 2007; 88: S330–1.
24. Ben-Meir A, Aboo-Dia M, Revel A et al. The benefit of human chorionic gonadotropin supplementation throughout the secretory phase of frozen-thawed embryo transfer cycles. *Fertil Steril* 2010; 93: 351–4.
25. Bider D, Amoday I, Yonesh M et al. Glucocorticoid administration during transfer of frozen-thawed embryos: A prospective, randomized study. *Fertil Steril* 1996; 66: 154–6.
26. Moffitt D, Queenan JT, Jr., Veeck LL et al. Low-dose glucocorticoids after *in vitro* fertilization and embryo transfer have no significant effect on pregnancy rate. *Fertil Steril* 1995; 63: 571–7.
27. Firouzabadi RD, Davar R, Hojjat F, Mahdavi M. Effect of sildenafil citrate on endometrial preparation and outcome of frozen–thawed embryo transfer cycles: A randomized clinical trial. *Iran J Reprod Med* 2013; 11: 151–8.
28. Morozov V, Ruman J, Kenigsberg D et al. Natural cycle cryo-thaw transfer may improve pregnancy outcome. *J Assist Reprod Genet* 2007; 24: 119–23.
29. Loh SK, Leong NK. Factors affecting success in an embryo cryopreservation programme. *Ann Acad Med Singapore* 1999; 28: 260–5.
30. Cattoli M, Ciotti PM, Seracchioli R et al. *A randomized prospective study on cryopreserved-thawed embryo transfer: Natural verses hormone replacement cycles. Abstracts of the 10th Annual Meeting of ESHRE*. Brussels, Belgium, June 27–29, 1994; 5: pp. 956–60.
31. Mounce G, McVeigh E, Turner K, Child TJ. Randomized, controlled pilot trial of natural versus hormone replacement therapy cycles in frozen embryo replacement *in vitro* fertilization. *Fertil Steril* 2015; 104: 915–20.
32. Wright KP, Guibert J, Weitzen S et al. Artificial versus stimulated cycles for endometrial preparation prior to frozen–thawed embryo transfer. *Reprod Biomed Online* 2006; 13: 321–5.
33. Peeraer K, Couck I, Debrock S et al. Frozen–thawed embryo transfer in a natural or mildly hormonally stimulated cycle in women with regular ovulatory cycles: A RCT. *Hum Reprod* 2015; 30: 2552–62.
34. Loh SKE, Ganesan G, Leong NK. Clomid verses hormone endometrial preperation in FET cycles. *Abstract Book of the 17th World Congress in Fertility and Sterility (IFFS)*, Melbourne, Australia, 25–30 November 2001: 3.
35. Coroleu B, Barri PN, Carreras O et al. The usefulness of ultrasound guidance in frozen–thawed embryo transfer: A prospective randomized clinical trial. *Hum Reprod* 2002; 17: 2885–90.
36. Brown J, Buckingham K, Buckett W, Abou-Setta AM. Ultrasound versus "clinical touch" for catheter guidance during embryo transfer in women. *Cochrane Database Syst Rev* 2016; 3: CD006107.
37. Check JH, Dietterich C, Graziano V et al. Effect of maximal endometrial thickness on outcome after frozen embryo transfer. *Fertil Steril* 2004; 81: 1399–400.
38. Coulam CB, Bustillo M, Soenksen DM, Britten S. Ultrasonographic predictors of implantation after assisted reproduction. *Fertil Steril* 1994; 62: 1004–10.
39. El-Toukhy T, Coomarasamy A, Khairy M et al. The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. *Fertil Steril* 2008; 89: 832–9.
40. Ng EH, Chan CC, Tang OS et al. The role of endometrial and subendometrial vascularity measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during frozen–thawed embryo transfer cycles. *Hum Reprod* 2006; 21: 1612–17.
41. Check JH, Dietterich C, Nazari A et al. Nonhomogeneous hyperechogenic echo pattern three days after frozen embryo transfer is associated with lower pregnancy rates. *Clin Exp Obstet Gynecol* 2005; 32: 15–8.
42. Sardana D, Upadyhah AJ, Deepika K et al. Correlation of subendometrial–endometrial blood flow assessment by two dimensional power Doppler with pregnancy outcome in frozen-thawed embryo transfer cycles. *J Hum Reprod Sci* 2014; 7: 130–5.

43. Senn A, Vozzi C, Chanson A et al. Prospective randomized study of two cryopreservation policies avoiding embryo selection: The pronucleate stage leads to a higher cumulative delivery rate than the early cleavage stage. *Fertil Steril* 2000; 74: 946–52.
44. Veeck LL. Does the developmental stage at freeze impact on clinical results post-thaw? *Reprod Biomed Online* 2003; 6: 367–74.
45. Queenan JT, Jr., Veeck LL, Toner JP et al. Cryopreservation of all prezygotes in patients at risk of severe hyperstimulation does not eliminate the syndrome, but the chances of pregnancy are excellent with subsequent frozen–thaw transfers. *Hum Reprod* 1997; 12: 1573–6.
46. Jaroudi K, Al-Hassan S, Sieck U et al. Zygote transfer on day 1 versus cleavage stage embryo transfer on day 3: A prospective randomized trial. *Hum Reprod* 2004; 19: 645–8.
47. Hartshorne GM, Wick K, Elder K, Dyson H. Effect of cell number at freezing upon survival and viability of cleaving embryos generated from stimulated IVF cycles. *Hum Reprod* 1990; 5: 857–61.
48. Salumets A, Tuuri T, Makinen S et al. Effect of developmental stage of embryo at freezing on pregnancy outcome of frozen–thawed embryo transfer. *Hum Reprod* 2003; 18: 1890–5.
49. Sifer C, Sellami A, Poncelet C et al. Day 3 compared with day 2 cryopreservation does not affect embryo survival but improves the outcome of frozen–thawed embryo transfers. *Fertil Steril* 2006; 86: 1537–40.
50. Horne G, Critchlow JD, Newman MC et al. A prospective evaluation of cryopreservation strategies in a two-embryo transfer programme. *Hum Reprod* 1997; 12: 542–7.
51. Glujovsky D, Blake D, Bardach A, Farquhar C. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev* 2016; 4: CD002118.
52. Kader AA, Choi A, Orief Y, Agarwal A. Factors affecting the outcome of human blastocyst vitrification. *Reprod Biol Endocrinol* 2009; 7: 99.
53. Youssry M, Ozmen B, Zohni K et al. Current aspects of blastocyst cryopreservation. *Reprod Biomed Online* 2008; 16: 311–20.
54. Berin I, McLellan ST, Macklin EA et al. Frozen–thawed embryo transfer cycles: Clinical outcomes of single and double blastocyst transfers. *J Assist Reprod Genet* 2011; 28: 575–81.
55. Yanaihara A, Yorimitsu T, Motoyama H et al. Clinical outcome of frozen blastocyst transfer; single vs. double transfer. *J Assist Reprod Genet* 2008; 25: 531–4.
56. Scott LA, Smith S. The successful use of pronuclear embryo transfers the day following oocyte retrieval. *Hum Reprod* 1998; 13: 1003–13.
57. Garello C, Baker H, Rai J et al. Pronuclear orientation, polar body placement, and embryo quality after intra-cytoplasmic sperm injection and *in-vitro* fertilization: Further evidence for polarity in human oocytes? *Hum Reprod* 1999; 14: 2588–95.
58. Tesarik J, Greco E. The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology. *Hum Reprod* 1999; 14: 1318–23.
59. Hammitt DG, Sattler CA, Manes ML, Singh AP. Selection of embryos for day-3 transfer at the pronuclear-stage and pronuclear-stage cryopreservation results in high delivery rates in fresh and frozen cycles. *J Assist Reprod Genet* 2004; 21: 271–8.
60. James AN, Hennessy S, Reggio B et al. The limited importance of pronuclear scoring of human zygotes. *Hum Reprod* 2006; 21: 1599–604.
61. Miller KF, Goldberg JM. *In vitro* development and implantation rates of fresh and cryopreserved sibling zygotes. *Obstet Gynecol* 1995; 85: 999–1002.
62. Senn A, Urner F, Chanson A et al. Morphological scoring of human pronuclear zygotes for prediction of pregnancy outcome. *Hum Reprod* 2006; 21: 234–9.
63. Edgar DH, Bourne H, Jericho H, McBain JC. The developmental potential of cryopreserved human embryos. *Mol Cell Endocrinol* 2000; 169: 69–72.
64. Sunkara SK, Siozos A, Bolton VN et al. The influence of delayed blastocyst formation on the outcome of frozen–thawed blastocyst transfer: A systematic review and meta-analysis. *Hum Reprod* 2010; 25: 1906–15.
65. Goto S, Kadowaki T, Tanaka S et al. Prediction of pregnancy rate by blastocyst morphological score and age, based on 1,488 single frozen–thawed blastocyst transfer cycles. *Fertil Steril* 2011; 95: 948–52.
66. Carroll J, Depypere H, Matthews CD. Freeze–thaw-induced changes of the zona pellucida explains decreased rates of fertilization in frozen–thawed mouse oocytes. *J Reprod Fertil* 1990; 90: 547–53.
67. Carney SK, Das S, Blake D et al. Assisted hatching on assisted conception (*in vitro* fertilization and intracytoplasmic sperm injection). *Cochrane Database Syst Rev* 2012; 12: CD001894.
68. Kumasako Y, Otsu E, Utsunomiya T, Araki Y. The efficacy of the transfer of twice frozen–thawed embryos with the vitrification method. *Fertil Steril* 2009; 91: 383–6.
69. Check JH, Summers-Chase D, Yuan W et al. Pregnancy rates following the exclusive transfer of twice frozen twice thawed embryos using a modified slow cool cryopreservation technique. *Clin Exp Obstet Gynecol* 2013; 40: 20–1.
70. Murakami M, Egashira A, Murakami K et al. Perinatal outcome of twice-frozen–thawed embryo transfers: A clinical follow-up study. *Fertil Steril* 2011; 95: 2648–50.
71. Winston RM, Hardy K. Are we ignoring potential dangers of *in vitro* fertilization and related treatments? *Nat Cell Biol* 2002; (4 Suppl): s14–8.
72. Pelkonen S, Gissler M, Koivurova S et al. Physical health of singleton children born after frozen

embryo transfer using slow freezing: A 3-year follow-up study. *Hum Reprod* 2015; 30: 2411–8.
73. Wennerholm UB, Soderstrom-Anttila V, Bergh C et al. Children born after cryopreservation of embryos or oocytes: A systematic review of outcome data. *Hum Reprod* 2009; 24: 2158–72.
74. Maheshwari A, Pandey S, Shetty A et al. Obstetric and perinatal outcomes in singleton pregnancies resulting from transfer of frozen thawed versus fresh embryos generated through *in vitro* fertilization treatment: A systematic review and meta-analysis. *Fertil Steril* 2012; 98: 368–77.
75. Pinborg A, Henningsen AA, Loft A et al. Large baby syndrome in singletons born after frozen embryo transfer (FET): Is it due to maternal factors or the cryotechnique? *Hum Reprod* 2014; 29: 618–27.

Anesthesia for *in vitro* fertilization

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INTRODUCTION

Although *in vitro* fertilization (IVF) is considered a minor surgical procedure, it might be very painful and associated with fear and anxiety (1).

IVF techniques include (2):

1. Ovarian stimulation and monitoring
2. Transvaginal ultrasound-guided oocyte retrieval (TUGOR)
3. Fertilization in the laboratory and transfer of embryos into the uterus

Anesthesiologists are mainly involved in TUGOR.

Women undergoing transvaginal oocyte retrieval experience mild to moderate pain caused by the puncture of the vaginal wall and ovarian capsule with a needle necessary to aspirate the follicles. The majority of the patients are young and healthy, but exhibit stress, anxiety, and other psychological disturbances associated with infertility. It is particularly important for the anesthesiologist to understand the patient's psychological stress and take appropriate measures to allay it. Repeated interventions are often necessary and therefore the need for mitigating the pain associated with the procedure is a major consideration (3).

Sedation alone or combined with analgesia, as well as different anesthetic techniques including general anesthesia (GA), regional anesthesia, and alternative medicine approaches, have all been used for these procedures. All of the above techniques demand the active involvement of an anesthesiologist to make transvaginal oocyte retrieval a safe procedure.

The following sections describe what the anesthesiologist should consider in caring for the women undergoing TUGOR.

GENERAL CONSIDERATIONS

Comorbidities

Patients may be suffering from morbid obesity, severe renal, cardiac, or pulmonary disease, or other chronic illnesses (4–7). In cancer patients, oocyte retrieval is usually being performed prior to chemo/radiotherapy (8). These conditions may require special patient preparation and/or affect the anesthetic plan.

Medications

Patients may receive chronic treatment with anticoagulants (9), thyroid medications, antidepressants/anxiolytics (10), analgesics, or other medications. These may be associated with excessive bleeding from the procedure or interact with the effect of the anesthetic agents.

ANESTHETIC CONSIDERATIONS

The anesthetic options available today for TUGOR are conscious sedation with local anesthesia or epidural, spinal, and GA.

In the recent years, acupuncture has been gaining more popularity (11).

According to a Cochrane review in 2013 (12), conscious sedation is used in 84% of IVF clinics in the U.K. (13) and in 95% of IVF clinics in the U.S.A. (14). The current standard of care for the various methods of pain relief has not been defined. This was shown by a recent Cochrane review (12) identifying 390 reports, of which only 12 papers were included. It was concluded that no particular pain relief method appeared more effective and no significant differences were found in regard to pregnancy rate or patient satisfaction.

Monitored anesthesia care or conscious sedation

Conscious sedation allows patient cooperation to be maintained and the procedure to be conveniently performed in the outpatient setting. With this technique, patient consciousness is minimally depressed, and the patient is able to respond to verbal commands and cooperate (15), while receiving appropriate analgesia and eventually amnesia, with a patent airway throughout the surgical process. As written above, conscious sedation remains the most commonly used method of providing analgesia and anesthesia during transvaginal oocyte retrieval (14,16). By comparison, 16% of U.K. clinics use general anesthesia for IVF procedures (13,17).

Monitored anesthesia care (MAC) is relatively easy to deliver; anesthetic/analgesic drugs are well-tolerated and best suited for day care settings. MAC may diminish the potentially harmful effects of anesthetic drugs on oocytes. Different methods of conscious sedation and analgesia have been used for oocytes recovery. Drugs used for these procedures are selected by the quality of sedation and analgesia and their potential deleterious effects on reproductive outcomes (18). According to an updated Cochrane review conducted in 2013 (22), the various approaches for MAC or conscious sedation used for IVF appeared to be acceptable and were associated with a high degree of women's satisfaction. Simultaneous use of more than one method of sedation and analgesia resulted in better pain relief than one modality alone (12). To cope with changing patient requirements for pain relief, a patient-controlled analgesia (PCA) device has been developed, which facilitates a patient's control over pain relief (19).

Administration of conscious sedation should be started only after obtaining verbal and written patient consent.

The most popular anesthetic agent used is propofol or its combination with midazolam and fentanyl. Personnel not trained in anesthesia prefer to use a midazolam–meperidine combination. According to Ditkoff et al. (14), the non-anesthesiologists providing sedation for IVF mainly used meperidine and midazolam, while 90% of the anesthesiologists preferred to employ midazolam and/or propofol with fentanyl. The complication rate and recovery time for both groups were similar (90–120 minutes), but the cost of drugs used by anesthesiologists was higher.

Monitoring the patient's condition during conscious sedation should include the standard American Society of Anesthesiologists (ASA) monitoring of noninvasive blood pressure, heart rate, oxygen saturation, electrocardiogram, capnography, respiratory rate, and level of consciousness. To prevent the local burning sensation caused by propofol injection, pretreatment with 1 mg/kg intravenous (i.v.) lidocaine may be helpful.

A Cochrane review published in 2013 (12) assessed the efficacy of conscious sedation and analgesia versus alternative methods on pregnancy outcomes and pain relief. There were no significant differences in the pregnancy rate or patient satisfaction. There were conflicting results concerning pain treatment due to methodological variability in terms of anesthesia mode or the dose and type of the drug used. Therefore, a meta-analysis could not be done.

Pregnancy rate

Live birth rate following conscious sedation was only reported in a single trial (20), whereas seven trials referred to ongoing pregnancy rates (21–26). Overall, various methods of conscious sedation/analgesia did not result in marked differences in pregnancy rates. Three trials compared i.v. alfentanil (an opiate analgesic) plus paracervical block (PCB) with electroacupuncture (plus PCB) (20,21,26) and found no significant difference in clinical pregnancy rates (26% vs. 37%, 36% vs. 32%, and 50% vs. 46%, respectively). Combining clinical pregnancy data from these three trials resulted in an odds ratio (OR) of 1.01 (95% confidence interval [CI] = 0.73–1.40). There were no significant differences between i.v. alfentanil and electroacupuncture in terms of ongoing pregnancy rate and live birth rate per woman (20,21) (31% vs. 27% and 26% vs. 33%; combined OR = 0.99, 95% CI = 0.65–1.51).

Pain control during TUGOR

The pain expressed during aspiration of oocytes is identical to intensive menstrual pain and is produced by the needle inserted through the vaginal wall and by mechanical stimulation of the ovary (27). The number of follicles and duration of the oocyte retrieval procedure may affect the pain intensity. Single-follicle aspiration would take less time and cause less pain as compared to multiple-follicle aspiration (27). A favorable analgesic regimen for oocyte retrieval must have no toxic effects on the oocytes, with rapid onset, rapid recovery, and ease of administration and monitoring.

Kwan et al. (12) identified 21 randomized controlled trials involving 2974 women comparing the effects of five different methods of conscious sedation and pain relief, including general anesthesia. The review found insufficient evidence to support any one method as being superior to others in terms of pain relief or pregnancy outcomes. The mainstay of pain relief is the use of opioid drugs. Most of the methods seemed to work well and the effect was usually enhanced by addition of another method such as pain relief with PCB, which involves a local anesthetic agent being injected into the cervix prior to egg retrieval.

Patients who received sedation in addition to PCB reported lower pain scores as compared to those who received PCB with placebo. The response to pain in oocyte retrieval during conscious sedation was more intense when compared to GA, but postoperative abdominal pain was significantly lower in the sedation group than in the GA group (22).

Less pain perception was reported by patients in the physician-controlled group. In a randomized controlled trial, Lok et al. (24) compared the fertility outcomes and anesthetic parameters in two groups: a PCA group who received propofol with alfentanil and a second patient group who received i.v. pethidine with diazepam, plus additional doses of pethidine administered by the anesthesiologist. Levels of sedation, cooperation, and fertility outcomes were similar. Pain scores were higher throughout the procedure in the PCA group, although their future preference for this mode of analgesia was higher. Edwards et al. (28) reported on 4342 patients in the U.K. who were administered propofol (target controlled infusion) and alfentanil boluses by non-anesthetists during oocyte retrieval. According to the study design, safety was acceptable, with a respiratory adverse incident rate of 0.5/1000. In this study, unplanned, direct anesthetic assistance was required in 3.5/1000 cases and anesthetic advice was required in 7.5% cases.

We can conclude that no single method of conscious sedation delivery system appeared superior in terms of pregnancy rates and pain relief. Future studies need to be consistent in the choice of tools used to measure pain and the timing of such evaluations.

Complications of conscious sedation

A Cochrane review in 2013 (12) reported no serious adverse effects or oocyte retrieval procedure cancelations attributed to conscious sedation. The rates of postoperative nausea and vomiting were similar in all conscious sedation groups. Loss of airway control was very rare (25). In the trial that compared patient-controlled sedation with propofol or midazolam, two women suffered syncope after propofol and one woman became transiently unresponsive after midazolam.

Drugs used in conscious sedation

Midazolam (a benzodiazepine) is a commonly used drug in conscious sedation because of its sedative and anxiolytic effects (29). Additionally, it has anticonvulsant, amnesic (produces anterograde amnesia), and mild muscle relaxation effects. When combined with opioids (mainly with fentanyl),

the synergistic effect with midazolam may enhance sedation, perhaps leading to respiratory depression or even apnea; therefore, reduced doses of both drugs are mandatory.

Opioids (meperidine, fentanyl, alfentanil, and remifentanil) are narcotic agents widely used for GA and conscious sedation, mainly for their potent analgesic effects. Remifentanil is a potent synthetic, ultra-short-acting opioid with a fast onset and short elimination time (30). In high doses or rapid administration, opioids may cause respiratory depression, bradycardia, and muscle rigidity. Apnea or stiff chest may necessitate manual ventilation or administration of naloxone or a muscle relaxant, followed by tracheal intubation. Other adverse effects of opioids are nausea, vomiting, and pruritus. Most adverse effects can be reversed by naloxone administration.

Propofol is the most popular anesthesia induction agent, with a fast onset and short elimination time (28). It is used for GA and conscious sedation (28). Its administration is associated with decreased postoperative nausea and vomiting, especially when used alone. When administered through a peripheral vein, it causes a local burning pain sensation. This side effect may be mitigated by a prior injection of i.v. lidocaine and slow administration of propofol. The use of propofol in conscious sedation for oocyte retrieval necessitates an anesthesiologist or personnel skilled in airway management (28). Depending on the dose, it may cause respiratory and myocardial depression. The use of propofol in combination with fentanyl or alfentanil in such a setting was found to be beneficial (13). Goutziomitrou et al. (31) compared clinical outcomes of IVF cycles using propofol or thiopental sodium as anesthetic agents for oocyte retrieval. The study revealed that the use of propofol compared with thiopental sodium for general anesthesia during oocyte retrieval results in similar fertilization rates and IVF outcomes.

Until recently, egg allergy was a contraindication to propofol administration. However, according to the recent medical literature, propofol is likely to be safe in the majority of egg-allergic patients who do not have a history of egg anaphylaxis (32,33).

Ketamine is an old induction agent used in GA and as a sedative and analgesic agent in conscious sedation. It belongs to the phencyclidine family of drugs that cause, through their central nervous system (CNS) effect, dissociative anesthesia (a cataleptic condition with eyes open and slow nystagmus gaze). It was considered an ideal anesthetic agent due to several properties required for GA, such as analgesia, loss of consciousness, and anterograde amnesia, without cardiorespiratory system-depressant effects and preserved laryngeal reflexes. However, this drug is not popular anymore because of its postoperative psychological adverse effects in 5%–30% of patients, such as hallucinations, vivid dreaming, and feelings of excitement or fear that may last for several hours (22). However, when combined with midazolam, which minimizes its CNS side effects, it may be a good alternative to GA with propofol and midazolam (22).

Ketamine administration may increase prolactin and β-endorphin levels, with no clear impact of these changes on IVF results (34).

Controversy exists regarding the effects of anesthetic drugs administered during transvaginal puncture procedures for oocyte retrieval on conception rates (35). Traces of anesthetic drugs have been detected in follicular fluid, and studies suggest that these drugs may adversely affect oocyte fertilization and embryonic development.

Continuous propofol infusion may cause a gradual, time-dependent, linear increase of propofol concentration in the follicular fluid (36–38). Nevertheless, this did not affect either the ratio between mature to immature oocytes or the fertilization and cleavage rates, as well as embryo cell number. In addition, a minimal concentrations of midazolam found in the follicular fluid has no detrimental effects on fertilization in animal or human studies (39–41). All the midazolam-related adverse effects on the woman can be reversed by Anexate (flumazenil).

General anesthesia

GA is the second most common technique for transvaginal oocyte retrieval and the most common technique for laparoscopic zygote or gamete intrafallopian transfer (42–44).

The ideal GA regimen would reduce pain to a tolerable level in all patients without the risk of adverse respiratory or cardiovascular events (22).

GA is induced and maintained in a hospital setting by specially trained personnel using either i.v. or inhalational agents. The i.v. agents include propofol, narcotics, and sedatives. The inhalational agents include nitrous oxide (N_2O) and volatile anesthetics such as isoflurane, desflurane, or sevoflurane. Spontaneous ventilation is usually maintained through a face mask or laryngeal mask airway. Rarely, mechanical ventilation through endotracheal tube or laryngeal mask airway is required.

Drawbacks of GA are prolonged recovery, drowsiness, nausea, and vomiting, as well as possible detrimental effects on reproduction.

Anesthetic agents have been found in the follicular fluid, and these drugs may have adverse effects on oocyte fertilization and embryonic development. Prolonged periods of exposure to GA agents can lead to lower pregnancy and delivery rates (45). Wilhelm et al. compared the outcomes of assisted reproductive technology procedures in 251 women who underwent MAC with remifentanil versus GA with alfentanil, propofol, isoflurane, and nitrous oxide (44). They concluded that the pregnancy rates in women undergoing transvaginal oocyte retrieval for assisted reproductive technologies were significantly higher with a remifentanil-based MAC technique than with a balanced GA technique involving nitrous oxide. Since there was a trend toward lower fertilization rate with prolonged GA drugs, the recommendation, therefore, was to minimize anesthetic drug exposure (45). However, Hadimioglu et al. (46) demonstrated that N_2O increased the success rate of IVF by lowering the concentration of other potentially toxic and less diffusible anesthetic drugs. Thus, the effect of N_2O on IVF outcome still remains questionable.

Despite the apparent concerns regarding GA, a recent Cochrane review (12) concluded that no particular pain

relief method appeared to be more effective for IVF, with no significant differences in regard to pregnancy rate or patient satisfaction.

Our personal experience in anesthesia for TUGOR (unpublished data) includes 1500 patients, who were given balanced GA (without premedication), with a pre-induction dose of 1 mg midazolam or 20 mg propofol followed by 50 μg fentanyl, maintaining spontaneous ventilation with face mask and maintaining anesthesia with a 50% O_2/N_2O mixture and repetitive boluses of 20–40 mg propofol. Postoperative analgesia is provided with non-steroidal anti-inflammatory medications until discharge from hospital within one to two hours.

Neuraxial anesthesia

Neuraxial anesthesia is an effective method of analgesia for TUGOR. It can be achieved by injection of local anesthetics into the epidural or spinal space. Neuraxial anesthesia has the advantage of minimal local anesthetic absorption, and therefore minimal follicular accumulation (47–51).

We are aware of two studies that compared pregnancy outcomes using GA versus spinal anesthesia for IVF. One of them was a cohort study (47) and the second one was a randomized controlled trial (49).

The results of the both studies demonstrate that spinal anesthesia increases the chance of fertilization success. However, both studies were performed in the same academic center and so more randomized controlled trials should probably be performed.

Adverse effects of epidural anesthesia are spinal headache, urinary retention, and accidental intravascular injection of local anesthetics. A high spinal block can cause respiratory depression. Local infection at the injection site, coagulopathy, increased intracranial pressure, and patient refusal are contraindications to epidural or spinal anesthesia.

Paracervical and pre-ovarian block

In PCB, a local anesthetic (usually lidocaine 150–200 mg) is injected at two to six points and a depth of 3–7 mm alongside the vaginal portion of the cervix in the vaginal fornices (52).

Cerne et al. (53) studied pre-ovarian block (POB) where the local anesthetic is infiltrated under ultrasound guidance between the vaginal wall and peritoneal surface near the ovary. The follicle aspiration needle is then inserted in exactly the same location where the lidocaine was deposited. In this study, the authors analyzed POB versus PCB for oocyte retrieval in a prospective, randomized, multicenter study of 183 patients. They concluded that both techniques provided comparable pain relief and both POB and PCB in combination with i.v. alfentanil may be considered safe methods with rapid onset and recovery and easy administration (54). Three randomized controlled trials have compared the effects of PCB when combined with conventional analgesics to PCB plus electroacupuncture (20,21,26). There were no significant differences regarding clinical pregnancy rates, but the intraoperative analgesic scores were lower in the group that received conventional analgesics with PCB. A possible risk associated with PCB is the potential of toxicity from absorbed lidocaine or bupivacaine (18,55). No adverse effects on fertilization, cleavage, or pregnancy rates were shown using PCB (18). PCB with different doses of lidocaine has been studied, and no differences were found in pain levels during oocyte retrieval when 50, 100, or 200 mg of lidocaine were used (52,54,55). Thus, the lowest dose should be recommended.

Alternative and non-pharmacological pain management for IVF

Acupuncture has had a prominent place in Chinese medicine for thousands of years and it has garnered a growing awareness in Western countries as a modality for successful treatment in chronic pain, nausea and vomiting, fibromyalgia, and drug addiction (56,57).

Recently, electroacupuncture, which activates the endogenous opioid system responsible for pain, has been reported to decrease pain during oocyte retrieval with few negative side effects (58). In a prospective, randomized study of 200 women, Humaidan and Stener-Victorin (26) investigated the role of electroacupuncture as an alternative to a conventional method of sedation and analgesia for IVF (benzodiazepine premedication and alfentanil boluses). They found that the procedure was well tolerated in both groups; however, higher pain scores were observed in the electroacupuncture group. In a randomized study of 160 women, Gejervall et al. (27) have found that electroacupuncture cannot be generally recommended as a pain-relieving method of oocyte aspiration, but it may be an alternative for women desiring a non-pharmacological method. In a similar study, Stener-Victorin et al. (20) concluded that the analgesic effects produced by electroacupuncture are as good as those produced by conventional analgesics. Three meta-analyses have recently been published concerning the effects of acupuncture on IVF outcome (59–61). The meta-analysis published by Manheimer et al. (59) indicated that acupuncture at the time of embryo transfer improved clinical pregnancy rates. In two other meta-analyses (60,61), no beneficial effects of acupuncture had been shown on clinical pregnancy rates or live birth rates. The authors of all of these meta-analyses indicated bias effects as a result of the heterogeneity of the trials reviewed, use of sham acupuncture (use of non-selected points) as control or lack of control, the timing of acupuncture, and the heterogeneity of procedures. However, acupuncture is devoid of toxicity and may be appropriate in case of patient refusal or contraindication to conventional anesthesia.

CONCLUSIONS

The role of the anesthesiologist in IVF is to provide adequate comfort and pain relief to patients during oocyte retrieval and embryo transfer procedures. The modality of providing adequate anesthesia assistance during the procedure depends on patient cooperation. If the patient is comfortable and cooperative, conscious sedation is a good option. However, in some cases, regional anesthesia or GA may be requested or necessary. Numerous studies have

explored the effects of anesthesia on IVF outcomes, but have yielded contradictory findings. These differences may be attributed to differences in study design and randomization, the anesthetic drugs used, or the anesthetic technique employed. Nevertheless, duration of anesthesia should be as short as possible. Comorbidities and concurrent medication may affect the choice of anesthesia for IVF.

REFERENCES

1. Tang J, Gibson SJ. A psychophysical evaluation of the relationship between trait anxiety, pain perception, and induced state anxiety. *J Pain* 2005; 6: 612–9.
2. Gindoff PR, Hall JL, Stillman RJ. Utility of *in vitro* fertilization at diagnostic laparoscopy. *Fertil Steril* 1994; 62: 237–41.
3. Vlahos, NF, Giannakikou, I, Vlachos, A, Vitoratos, N. Analgesia and anesthesia for assisted reproductive technologies. *Int J Gynecol Obstet* 2009; 105: 201–5.
4. Zaig I, Azem F, Schreiber S, Gottlieb-Litvin Y, Meiboom H, Bloch M. Women's psychological profile and psychiatric diagnoses and the outcome of *in vitro* fertilization: Is there an association? *Arch Womens Ment Health* 2012; 15: 353–9.
5. Metzler E, Ginsburg E, Tsen LC. Use of assisted reproductive technologies and anesthesia in a patient with primary pulmonary hypertension. *Fertil Steril* 2004; 81: 1684–7.
6. Sioulas VD, Gracia CR. Ovarian stimulation and embryo banking for fertility preservation in a woman with severe mixed connective tissue disease: Is it safe? *J Assist Reprod Genet* 2012; 29: 271–5.
7. Creatsas G, Deligeoroglou E, Tsimaris P, Pantos K, Kreatsa M. Successful pregnancy in a Swyer syndrome patient with preexisting hypertension. *Fertil Steril* 2011; 96: e83–5.
8. Barton SE, Missmer SA, Berry KF, Ginsburg ES. Female cancer survivors are low responders and have reduced success compared with other patients undergoing assisted reproductive technologies. *Fertil Steril* 2012; 97: 381–6.
9. Fleming T, Sacks G, Nasser J. Internal jugular vein thrombosis following ovarian hyperstimulation syndrome. *Aust N Z J Obstet Gynaecol* 2012; 52: 87–90.
10. Hashemi S, Simbar M, Ramezani-Tehrani F, Shams J, Majd HA. Anxiety and success of *in vitro* fertilization. *Eur J Obstet Gynecol Reprod Biol* 2012; 164: 60–4.
11. Rowe T. Acupuncture and reproduction. *J Obstet Gynaecol Can* 2010; 32: 1023–7.
12. Kwan I, Bhattacharya S, Knox F, McNeil A. Pain relief for women undergoing oocyte retrieval for assisted reproduction. *Cochrane Database Syst Rev* 2013; 1: CD004829.
13. Elkington NM, Kehoe J, Acharya U. Intravenous sedation in assisted conception unit: A UK survey. *Hum Fertil* 2003; 6: 74–6.
14. Ditkoff EC, Plumb J, Selick A, Sauer MV. Anesthesia practices in the United States common to *in vitro* fertilization (IVF) centers. *J Assist Reprod Genet* 1997; 14: 145–7.
15. White DC. Anaesthesia: A privation of the senses. In: *Conscious Awareness and Pain in General Anaesthesia*. Rosen M, Lunn JN (eds). London, U.K.: Butterworth, 1987; 1–9.
16. Trout S, Vallerand SH, Kemmann E. Conscious sedation for *in vitro* fertilisation. *Fertil Steril* 1998; 69: 799–808.
17. Andersen AN, Goossens V, Ferraretti AP et al. Assisted reproductive technology in Europe, 2004: Results generated from European registers by ESHRE. *Hum Reprod* 2008; 23: 756–71.
18. Matsota P, Kaminioti E, Kostopanagiotou G. Anesthesia related toxic effects on *in vitro* fertilization outcome: Burden of proof. *Biomed Res Int* 2015; 2015: 475362.
19. Dell RG, Cloote AH. Patient-controlled sedation during transvaginal oocyte retrieval: an assessment of patient acceptance of patient-controlled sedation using a mixture of propofol and alfentanil. *Eur J Anaesthesiol* 1998; 15: 210–5.
20. Stener-Victorin E, Waldenstrom U, Nilsson L, Wikland M, Janson PO. A prospective randomized study of electro-acupuncture versus alfentanil as anaesthesia during oocyte aspiration in *in-vitro* fertilization. *Hum Reprod* 1999; 14: 2480–4.
21. Stener-Victorin E, Waldenstrom U, Wikland M, Nilsson L, Hägglund L, Lundeberg T. Electroacupuncture as a perioperative analgesic and its effect on implantation rate and neuropeptide Y concentrations in follicular fluid. *Hum Reprod* 2003; 18: 1454–60.
22. Ben-Shlomo I, Moskovich R, Katz Y, Shalev E. Midazolam/ketamine sedative combination compared with fentanyl/propofol/isoflurane anaesthesia for oocyte retrieval. *Hum Reprod* 1999; 14: 1757–9.
23. Ng EH, Chui DK, Tang OS, Ho PC. Paracervical block with and without conscious sedation: A comparison of the pain levels during egg collection and the postoperative side effects. *Fertil Steril* 2001; 75: 711–7.
24. Lok IH, Chan MTV, Chan DLW, Cheung LP, Haines CJ, Yuen PM. A prospective randomized trial comparing patient-controlled sedation using propofol and alfentanil and physician-administered sedation using diazepam and pethidine during transvaginal ultrasound-guided oocyte retrieval. *Hum Reprod* 2002; 17: 2101–6.
25. Thompson N, Murray S, MacLennan F et al. A randomized controlled trial of intravenous versus inhalational analgesia during outpatient oocyte recovery. *Anaesthesia* 2000; 55: 770–3.
26. Humaidan P, Stener-Victorin E. Pain relief during oocyte retrieval with a new short duration electroacupuncture technique—An alternative to conventional analgesic methods. *Hum Reprod* 2004; 19: 1367–72.

27. Gejervall AL, Stener-Victorin E, Möller A, Janson PO, Werner C, Bergh C. Electro-acupuncture versus conventional analgesia: A comparison of pain levels during oocyte aspiration and patients' experiences of well-being after surgery. *Hum Reprod* 2005; 20: 728–35.
28. Edwards JA, Kinsella J, Shaw A, Evans S, Anderson KJ. Sedation for oocyte retrieval using target controlled infusion of propofol and incremental alfentanil delivered by nonanesthetists. *Anaesthesia* 2010; 65: 453–61.
29. Trout S, Vallerand A, Kemmann E. Conscious sedation for *in vitro* fertilization. *Fertil Steril* 1998; 69: 799–808.
30. Arndt M, Kreienmeyer J, Vagts DA, Nöldge-Schomburg GH. Remifentanil analgesia for aspiration of follicles for oocyte retrieval. *Anaesthesiol Reanim* 2004; 29: 69–73.
31. Goutziomitrou E, Venetis CA, Kolibianakis EM, Bosdou JK, Parlapani A, Grimbizis G, Tarlatzis BC. Propofol versus thiopental sodium as anaesthetic agents for oocyte retrieval: A randomized controlled trial. *Reprod Biomed Online* 2015; 31: 752–9.
32. Murphy A, Campbell DE, Baines D, Mehr S. Allergic reactions to propofol in egg-allergic children. *Anesth Analg* 2011; 113: 140–4.
33. Bradley A, Tober K, Brown R. Use of propofol in patients with food allergies. *Anaesthesia* 2008; 63: 439.
34. Garfield JM, Garfield FB, Stone JG, Hopkins D, Johns LA. A comparison of psychologic responses to ketamine and thiopental–nitrous oxide–halothane anesthesia. *Anesthesiology* 1972; 36: 329–38.
35. Sterzik K, Nitsch CD, Korda P et al. The effect of different anesthetic procedures on hormone levels in women. Studies during an *in vitro* fertilization–embryo transfer (IVF-ET) program. *Anaesthesist* 1994; 43: 738–42.
36. Christiaens F, Janssenswillen C, Verborgh C et al. Propofol concentration in follicular fluid during general anesthesia for transvaginal oocyte retrieval. *Hum Reprod* 1999; 14: 345–8.
37. Janssenswillen C, Christiaens F, Camu F, Van Steirteghem A. The effect of propofol on parthenogenetic activation, *in vitro* fertilization and early development of mouse oocytes. *Fertil Steril* 1997; 67: 769–74.
38. Ben–Shlomo I, Moskovich R, Golane J, Eyali V, Tabak A, Shalev E. The effect of propofol anaesthesia on oocyte fertilization and early embryo quality. *Hum Reprod* 2000; 10: 2197–9.
39. Swanson R, Leavitt M. Fertilization and mouse embryo development in the presence of midazolam. *Anesth Analg* 1992; 75: 549–54.
40. Chapineau J, Brazin JE, Terrisse MP et al. Assay for midazolam in liquor folliculi during *in vitro* fertilization under anesthesia. *Clin Pharm* 1993; 12: 770–3.
41. Jennings J, Moreland K, Petersen CM. *In vitro* fertilisation: A review of drug therapy and clinical management. *Drugs* 1996; 52: 313–43.
42. Stener-Victorin E. The pain-relieving effect of electro-acupuncture and conventional medical analgesic methods during oocyte retrieval: A systemic review of randomized controlled trials. *Hum Reprod* 2005; 20: 339–49.
43. Hayes MF, Sacco AG, Savoy-Moore RT, Magyar DM, Endler GC, Moghissi KS. Effect of general anesthesia on fertilization and cleavage of human oocytes *in vitro*. *Fertil Steril* 1987; 48: 975–81.
44. Wilhelm W, Hammadeh ME, White PF, Georg T, Fleser R, Biedler A. General anesthesia versus monitored anesthesia care with remifentanil for assisted reproductive technologies: Effect on pregnancy rate. *J Clin Anesth* 2002; 14: 1–5.
45. Coetsier T, Dhont M, De Sutter P, Merchiers E, Versichelen L, Rosseel MT. Propofol anaesthesia for ultrasound-guided oocyte retrieval: Accumulation of the anaesthetic agent in follicular fluid. *Hum Reprod* 1992; 7: 1422–4.
46. Hadimioglu N, Aydogdu Titiz T, Dosemeci L, Erman M. Comparison of various sedation regimens for transvaginal oocyte retrieval. *Fertil Steril* 2002; 78: 648–9.
47. Aghaamoo S, Azmoodeh A, Yousefshahi F et al. Does spinal analgesia have advantage over general anesthesia for achieving success in *in-vitro* fertilization? *Oman Med J* 2014; 29: 97–101.
48. Kogosowsky A, Lessing JB, Amit A, Rudick V, Peyser MR, David MP. Epidural block: A preferred method of anesthesia for ultrasonically guided oocyte retrieval. *Fertil Steril* 1987; 47: 166–8.
49. Azmude A, Agha'amou S, Yousefshahi F et al. Pregnancy outcome using general anesthesia versus spinal anesthesia for *in vitro* fertilization. *Anesth Pain Med* 2013; 3: 239–42.
50. Lehtinen AM, Laatikainen T, Koskimies AI, Hovorka J. Modifying effects of epidural analgesia or general anesthesia on the stress hormone response to laparoscopy for *in vitro* fertilization. *J In Vitro Fert Embryo Transf* 1987; 4: 23–9.
51. Botta G, D'Angelo A, D'Ari G, Merlino G, Chapman M, Grudzinskas G. Epidural anesthesia in an *in vitro* fertilization and embryo transfer program. *J Assist Reprod Genet* 1995; 12: 187–90.
52. Ng EHY, Tang OS, Chui DKC, Ho PC. Comparison of two different doses of lidocaine used in paracervical block during oocyte collection in an IVF programme. *Hum Reprod* 2000; 15: 2148–51.
53. Cerne A, Bergh C, Borg K et al. Pre-ovarian block versus paracervical block for oocyte retrieval. *Hum Reprod* 2006; 21: 2916–21.
54. Ng EHY, Tang OS, Chui DKC, Ho PC. A prospective, randomized, double-blind and placebo-controlled study to assess the efficacy of paracervical block in the pain relief during egg collection in IVF. *Hum Reprod* 1999; 14: 2783–7.

55. Corson L, Batzer FR, Gocial B et al. Is paracervical block anesthesia for oocyte retrieval effective? *Fertil Steril* 1994; 62: 133–6.
56. Andersson S, Lundberg T. Acupuncture—From empiricism to science: Functional background to acupuncture effects in health and disease. *Med Hypotheses* 1995; 45: 271–81.
57. Manheimer E, White A, Berman B, Forys K, Ernst E. Meta-analysis: Acupuncture for low back pain. *Ann Intern Med* 2005; 142: 651–63.
58. Han JS. Acupuncture: Neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neurosci* 2003; 26: 17–22.
59. Manheimer E, Zhang G, Udoff L et al. Effects of acupuncture on rates of pregnancy and live birth among women undergoing *in vitro* fertilisation: Systemic review and meta-analysis. *BMJ* 2008; 336: 545–9.
60. El-Toukhy T, Sunkara SK, Khairy M, Dyer R, Khalaf Y, Coomarasamy A. A systemic review and meta-analysis of acupuncture in *in vitro* fertilisation. *BJOG* 2008; 115: 1203–13.
61. Cheong Y, Nardo LG, Rutherford T, Ledger W. Acupuncture and herbal medicine in *in vitro* fertilisation: A review of the evidence for clinical practice. *Hum Fertil* 2010; 13: 3–12.

Medical considerations of single-embryo transfer

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OUTI HOVATTA

HEALTHIER *IN VITRO* FERTILIZATION CHILDREN FOLLOWING SINGLE-EMBRYO TRANSFER

The health of children born after *in vitro* fertilization (IVF) has been followed up since the beginning of clinical infertility treatment. In the beginning, the numbers of children were small, but then national and international registers were established. Accumulated data showed that children born as a result of IVF had a higher risk of abnormalities when compared to conventionally born children, as described in a review article by Edwards and Ludwig (1). The risk ratio of having some abnormality was 1.2–1.4 in the reviewed literature.

There are many possible causes of such abnormalities. Parental factors are the most likely ones (1), but multiple pregnancies and their consequences have appeared to be the most common.

There is now more recent information mainly confirming the earlier findings. Using a large registry-based analysis in the U.S.A., Styer et al. (2) showed that the ideal outcome of assisted reproduction, as defined by non-low birth weight singleton live births, was increased from 45% to 52% with elective single-embryo transfer (eSET). There are also recent data from Japan where SET policy resulted in extraordinary clinical success (3,4). The improvement in eSET cumulative pregnancy outcomes seen in young women could not be seen in women of advanced reproductive age (5).

RISKS OF MULTIPLE PREGNANCIES FOR THE CHILDREN

The increased risk of premature births even among twin pregnancies, not to mention triplets and higher-order multiple pregnancies, was the main cause of morbidity and mortality among IVF children. This became very clear in a Swedish nationwide analysis of all 5856 IVF children born in 1982–1995 in this country (6,7). Data regarding all the IVF children born were compared to those of the children born in the general population during the same time (1,505,724 children) using the Swedish Medical Birth Registry and the Registry of Congenital Malformations. There were 27% multiple births after IVF, but only 1% in the control population. The rate of preterm births was 30.3% among the IVF infants, while it was only 6.3% in the control population. The percentage of low-birthweight (<2500 g) infants after IVF was 27.4%, while it was 4.6% among control infants. The perinatal mortality rate was 1.9% in the IVF group and 1.1% in the controls. The high frequency of multiple births and maternal characteristics were regarded as the main factors underlying adverse outcomes, and not the IVF technique itself.

A closer analysis of the abnormalities among the IVF children was very alarming (7). From the same population of Swedish IVF children of 2060 twins out of 5680 IVF children, it became clear that the risk of a neurological diagnosis was significantly higher than that among control children. This did not differ from the risk in control twins. The most common neurological diagnosis was cerebral palsy, for which IVF children had an increased risk of 3.7 (95% confidence interval [CI]] 2.0–6.6). The risk for IVF singletons was 2.8 (95% CI 1.3–5.8). Also, the risk of developmental delay was four-fold greater among the IVF children.

After these results, Swedish IVF clinics have been allowed to transfer only one embryo at a time, or two in exceptional cases.

In Denmark, a large register study including all 8602 infants born after IVF and intracytoplasmic sperm injection between 1995 and 2000 was carried out (8). The cohort included 3438 twins and 5164 singletons. A significantly increased risk of premature delivery was found between twins and singletons. There was a 10-fold increase among all births before 37 completed weeks and a 7.4-fold increase in births before 32 completed weeks. The stillbirth rate was doubled in twins (13.1/1000) when compared to that in singletons (6.6/1000).

In addition to premature births, congenital anomalies are also more common in twin pregnancies (9). A 2.3-fold higher prevalence of major malformation (9.3%) was found among IVF infants when compared to control infants. Of the IVF infants with malformation, 70% were born from twin or triplet pregnancies (10). Congenital heart disease is more common among twins than among singletons (11,12). In the large Danish register study (8), the total malformation rate (minor and major, 73.7/100) was significantly higher than that in singletons (55.0/1000). Patent ductus arteriosus, which is typical of premature birth, was very common among twins. An increased risk of anencephaly among twin infants born after assisted reproduction was also found (13). Other perinatal complications typical of twin pregnancies also occurred in IVF twin pregnancies.

Another reason why IVF singleton pregnancies also have more growth retardation and other perinatal problems is the vanishing twin syndrome, which is actually a consequence of an IVF twin pregnancy (14–16).

Risk of premature birth is further enhanced in other conditions predisposing to prematurity, such as uterine malformations. Twin pregnancies should not be induced among individuals who have any known increased risk factor of premature deliveries.

MULTIPLE PREGNANCIES ARE ALSO RISKY FOR THE MOTHERS

Multiple pregnancies are not risk free for the mother, either. The risks of pre-eclampsia, gestational hypertension, placental abruption, and placenta previa are higher among twin pregnancies (17–19). These cause consequences for the mother's health.

Impaired glucose tolerance and pregnancy-induced diabetes (20) are more common during multiple pregnancies. A diabetic mother's pregnancy is always a high-risk condition, both for the mother and the fetus, and all possible actions should be taken to diminish such risks (21). Obesity further increases the risks of gestational diabetes and hypertension (22). Increasing the risk of diabetic complications by inducing a twin pregnancy due to transferring more than one embryo is not acceptable. For a diabetic woman, eSET is always indicated, irrespective of the embryo quality. The same relates to all other chronic disorders. A non-complicated singleton pregnancy after kidney transplantation and SET has been reported (23).

Turner's syndrome is a medical situation in which the majority of women with the condition need donated oocytes (24,25). These women often have cardiac anomalies and hypertension, and it is not justified to make these pregnancies more risky by transferring more than one embryo at a time. The most dangerous complication among Turner's syndrome women is aortic dissection, and several cases have occurred during pregnancy (26). In Turner's syndrome, only eSET can be accepted.

Oocyte donation is a situation in which the pregnancy rates are relatively high. eSET gives excellent pregnancy rates in oocyte donation (27,28). eSET is further motivated in this group of women because they have an increased risk of hypertension in pregnancy (29). The likelihood of operative delivery with possible complications is higher in multiple pregnancies (8), which is another maternal indication not to transfer more than one embryo at a time (30).

eSET AS A METHOD TO AVOID MULTIPLE PREGNANCIES

During the early days of IVF, the evolving embryo culture techniques did not allow high pregnancy rates. In early statistics, the pregnancy rate per SET was clearly lower than that after transfer of more than one embryo. This urged clinicians to try to improve the treatment results by transfer of multiple embryos. The consequence was the well-known increase in multiple pregnancies. However, pediatricians who saw the increase in complications caused by this new epidemic of multiple births warned the IVF community relatively early. They were particularly active in Northern Europe. In Finland, we started eSETs in the mid-1990s. We then analyzed the pregnancy rates after SET during one year in two IVF units in Helsinki (31). These results very clearly demonstrated the difference between non-eSET and eSET. If only one embryo was available for transfer, the pregnancy rate was 20% per transfer, but if an eSET was carried out in this non-selected patient population, a pregnancy rate of 29.7% was achieved. This was similar to that in two-embryo transfer (29.4%). That eSET results in much better pregnancy rates than non-eSET cases and that such a pregnancy rate is similar to that achieved in the transfer of two embryos when top- or good-quality embryos were available was soon demonstrated in two prospective randomized trials. Gerris et al. (32) randomized 53 couples with a female partner aged <34 years and who had at least two top-quality embryos for eSET or two-embryo transfer. The pregnancy rate after eSET was 42.3%, and the pregnancy rate among the couples with double-embryo transfer was 48.1%, with 30% twins. We carried out a multicenter study in Finland (33) in which 144 couples with good-quality embryos were randomized to eSET or double-embryo transfer. The pregnancy rate per transfer was 32.4% in the eSET group and 47.1% in the double-embryo transfer group. The difference was not statistically significant. The cumulative pregnancy rates after frozen embryo transfers were 47.3% in the eSET group and 58.6% in the double-embryo transfer group. After double-embryo transfer, there were 39% twins.

A large North European multicenter study was then carried out (34). Couples where the female partner was younger than 36 years of age and who had at least two good-quality embryos were randomized to receiving either two embryos or first a single fresh embryo and then a frozen–thawed single embryo. A pregnancy resulting in at least one live birth was encountered in 142 of the 331 women (42.9%) who received two fresh embryos, and in 128 women (38.8%) who received first one fresh and then one frozen embryo. The pregnancy rate after SETs was lower, but not substantially lower than that after double-embryo transfer. However, there was a highly significantly ($p < 0.001$) lower twinning rate after SET (0.8%) compared to that after double-embryo transfer (33.1%).

Pregnancy outcome was not affected when blastocysts were transferred, as shown in an analysis of couples undergoing single-blastocyst transfer ($n = 52$, live birth rate 53.8%) or double-blastocyst transfer ($n = 187$, live birth rate 54.4%). In single-blastocyst transfer, the twin rate was 3.1%, while it was 51% in double-blastocyst transfer (35).

Effective and active cryopreservation policy, particularly in connection with SET, increases pregnancy rates per oocyte retrieval (36). eSET also reduces multiple pregnancies in frozen embryo transfers (37).

eSET has proved to be a method by which multiple pregnancies can be reduced without decreasing overall pregnancy rates (38). In real life, it has not only been equally effective as double-embryo transfer, but has also been economically substantially cheaper than double-embryo transfer with all its complications (39,40). In a non-selected population, SET has resulted in a lower pregnancy rate than double-embryo transfer. Hence, some criteria have been used to select couples for eSET (41). Effective cryopreservation methods have probably greatly reduced the need for strict selection (42,43). All of the obtained embryos can be transferred anyway, but a longer time may be required for achieving a pregnancy.

There are now data available in this area from Sweden and Belgium. These countries have laws and guidelines

for regular SET, and the pregnancy rates have remained similar in these countries, while the rate of multiple pregnancies has dropped significantly (44,45). After the publication of the Swedish data, which cover all of the cycles in Sweden, a similar follow-up has continuously been collected by the Swedish authorities, and the pregnancy rate has remained constant during the many years of SET use, and the twin rate has remained at approximately 5%. At the same time, the proportions of children weighing less than 2500 g or born prematurely have significantly decreased, hence bringing about better health for the infants (45). In addition, the costs of assisted reproduction technology (ART) using SET are lower, as shown in a recent national survey in the U.S.A. (46). Sequential SETs, when clinically appropriate, can reduce total ART treatment and pregnancy/infant-associated medical costs by reducing multiple births without lowering live birth rates.

OPTIMIZING CRITERIA FOR SET

To achieve equal pregnancy rates within the same time period for SETs and double-embryo transfers, some kind of selection has to be made in order to balance the likelihood of pregnancy and to minimize the risk of twins (41). The criteria presented in all the above-mentioned articles include the age of the female partner, the number of earlier unsuccessful cycles, and embryo quality. In Sweden, these criteria have in most clinics become stricter during the period in which SET has been practiced. The National Board of Health and Welfare in Sweden has collected statistics from all IVF clinics (45), and the units by themselves have agreed upon these criteria, using which a twin rate of <5% and an unchanged pregnancy rate can be achieved. The recommended criteria for eSET at that time were: the age of the woman <40 years; not more than three failed ART cycles; and at least two good-quality embryos. Regarding cleavage-stage embryos, the criteria are: less than 20% fragmentation; the embryo fills an even zona; no multinuclear blastomeres; four cells on day 2; and eight cells on day 3. An exception to one of these parameters still counts as a good-quality embryo. Early cleavage is an additional sign recommending SET. At the blastocyst stage, an expanded blastocyst on day 5 is of good quality. In frozen embryo transfers, intact embryo after thawing indicates SET. However, there are now also more advanced systems for embryo selection. A computer-assisted scoring system proved to be better than the conventionally used one (47). Using gene expression or biochemical information from cumulus cells may become more widely used (48,49). Glucose consumption by an embryo may be measured (50). Sequential fresh and frozen embryo transfers increase the rate of pregnancy per transfer (43).

CONCLUSIONS

Multiple pregnancies, including twins, lead to high risks for the health of the infant and the mother. These risks are largely caused by the high incidence of premature birth, but also congenital anomalies are increased among twins. Twin pregnancies can be almost completely avoided by carrying out eSET. Even though there are no substantial differences in cumulative pregnancy rates between the first fresh and the following frozen–thawed embryos transferred one at a time, or following the same process with frozen transfer in spite of embryo quality, many teams still search for optimal quality of embryos in order to give the highest possible pregnancy rate as soon as possible. Methods such as time-lapse follow-up in embryo selection (51) and measurement of serum concentration of human chorionic gonadotropin-β (52) are helpful in selecting the best embryo for transfer. The earlier results have been confirmed by many recent surveys in Europe, Japan, and the U.S.A. (2–4,49–54).

A twin rate of <5% can be achieved if a single good-quality embryo is transferred to a woman under <40 years of age during her three first ART cycles (53) who has at least two good-quality embryos, and two others for the rest. However, the cumulative pregnancy rate among women over 40 years of age is actually similar if she cumulatively receives all of her embryos either one or two at a time. In addition, if the embryo is intact after thawing, a single frozen embryo should be transferred.

Irrespective of the woman's age, number of earlier cycles, or embryo quality, a SET should be carried out in situations with a known high risk of premature birth, such as uterine abnormalities. The same relates to maternal contraindications for multiple pregnancies, such as diabetes, hypertension, or Turner's syndrome (55).

REFERENCES

1. Edwards RG, Ludwig M. Are major defects in children conceived *in vitro* due to innate problems in patients or to induced genetic damage? *Reprod Biomed Online* 2003; 7: 131–82.
2. Styer AK, Luke B, Vitek W, Christianson MS, Baker VL, Christy AY, Polotsky AJ. Factors associated with the use of elective single-embryo transfer and pregnancy outcomes in the United States, 2004–2012. *Fertil Steril* 2016; 106: 80–9.
3. Morimoto Y. Extraordinary clinical success of a single-embryo transfer policy. What comes next? *Fertil Steril* 2016; 105: 302–3.
4. Takeshima K, Jwa SC, Saito H, Nakaza A, Kuwahara A, Ishihara O, Irahara M, Hirahara F, Yoshimura Y, Sakumoto T. Impact of single embryo transfer policy on perinatal outcomes in fresh and frozen cycles—Analysis of the Japanese Assisted Reproduction Technology registry between 2007 and 2012. *J Assist Reprod Genet* 2015; 32: 1773–9.
5. Fujimoto A, Morishima K, Harada M, Hirata T, Osuga Y, Fujii T. Elective single-embryo transfer improves cumulative pregnancy outcome in young patients but not in women of advanced reproductive age. *J Assist Reprod Genet* 2015; 32: 1773–9.
6. Bergh T, Ericson A, Hillensjö T, Nygren KG, Wennerholm UB. Deliveries and children born after *in-vitro* fertilisation in Sweden 1982–95: A retrospective cohort study. *Lancet* 1999; 354: 1579–85.

7. Strömberg B, Dahlquist G, Ericson A, Finnström O, Köster M, Stjernqvist K. Neurological sequelae in children born after *in-vitro* fertilisation: A population-based study. *Lancet* 2002; 359: 461–5.
8. Pinborg A, Loft A, Nyboe Andersen A. Neonatal outcome in a Danish national cohort of 8602 children born after *in vitro* fertilization or intracytoplasmic sperm injection: The role of twin pregnancy. *Acta Obstet Gynecol Scand* 2004; 83: 1071–8.
9. Sperling L, Kiil C, Larsen LU et al. Detection of chromosomal abnormalities, congenital abnormalities and transfusion syndrome in twins. *Ultrasound Obstet Gynecol* 2007; 29: 517–26.
10. Merlob P, Sapir O, Sulkes J, Fisch B. The prevalence of major congenital malformations during two periods of time, 1986–1994 and 1995–2002 in newborns conceived by assisted reproduction technology. *Eur J Med Genet* 2005; 48: 5–11.
11. Caputo S, Russo MG, Capozzi G et al. Congenital heart disease in a population of dizygotic twins: An echocardiographic study. *Int J Cardiol* 2005; 102: 293–6.
12. Hajdu J, Beke A, Marton T et al. Congenital heart diseases in twin pregnancies. *Fetal Diagn Ther* 2006; 21: 198–203.
13. Ben-Ami I, Vaknin Z, Reish O et al. Is there an increased rate of anencephaly in twins? *Prenat Diagn* 2005; 25: 1007–10.
14. Spellacy WN. Antepartum complications in twin pregnancies. *Clin Perinatol* 1988; 15: 79–86.
15. Pinborg A, Lidegaard O, Freiesleben NC, Andersen AN. Vanishing twins: A predictor of small-for-gestational age in IVF singletons. *Hum Reprod* 2007; 22: 2707–14.
16. Sazonova A, Källen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Factors affecting obstetric outcome of singletons born after IVF. *Hum Reprod* 2011; 26: 2878–86.
17. Duckitt K, Harrington D. Risk factors for pre-eclampsia at antenatal booking: Systematic review of controlled studies. *BMJ* 2005; 330: 565.
18. Erez O, Vardi IS, Hallak M et al. Preeclampsia in twin gestations: Association with IVF treatments, parity and maternal age. *J Matern Fetal Neonatal Med* 2006; 19: 141–6.
19. Allen VM, Wilson RD, Cheung A, Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC), Reproductive Endocrinology Infertility Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC). Pregnancy outcomes after assisted reproductive technology. *J Obstet Gynaecol Can* 2006; 28: 220–50.
20. Adler Levy Y, Lunenfeld E, Levy A. Obstetric outcome of twin pregnancies conceived by *in vitro* fertilization and ovulation induction compared with those conceived spontaneously. *Eur J Obstet Gynecol Reprod Biol* 2007; 133: 173–8.
21. Hod M, Jovanovic L. Improving outcomes in pregnant women with type 1 diabetes. *Diabetes Care* 2007; 30: e62.
22. Dokras A, Baredziak L, Blaine J, Syrop C, VanVoorhis BJ, Sparks A. Obstetric outcomes after *in vitro* fertilization in obese and morbidly obese women. *Obstet Gynecol* 2006; 108: 61–9.
23. Nouri K, Bader Y, Helmy S et al. Live birth after *in vitro* fertilization and single embryo transfer in a kidney transplant patient: A case report and review of the literature. *J Assist Reprod Genet* 2011; 28: 351–3.
24. Foudila T, Söderström-Anttila V, Hovatta O. Turner's syndrome and pregnancies after oocyte donation. *Hum Reprod* 1999; 14: 532–5.
25. Hovatta O. Pregnancies in women with Turner's syndrome. *Ann Med* 1999; 31: 106–10.
26. Bondy CA. Care of girls and women with turner syndrome: A guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab* 2007; 92: 10–25.
27. Söderström-Anttila V, Vilska S, Mäkinen S, Foudila T, Suikkari AM. Elective single embryo transfer yields good delivery rates in oocyte donation. *Hum Reprod* 2003; 18: 1858–63.
28. Söderström-Anttila V, Vilska S. Five years of single embryo transfer with anonymous and non-anonymous oocyte donation. *Reprod Biomed Online* 2007; 15: 428–33.
29. Söderström-Anttila V, Tiitinen A, Foudila T, Hovatta O. Obstetric and perinatal outcome after oocyte donation: Comparison with *in-vitro* fertilization pregnancies. *Hum Reprod* 1998; 13: 483–90.
30. De Sutter P. Single embryo transfer (SET) not only leads to a reduction in twinning rates after IVF/ICSI, but also improves obstetrical and perinatal outcome of singletons. *Verh K Acad Geneeskd Belg* 2006; 68: 319–27.
31. Vilska S, Tiitinen A, Hydén Granskog C, Hovatta O. Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. *Hum Reprod* 1999; 14: 2392–5.
32. Gerris J, De Neubourg D, Mangelschots K et al. Prevention of twin pregnancy after *in-vitro* fertilization or intracytoplasmic sperm injection based on strict embryo criteria: A prospective randomized clinical trial. *Hum Reprod* 1999; 14: 2581–7.
33. Martikainen H, Tiitinen A, Tomás C et al. One versus two embryo transfer after IVF and ICSI: A randomized study. *Hum Reprod* 2001; 16: 1900–3.
34. Thurin A, Hausken J, Hillensjö T et al. Elective single-embryo transfer versus double-embryo transfer in *in vitro* fertilization. *N Engl J Med* 2004; 351: 2392–402.
35. Styer AK, Wright DL, Wolkovich AM, Veiga C, Toth TL. Single-blastocyst transfer decreases twin gestation without affecting pregnancy outcome. *Fertil Steril* 2007; 330: 549–50.
36. Tiitinen A, Hydén-Granskog C, Gissler M. What is the most relevant standard of success in assisted reproduction? The value of cryopreservation on

cumulative pregnancy rates per single oocyte retrieval should not be forgotten. *Hum Reprod* 2004; 19: 2439–41.
37. Hydén Granskog C, Unkila KL et al. Single embryo transfer is an option in frozen embryo transfer. *Hum Reprod* 2005; 20: 2935–8.
38. Tiitinen A, Unkila Kallio L et al. Impact of elective single embryo transfer on the twin pregnancy rate. *Hum Reprod* 2003; 18: 1449–53.
39. Gerris J, De Sutter P, De Neubourg D et al. A real-life prospective health economic study of elective single embryo transfer versus two-embryo transfer in first IVF/ICSI cycles. *Hum Reprod* 2004; 19: 917–23.
40. Fiddelers AA, Severens JL, Dirksen CD et al. Economic evaluations of single- versus double-embryo transfer in IVF. *Hum Reprod Update* 2007; 13: 5–13.
41. van Montfoort AP, Fiddelers AA, Land JA et al. eSET irrespective of the availability of a good-quality embryo in the first cycle only is not effective in reducing overall twin pregnancy rates. *Hum Reprod* 2007; 22: 1669–74.
42. Sundström P, Saldeen P. Cumulative delivery rate in an *in vitro* fertilization program with a single embryo transfer policy. *Acta Obstet Gynecol Scand* 2009; 88: 700–6.
43. Veleva Z, Karinen P, Tomás C, Tapanainen JS, Martikainen H. Elective single embryo transfer with cryopreservation improves the outcome and diminishes the costs of IVF/ICSI. *Hum Reprod* 2009; 24: 1632–9.
44. Van Landuyt L, Verheyen G et al. New Belgian embryo transfer policy leads to sharp decrease in multiple pregnancy rate. *Reprod Biomed Online* 2006; 13: 765–71.
45. Karlström PO, Bergh C. Reducing the number of embryos transferred in Sweden-impact on delivery and multiple birth rates. *Hum Reprod* 2007; 22: 2202–7.
46. Crawford S, Boulet SL, Mneimneh AS, Perkins KM, Jamieson DJ, Zhang Y, Kissin DM. Costs of achieving live birth from assisted reproductive technology: A comparison of sequential single and double embryo transfer approaches. *Fertil Steril* 2016; 105: 444–50.
47. Paternot G, Debrock S, D'Hooghe T, Spiessens C. Computer-assisted embryo selection: A benefit in the evaluation of embryo quality? *Reprod Biomed Online* 2011; 23: 347–54.
48. Assou S, Haouzi D, De Vos J, Hamamah S. Human cumulus cells as biomarkers for embryo and pregnancy outcomes. *Mol Hum Reprod* 2010; 16: 531–8.
49. Gebhardt KM, Feil DK, Dunning KR, Lane M, Russell DL. Human cumulus cell gene expression as a biomarker of pregnancy outcome after single embryo transfer. *Fertil Steril* 2011; 96: 47–52.
50. Gardner DK, Wale PL, Collins R, Lane M. Glucose consumption of single post-compaction human embryos is predictive of embryo sex and live birth outcome. *Hum Reprod* 2011; 26: 1981–6.
51. Bodri D, Kawachiya S, Sugimoto T, Yao Serna J, Kato R, Matsumoto T. Time-lapse variables and embryo gender: A retrospective analysis of 81 live births obtained following minimal stimulation and single embryo transfer. *J Assist Reprod Genet* 2016; 33: 589–96.
52. Keane KN, Mustafa KB, Hinchliffe P, Conceicao J, Yovich JL. Higher β-HCG concentrations and higher birthweights ensue from single vitrified embryo transfers. *Reprod Biomed Online* 2016; 33: 149–60.
53. Heino A, Gissler M, Hindori-Mohangoo AD et al., Euro-Peristat Scientific Committee. Variations in multiple birth rates and impact on perinatal outcomes in Europe. *Fertil Steril* 2016; 105: 444–50.
54. Gleicher N, Kushnir VA, Barad DH. Risks of spontaneously and IVF-conceived singleton and twin pregnancies differ, requiring reassessment of statistical premises favoring elective single embryo transfer (eSET). *Reprod Biol Endocrinol* 2016; 14: 25.
55. Chen F, De Neubourg D, Debrock S, Peeraer K, D'Hooghe T, Spiessens C. Selecting the embryo with the highest implantation potential using a data mining based prediction model. *Reprod Biol Endocrinol* 2016; 14: 10.

Endometriosis and assisted reproduction technology

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INTRODUCTION

Endometriosis, as a clinical entity, has been recognized and intensely investigated for well over 100 years. Despite the accumulation of an enormous amount of information, uncertainty still exists regarding etiologies, clinical consequences, and treatment efficacy. The two most common complaints leading to a diagnosis of endometriosis are pelvic pain and infertility. The advancement of innovative medical and surgical approaches, such as gonadotropin-releasing hormone (GnRH) agonists and laparoscopically guided laser ablation, have proven quite effective at improving many of the symptoms associated with endometriosis. It does appear that assisted reproduction technology (ART) is becoming an indispensable asset in providing affected couples with viable pregnancies, and with the accumulation of randomized trials, the role of GnRH agonist and antagonist protocols is becoming clear.

ENDOMETRIOSIS AND INFERTILITY

There is little debate that the extensive anatomical distortion and tubal obstruction frequently attributed to severe endometriosis does impair fertility. Less clear is the reported association between minimal or mild endometriosis and infertility in the absence of any mechanical disruption. Although there is no conclusive evidence that minimal to moderate endometriosis actually causes infertility, several studies dating back to the 1930s have suggested that there is at least an association between the two (1). In the 1970s, three studies retrospectively compared the incidence of endometriosis in women undergoing laparoscopy for infertility or voluntary sterilization (2–4). The incidences of endometriosis ranged from 21% to 48% in infertile women, while endometriosis was noted in only 1.3%–5% of fertile women undergoing tubal ligation. More recent studies (5,6) including one prospective investigation (7) have demonstrated that among women undergoing insemination with donor sperm due to severe male factor infertility, those with coexisting endometriosis had markedly fewer conceptions per exposure than women who did not have the disease. Another recent prospective double-blind study (8), which looked specifically at women with mild endometriosis compared to women without endometriosis, was able to show a trend toward higher pregnancy rates in women without the disease. The results, however, did not reach statistical significance. This may be attributable to the fact that the number of patients enrolled did not meet the study's power calculation.

Although the above studies were methodologically imperfect and far from conclusive, virtually every area within the reproductive process has been intensely investigated in an attempt to describe a causal relationship between endometriosis and infertility. The results of several tangential lines of investigation have added to the confusion, as studies are frequently in direct contradiction to one another. Investigators have suggested that women with mild to moderate endometriosis have a higher incidence of endocrine abnormalities (9), anovulation (10), corpus luteum insufficiency (11), hyperprolactinemia (12), luteinized unruptured follicle syndrome (13), and spontaneous abortions (14). However, other well-organized, prospective studies have found most of these factors to be either normal or lacking in clinical significance (15–20).

Immune dysfunction in endometriosis has become the focus of more recent efforts, as it is hypothesized that immunity plays a role in the pathogenesis of the disease. Several immunologic abnormalities, which could potentially impair fertility, have been identified. Researchers have reported increased B-cell activity, with the production of specific antibodies against endometrial antigens, T-cell and macrophage dysfunction, and nonspecific polyclonal B-cell activation, which may negatively impact implantation (15,21). There has been evidence to suggest peritoneal fluid in patients suffering from endometriosis may be compromised by inflammatory mediators, which may negatively impact the fertilization of released oocytes (22). Recent evidence has shown that these cytokines and eicosanoids may impact sperm motility (23), sperm function (24), and even interaction between sperm and oocyte (25). As with other factors, many conflicting reports have emerged. Furthermore, it is not at all clear which is the cause and which the effect, or what role each abnormality actually plays in the pathogenesis of endometriosis-associated infertility.

Many investigators have proposed that endometriosis is actually caused by interplay between environmental and genetic factors. Many have also suggested that certain genetic polymorphisms associated with endometriosis could predispose a woman to infertility. In a review of the advances in the genetics of endometriosis, Dun et al. (26) reviewed the most commonly studied genes thought to be associated with endometriosis. Over 18 genes were implicated, with most relating to xenobiotic metabolism, steroid action and receptors, and inflammatory and angiogenic factors.

A direct association between these genetic polymorphisms and endometriosis-associated infertility has yet to be shown though.

Previous studies using magnetic resonance imaging of the uterus in patients with endometriosis have demonstrated up to a 90% prevalence rate of adenomyotic lesions in those patients with established pelvic endometriosis. This association between endometriosis and adenomyosis may also contribute to the infertility seen in these patients, particularly those with severe disease (27).

As stated, one argument that has been proposed against a causal relationship between endometriosis and infertility is the outright failure of medical or surgical treatment to significantly improve pregnancy success in these patients. The use of medical treatments, otherwise successful in alleviating the non-reproductive symptoms of endometriosis, has failed to demonstrate a reasonable improvement in fertility (28). Most studies investigating the effect of surgical ablation of endometriotic lesions, by any one of a number of techniques, have failed to show increased fecundity. One randomized study, however, did show an improved rate of pregnancy for women with minimal/mild endometriosis treated with ablation of endometriotic lesions, when compared with a control group receiving diagnostic laparoscopy alone (29). However, this study has been criticized for having a lower fecundity rate among untreated patients than would normally be expected, for notifying patients of their treatment status, and for following pregnancies to only 20 weeks. Subsequently, another randomized study, which looked at actual birth rates, failed to demonstrate a reproductive benefit for patients whose lesions were ablated, but had lower power than the first study (30). When the results were combined, no significant statistical heterogeneity was noted and the increased chance of achieving pregnancy after surgery was found to be only 8.6% (95% confidence interval [CI] 2.1%–15.0%) (31). Thus, surgically ablating visible endometriosis lesions only potentially benefits pregnancy outcomes minimally.

With regard to endometriomas, a recent Cochrane review of four trials concluded that surgery (aspiration or cystectomy) versus expectant management showed no evidence of a benefit for clinical pregnancy with either technique (32).

OVULATION INDUCTION AND INSEMINATION

Controlled ovarian stimulation (COS), in combination with intrauterine insemination (IUI), has proven to be a cost-effective and appropriate first-line treatment for many infertility diagnoses (33). However, it is not entirely clear that this approach is as effective for patients with endometriosis. Deaton et al. (34) demonstrated increased fecundity in patients treated with clomiphene citrate and IUI. However, Fedele et al. (35) reported that the increased conception rate with COS and IUI did not lead to a significantly different pregnancy rate at six months. Furthermore, a retrospective comparison of COS and IUI reported per-cycle pregnancy rates of 6.5%, 11.8%, and 15.3% for endometriosis, male factor, and unexplained infertility, respectively (36). Similarly, although with more optimistic results, a prospective, observational study reported pregnancy rates of 16.3% and 33.6% following COS/IUI in patients with endometriosis and unexplained infertility, respectively (37). In a meta-analysis, Hughes (38) reported that a diagnosis of endometriosis decreased the per-cycle COS/IUI conception rate by half. Also, a later prospective, randomized study reported live birth rates of 11% and 2% for endometriosis patients undergoing COS/IUI and no treatment, respectively (39). While this demonstrates a live birth odds ratio (OR) of 5.5 for the treatment group, the actual percentage of live births after treatment remains relatively low. Failure of COS/IUI has recently been correlated with advanced endometriosis. A retrospective study of 92 patients found that more than a third of patients failing to conceive after four ovulatory cycles of clomiphene citrate had stage III or IV disease, an endometrioma, pelvic adhesions, and/or tubal disease (40). Most recently, however, a retrospective, controlled cohort study of 259 COS/IUI cycles found no difference in cycle pregnancy rate and cumulative live birth rate between women with surgically treated minimal to mild endometriosis and women with unexplained infertility, indicating potentially improved outcomes after surgical treatment (41).

The advent of aromatase inhibitors has added to the armamentarium of therapeutic modalities for the treatment of endometriosis. With its efficacy in treating endometriosis-associated pain, more formally established studies (42,43) are underway to evaluate its utility in ovulation induction. Wu et al. (44) found that a third-generation aromatase inhibitor was able to achieve a reasonable pregnancy rate, with a thicker endometrium but fewer ovulatory follicles, when randomized and compared with clomiphene citrate. However, a recent study by Abu Hashim et al. showed no significant difference in the clinical pregnancy rate per cycle in groups randomized to receive either letrozole (a third-generation aromatase inhibitor) or clomiphene citrate for controlled ovarian hyperstimulation (15.9% for letrozole and 14.5% for clomiphene citrate) (45).

The use of GnRH antagonists in IUI cycles with COS has also been studied. A recent randomized, double-blinded, placebo-controlled trial showed no difference in live birth rates for women with minimal or mild endometriosis when comparing women who were treated with GnRH antagonist to those who received a placebo (46).

ENDOMETRIOSIS AND ART

Treatment strategies for the infertile couple must be based on the specific situation. For young women with only minimal or mild endometriosis, expectant management may be the most appropriate course. However, for women approaching the end of their reproductive age, the chances of conceiving drop precipitously. In these women, intervention, in the form of COS/IUI or *in vitro* fertilization (IVF), may be warranted more expeditiously (47). The lower cost and low complication rate of ovulation induction and IUI make the combination an attractive first step. However, for women with severe endometriosis or tubal disease, or when male factor or a combination of etiologies is involved,

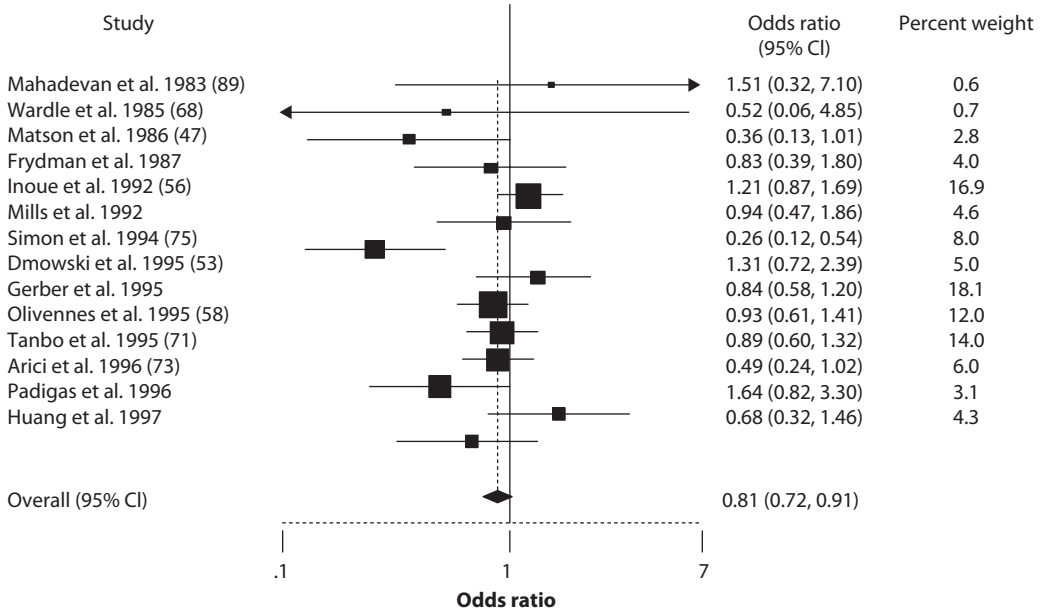

Figure 59.1 Unadjusted meta-analysis of the odds of pregnancy in endometriosis patients versus tubal factor controls. (From Barnhart K et al. *Fertil Steril* 2002; 77: 1148–55.)

assisted reproduction such as IVF may be pursued sooner. In addition, IVF offers the added benefit of being able to directly observe key events in the conception process, such as the assessment of gamete quality, the observation of fertilization, and the evaluation of early embryo development. As a result, the increasing use of ART in the treatment of endometriosis-associated infertility may help to answer some of the questions regarding this elusive association. It is thought that the use of IVF–embryo transfer (ET) in the infertile patient with endometriosis removes critical steps in reproduction, such as fertilization and early embryo development, from an *in vivo* environment that some have suggested is hostile to these processes. Thus, it has been anticipated that endometriosis patients will have IVF outcomes approaching those of other infertility etiologies. Recent studies, however, confirm that endometriosis patients, particularly those with moderate to severe disease, had lower pregnancy rates (48,49). Certainly, the development of GnRH agonists and transvaginal oocyte retrieval has been associated with increased success in the use of IVF for endometriosis-associated infertility. However, the value of reported ART results must be considered along with the understanding that there is great clinical and laboratory variability among centers, leading to a wide range of reported pregnancy rates. Furthermore, most studies are retrospective and observational and are therefore of limited value in reaching definitive conclusions regarding therapy efficacy. Barnhart et al. (50) performed a meta-analysis on the studies evaluating the effects of endometriosis on the outcomes of ARTs. They evaluated a total of 22 articles and concluded that, overall, patients with endometriosis had lower pregnancy rates, decreased fertilization and implantation rates, and a decreased number of oocytes retrieved compared to controls of tubal factor infertility (Figure 59.1). A more recent meta-analysis looking at the association between endometriosis and ART outcomes, which assessed results from 36 studies, found that when compared to women without endometriosis, those with the disease had lower clinical pregnancy rates per patient (OR 0.78, 95% CI 0.65–0.94) and lower mean numbers of oocytes retrieved per cycle (mean difference −1.98, 95% CI −2.87 to −1.09); however, they found similar live birth rates (OR 0.94, 95% CI 0.84–1.06) and therefore comparable success with IVF/intracytoplasmic sperm injection (ICSI) (51).

In a recent review article on the treatment of infertility associated with deep endometriosis, the authors looked at six studies that investigated the outcomes of IVF in patients with severe endometriosis. They found that the pregnancy rate per patient varied between 29% and 68%, with an aggregated rate per patient of 51% (95% CI 45%–56%) (52).

COS and oocyte retrieval

As the practice of assisted reproduction has evolved over the past three decades, so has the efficacy of IVF in the treatment of endometriosis. With regard to the effect of endometriosis on COS and oocyte retrieval, an obvious divide exists between earlier studies using clomiphene citrate with laparoscopic oocyte retrieval and more recent investigations benefiting from the development of GnRH agonists and ultrasound-guided transvaginal retrieval. Earlier studies did in fact report a reduced oocyte yield in patients with endometriosis undergoing IVF. In one small study, Chillik et al. (53) compared patients with either no endometriosis, mild to moderate endometriosis, or severe disease, and reported that oocyte yield was reduced in those patients of advanced stage. Oehninger et al. (54) reported a similar effect on oocyte retrieval for patients with stage III or IV

endometriosis. Both studies suggested that oocyte yield was impaired in this group of patients due to technical difficulties at the time of laparoscopic oocyte retrieval. Alternatively, other researchers have reported decreased folliculogenesis in patients with endometriosis (55–58). Furthermore, Dlugi et al. (59) and, more recently, Somigliana et al. (60) reported a significantly lower number of preovulatory follicles in patients with endometriomas when compared to patients with hydrosalpinges. Additionally, a recent review suggests that endometriomas may have deleterious effects on folliculogenesis and oocyte quality, independent of stretching/mass effect by the cyst (61).

Several contemporary studies utilizing GnRH agonists and transvaginal retrieval have not confirmed that endometriosis has a significant effect on oocyte yield. Dmowski et al. (62) retrospectively analyzed 237 IVF cycles and found no difference in either folliculogenesis or in the number of oocytes obtained for women with or without endometriosis. In a case–control study comparing 65 cycles of IVF for women with endometriosis to 98 cycles of IVF in patients with tubal infertility, Bergendal et al. (63) found no difference in folliculogenesis or oocyte retrieval. Several recent studies have further concluded that there is no difference in the number of oocytes obtained in patients with mild to moderate endometriosis when compared to patients with more severe disease (64–67). Barnhart et al. demonstrated a lower number of oocytes retrieved (OR 0.82, 95% CI 0.75–0.90) for patients with endometriosis when compared to patients with tubal factor (50).

The improvement in IVF outcomes brought about by the development of GnRH agonists is largely undisputed.

Olivennes et al. (67) reported a significantly improved clinical pregnancy rate for patients treated with GnRH agonists when compared with standard, gonadotropin-only ovarian stimulation protocols (Figure 59.1). Other investigations have reported similar results (68). Long-term GnRH agonist suppression has been thought to repress further endometriotic lesions and improve IVF outcome for patients with endometriosis. Dicker and associates (69), as well as Rickes et al. (70), reported a significantly higher clinical pregnancy rate after six months of GnRH agonist therapy compared with ovarian stimulation with gonadotropins alone. Chedid et al. (71) also investigated the use of a three-month and a three-week GnRH agonist down-regulation protocol and reported a significantly increased oocyte yield when compared with controls receiving only gonadotropins. Although they noted an improved pregnancy rate, it did not reach statistical significance.

Nakamura et al. (72) compared GnRH agonist suppression for 60 days with a shorter, mid-luteal down-regulation prior to ovulation induction. They reported pregnancy rates of 67% and 27% for the longer and shorter protocols, respectively. Marcus and Edwards (73) also reported a significantly higher pregnancy rate for patients treated with longer GnRH agonist protocols (Table 59.1) (53–56,62–67,74–77), although they used different GnRH agonists for the two groups and assigned patients based on their refusal to accept the longer regimen. Surrey et al. (78) investigated a three-month course of GnRH agonist therapy prior to IVF–ET and found the agonist therapy to be associated with a significantly higher ongoing pregnancy rate. Conversely, Chedid et al. (71) found no difference between long and short GnRH agonist administrations.

The use of continuous oral contraceptive pills prior to assisted reproduction treatment has also been examined. de Ziegler et al. (79) found that six to eight weeks of continuous use of oral contraceptive pills before IVF–ET for patients with endometriosis had similar outcomes to age-matched controls without endometriosis.

Recent studies have also analyzed the use of GnRH antagonist protocols for IVF in patients with endometriosis. A recent prospective randomized trial compared GnRH agonist and antagonist protocols for women with mild to moderate endometriosis (80). This study showed similar implantation and clinical pregnancy rates for patients treated with both GnRH agonist and antagonist protocols. Patients treated with a GnRH agonist, however, had a significantly higher number of additional embryos available for cryopreservation, making the cumulative fecundity rate higher with the agonist protocol.

For now, it appears that endometriosis patients respond to ovarian stimulation in a manner that is similar to other infertility etiologies. Although standard gonadotropin stimulation protocols work reasonably well, the addition of longer GnRH agonist down-regulation or the use of continuous oral contraceptive pills may increase IVF success and should be considered on a case-by-case basis.

Fertilization and early embryo development

It is unclear as to the degree to which endometriosis is a detriment to the process of fertilizing oocytes *in vitro*, as several investigations have now reported significantly impaired fertilization rates for these patients. One early study noted fertilization rates per oocyte of 33%, 63%, and 68% for patients with endometriosis, unexplained infertility, and tubal infertility, respectively (75), while another reported a marked impairment in fertilization with the presence of an endometrioma (59). Bergendal et al. (63) reported fertilization rates of 60% and 78% for patients with endometriosis and tubal factor, respectively ($p < 0.0001$). Other investigators have reported significantly lower fertilization success for stage III or IV endometriosis when compared with stage I or II endometriosis (66,67). With regard to early embryo development, researchers have reported fewer embryos reaching the four-cell stage at 48 hours (81), a reduced number of blastomeres at 72 hours (82), and lower cleavage rates when endometriosis is compared with tubal factor or unexplained infertility (83). Furthermore, Brizek et al. (84) retrospectively analyzed video records of 235 embryos and found a statistically significant increase in the incidence of aberrant nuclear and cytoplasmic morphology within embryos from patients with endometriosis.

Conversely, there have been several large studies that have failed to detect an impairment in fertilization. Dmowski et al. (62) analyzed 237 cycles and found no difference in either the fertilization rate or the early cleavage rate among

Table 59.1 Comparison of *in vitro* fertilization–embryo transfer outcomes for women with and without endometriosis

Study	Group	Number of cycles	Clinical pregnancies (% cycle)	Study	Group	Number of cycles	Clinical pregnancies (% cycle)
Mahadevan (74)	I–IV	14	14	Inoue (65)	I	111	40
	Tubal	261	10		II	78	42
Wardle (75)	I–IV	17	6		III	51	47
	Tubal	47	11		IV	69	42
					Other	372	44
Chillik (53)	I/II	10	60		I–IV	360	29
	III/IV	14	7	Olivennes (67)	Tubal	160	36
Matson (55)	I	24	13				
	II	37	14	Geber (64)	I/II	100	29
	III	36	6		III/IV	29	52
	VI	57	2		Tubal	1139	41
	Tubal	40	18	Dmowski (62)	I/II	89	25
Sharma (76)	I/II	135	16		III/IV	30	30
	III/IV	141	8		Other	118	21
	Tubal	994	13	Arici (77)	I/II	43	12
Oehninger (54)	I/II	191	24		III/IV	46	15
	III/IV	35	20		Tubal	147	24
Yovich (56)	I/II	61	13	Bergendal (63)	I–IV	65	28
	III/IV	93	3		Tubal	98	30
	Tubal	49	14	Pal (66)	I/II	45	44
					III/IV	39	33

patients with endometriosis or tubal factor infertility. Another case–control study, also comparing endometriosis with tubal factor, found no evidence of either impaired fertilization or a decrease in embryo quality (77). In comparing the effect of progressive endometriosis stages on fertilization and embryo development, Inoue et al. (65) found no differences in either the fertilization rate or the ET rate for 309 patients with stage I–IV endometriosis. Furthermore, Bergendal et al. (63), although reporting impaired fertilization for women with endometriosis, noted no difference in either the cleavage rate or the morphologic embryo score, when compared with tubal infertility.

As it stands, the question of a significant effect by endometriosis on fertilization and *in vitro* embryo development has yet to be answered. Barnhart et al. (50) showed an overall decrease in fertilization rate when all endometriosis patients were compared to patients with tubal infertility, but when stratified by stage of disease, patients with severe endometriosis actually had an increase in fertilization rates. However, more recent studies have shown that any impaired fertilization has little or no effect on the ultimate outcome of IVF, as pregnancy rates for patients with endometriosis are comparable with other etiologies. Suzuki et al. (85) found that endometriosis affects oocyte number but not embryo quality or pregnancy outcome, irrespective of the presence of an ovarian endometrioma. Perhaps the clinical insignificance of impaired fertilization is due to the fact that improved ovarian stimulation and oocyte recovery techniques have led to a surplus of available oocytes for fertilization. An increased oocyte yield can readily sustain a slight decrease in fertilization capacity to produce enough embryos for implantation. It is unclear what role, if any, ICSI may play in the fertilization of oocytes from women with endometriosis.

Implantation, pregnancy, and loss

Assuming a minimum number of good-quality embryos are available for transfer, a successful live birth is dependent on adequate implantation and a low rate of spontaneous abortion. However, as a result of the transfer of multiple embryos, a lower rate of implantation has not necessarily translated into a low pregnancy rate. Although a few contemporary studies have in fact reported reduced implantation rates, most have failed to demonstrate a correspondingly low pregnancy rate for patients with endometriosis. Some early studies have shown a decrease in the implantation rate with a subsequent decrease in the pregnancy rate (54,55,74). In a small study, Chillik et al. (53) reported a significantly lower implantation and pregnancy rate for patients with stage III or IV endometriosis when compared to patients with tubal factor or endometriosis of a lesser severity. Matson and Yovich (55) demonstrated pregnancy rates of 18%, 13%, 14%, 6%, and 2%, for patients with tubal factor and stage I–IV endometriosis, respectively. In a case–control study of 284 IVF cycles, Arici et al. (77) reported a significantly lower implantation rate of 3.9% for patients with endometriosis compared with 8.1% and 7.2%

for tubal infertility and unexplained infertility, respectively. They also demonstrated a trend toward a lower pregnancy rate in patients with endometriosis, although this did not reach significance. More recent studies have taken this finding and added live birth and cumulative pregnancy rates. Omland et al. (49) found the live birth rate after transfer of two embryos to be 66.0% compared with 78.8% for unexplained etiology of infertility. Kuivasaari et al. (48) found a significantly lower cumulative pregnancy rate after one to four IVF/ICSI treatments in women with stage III/IV endometriosis compared to women with stage I/II endometriosis and a control group of women with tubal infertility.

Errors in implantation may be attributed to the relationship between endometriosis and adenomyosis. Recent studies have suggested that treatment with either prolonged down-regulation with GnRH agonists (86) or oral contraceptives (79) may help overcome the effects of adenomyosis on the endometrium. A recent systematic review found a four-fold increase in the odds of clinic pregnancy (OR 4.28, 95% CI 2.00–9.15) with administration of GnRH agonists for a period of three to six months prior to IVF in patients with endometriosis (87); however, the safety data from this analysis were not readily available.

While Simon et al. (88) also reported lower implantation and pregnancy rates for patients with endometriosis versus tubal infertility, they added a dimension to the data by analyzing the outcomes of oocyte donation from donors with and without endometriosis. They reported comparable implantation and pregnancy rates for women with and without endometriosis who received oocytes from donors without endometriosis. However, patients who received oocytes from endometriotic ovaries had significantly lower implantation rates. Another study reported on 239 oocyte donor cycles and found that the presence of endometriosis in the recipient had no effect on implantation or pregnancy rates, regardless of the disease stage (89). From this, it has been suggested that an endometriosis-associated impairment of implantation results from a compromise to the potential of the oocyte or early embryo, and not to the endometrium itself.

A recent matched case–control study was performed comparing the implantation rates for patients with stage III/IV endometriosis with those of women who are free of the disease. Transfers using matched sibling oocytes from the same donor demonstrated no statistically significant difference in pregnancy, implantation, miscarriage, and live rates (90). This study suggests that endometrial receptivity and subsequent implantation rate may not be affected by stage III/IV endometriosis.

Several large investigations have failed to demonstrate either an impaired implantation rate or a lower pregnancy rate for patients with endometriosis when comparing stage by stage or with other infertility etiologies (63–67). Geber et al. (64) reported pregnancy rates in 140 cycles of 40% and 45% for patients with endometriosis or tubal infertility, respectively. Olivennes et al. noted similar pregnancy rates of 29% for endometriosis and 36% for tubal factor (67), while another study reported rates of 28% and 30%, respectively (63). In a study of 681 women with and without endometriosis, Inoue et al. (65) found no difference in the IVF conception rate between the two groups. Several comparisons within endometriosis stages have reported similar pregnancy rates despite increasing disease severity (54,62,64,77). Pal et al. analyzed IVF cycles in endometriosis patients with either stage I/II or stage III/IV disease. Although they reported a lower fertilization rate for patients with stage III or IV endometriosis, clinical pregnancy rates did not differ significantly between the two groups (66). In their meta-analysis, Barnhart et al. (50) calculated that the adjusted OR of achieving pregnancy compared with the group of controls was 0.56, 0.79, and 0.46, respectively, for overall patients, stage I/II patients, and stage III/IV patients, respectively (Table 59.2).

A few studies have associated endometriosis with increased pregnancy loss during IVF cycles. Oehninger et al. (54) noted a higher miscarriage rate following IVF among patients with stage III or IV endometriosis when compared to those with less severe disease. Along with a diminished oocyte yield and poor embryo quality, Yanushpolsky et al. (81) reported a significantly higher early pregnancy loss when endometriomas were aspirated at the time of oocyte retrieval. However, another large study comparing patients with aspirated endometriomas to others with endometriosis

Table 59.2 Comparing stage III/IV disease with stage I/II disease: results of bivariate analysis and multiple logistic regression comparing endometriosis patients with stage III/IV disease with patients with stage I/II disease

Outcome	Endometriosis stage III/IV	Endometriosis stage I/II	p-value	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Pregnancy rate	13.84	21.12	<0.001	0.60 (0.42–0.87)	0.64 (0.34–1.17)
Fertilization rate	74.47	58.38	<0.001	1.11 (1.09–1.13)	Not interpretable
Implantation rate	10.23	11.31	0.003	0.93 (0.89–0.98)	0.21 (0.15–0.32)
Mean oocyte count	6.70	8.19	<0.001	0.83 (0.78–0.87)	0.31 (0.24–0.39)
Peak E2	1447.74	5813.38	<0.001	N/A	N/A

Source: From Barnhart K et al. *Fertil Steril* 2002; 77: 1148–55.
Note: Total number of observations: 699.
[a] Adjusted for publication date and age.
Abbreviations: CI, confidence interval; E2, estradiol; N/A, not applicable; OR, odds ratio.

found no difference in oocyte yield, embryo quality, pregnancy rate, or miscarriage (92). Furthermore, most studies have not reported a significant endometriosis-associated increase in pregnancy loss (63,64).

Endometriosis may also be associated with late pregnancy complications, such as preterm birth. Stephansson et al. (93) showed that, compared with women without endometriosis, women with endometriosis had a higher risk of preterm birth, with an adjusted OR of 1.33. Conversely, Fernando et al. (94) showed an increased risk of preterm birth only when endometrioma was present. Women with endometriosis, but without endometrioma, did not show an increased risk for preterm birth when compared to women without endometriosis.

Surgery and ART

As stated earlier, the data are conflicting regarding the effect of surgery on fertility in patients with endometriosis. Unfortunately, there have been no prospective, randomized studies investigating the effect of surgery for endometriosis on ART outcome. One retrospective study compared IVF with repeat surgery for patients with stage III or IV endometriosis (95). A Cochrane review of two randomized trials comparing the effectiveness of laparoscopic surgery in the treatment of subfertility associated with endometriosis versus other treatment modalities or placebo found that use of laparoscopic surgery may improve the chance of pregnancy by an OR of 1.6 (96). Pregnancy rates were reported as 70% over two cycles of IVF compared with 24% for the nine months following surgery. There are no similar randomized studies evaluating the effects of surgery on severe disease. A non-randomized study (31) demonstrated that the cumulative probability of pregnancy in 216 infertile patients with severe disease two years after surgery was significantly increased.

In another study, Garcia-Velasco et al. reported no difference in fertilization, implantation, or pregnancy rates for patients who had undergone removal of an endometrioma, as compared to patients with suspected endometriomas that were not removed (91). A meta-analysis (97) of five studies agreed with these results by concluding that surgical management of endometriomas has no significant effect on IVF pregnancy rates and ovarian response to stimulation compared with no treatment.

In another randomized study comparing patients with and without varying degrees of endometriosis undergoing ICSI for male factor infertility found no difference in either fertilization or pregnancy and implantation rates between women with and without endometriosis, although significantly fewer oocytes were retrieved from patients with endometriosis (98).

However, a recent study by Bianchi et al. looking at women with deep infiltrative endometriosis found that extensive laparoscopic excision of endometriotic lesions improved pregnancy outcomes significantly (OR 2.45) (99).

Until better data are available, however, no definitive conclusions can be drawn regarding the role of surgery for endometriosis prior to ART. In fact, one recent study (100) found that in the absence of tubal occlusion or severe male factor infertility, laparoscopy may still be considered for the treatment of endometriosis even after multiple failed IVF cycles.

Future directions

Some researchers have suggested that endometriosis is associated with impaired folliculogenesis and a decreased oocyte yield. Although the data are conflicting, it is possible that the introduction of aromatase inhibitors may represent another large step forward in improving ovarian stimulation protocols and increasing IVF success. Further study of GnRH antagonists may also show a benefit for patients with endometriosis. Furthermore, the use of donor oocytes has been suggested to improve efficacy in patients with endometriosis. As ovarian hyperstimulation protocols become more tolerable and as oocyte cryopreservation becomes efficacious and efficient, it is possible that an increased number of women with endometriosis who have failed standard IVF will benefit from donation.

There is evidence for and against an endometriosis-associated impairment of oocyte fertilization *in vitro*. One of the tremendous benefits of fertilizing an oocyte *in vitro* is the ability to assess the process on a case-by-case basis. For patients with endometriosis who are experiencing fertilization difficulty, it is likely that ICSI will prove to be a valuable addition to the technology of assisted reproduction for this disease. Indeed, ICSI has proven to be of tremendous worth in achieving pregnancy in couples with male factor infertility. Minguez et al. (98) analyzed 980 cycles of ICSI for couples with male factor infertility, of which 101 cycles were also complicated by endometriosis. They found no significant difference in fertilization, implantation, or pregnancy rates with co-existing endometriosis. Finally, there is an increasing interest in the prolongation of *in vitro* embryo maturation, with many investigators studying the efficacy of blastocyst development and implantation. An endometriosis-associated detriment to implantation may be responsible for some IVF failures. Although reports are conflicting, some have suggested an impaired early embryo development in patients with endometriosis. It is possible that the practice of *in vitro* maturation to the blastocyst stage in these patients may allow for the transfer of a more selected group of healthier embryos, thus improving the implantation rate. It is anticipated that the improvements from this approach will eventually raise the implantation rate to a point at which it will become routine to transfer no more than one or two embryos at a time, thereby significantly lowering the incidence of multiple pregnancies. Furthermore, the adoption of various techniques in embryo manipulation, such as assisted hatching, may also have a positive effect on the implantation rate for these patients. Finally, as preimplantation genetic screening increases in popularity as a means of assessing quality of embryos prior to transfer, it is reasonable to assume that the transfer of embryos that have been selected as being chromosomally normal will lead to an increase in the success of IVF in patients with endometriosis.

There may also be a role for endometrial receptivity assays to screen patients with endometriosis prior to proceeding with ET. Although a recent study showed minimal differences in the functional profiles of gene expression microarray testing in patients with and without endometriosis (101), as this technique is expanded and improved, it may eventually provide useful clues or potential treatments for treatment of infertility associated with endometriosis in the future.

CONCLUSION

It is important to stress the heterogeneous nature of the data that have been reviewed. Laboratory and clinical practices vary greatly from center to center, as do the corresponding IVF success rates. Randomized, prospective studies designed to answer key questions about the optimum algorithmic approach to the treatment of endometriosis-associated infertility simply do not exist. With the evidence evaluated as a whole, it does appear that IVF outcomes have improved significantly for endometriosis patients with the adoption of GnRH agonists and transvaginal oocyte retrieval.

Although ART procedure alterations are site specific, the vast majority of endometriosis patients undergo the same treatment protocol as for those patients with tubal factor or unexplained infertility. There is, to date, no compelling evidence that endometriosis patients benefit from significant alterations from standard ART protocols or procedures, with the notable exception of prolonged GnRH agonist down-regulation. Until large, randomized, prospective studies have answered questions regarding the optimum length of down-regulation, the use of *in vitro* maturation or manipulation, the role of autoantibodies and immunosuppression, and other controversies, it is likely that patients with endometriosis will continue to undergo the same treatment protocol as everyone else. At the very least, it can be said that ART represents a tremendous advancement for women who, for whatever reason, have been unable to achieve pregnancy. For the patient with endometriosis, evolving options in pharmacotherapy and assisted reproduction may finally offer the blessing of a pain-free and reproductive life.

REFERENCES

1. Counseller VS. Endometriosis: A clinical and surgical review. *Am J Obstet Gynecol* 1938; 36: 877–88.
2. Hasson HM. Incidence of endometriosis in diagnostic laparoscopy. *J Reprod Med* 1976; 16: 135–8.
3. Strathy JH, Molgaard CA, Coulam CB, Melton LJ. Endometriosis and infertility: A laparoscopic study of endometriosis among fertile and infertile women. *Fertil Steril* 1982; 38: 667–72.
4. Drake TS, Grunert GM. The unsuspected pelvic factor in the infertility investigation. *Fertil Steril* 1980; 34: 27–31.
5. Hammond MG, Jordan S, Sloan CS. Factors affecting pregnancy rates in a donor insemination program using frozen semen. *Am J Obstet Gynecol* 1986; 155: 480–5.
6. Yeh J, Seibel MM. Artificial insemination with donor sperm: A review of 108 patients. *Obstet Gynecol* 1987; 70: 313–6.
7. Jansen RPS. Minimal endometriosis and reduced fecundibility: Prospective evidence from an artificial insemination by a donor program. *Fertil Steril* 1986; 46: 141–3.
8. Matorras R, Corcostegui B, Esteban J et al. Fertility in women with minimal endometriosis compared with normal women was assessed by means of a donor insemination program in unstimulated cycles. *Am J Obstet Gynecol* 2010; 203: 345.e1–6.
9. Bancroft K, Vaughan-Williams CA, Elstein M. Pituitary–ovarian function in women with minimal or mild endometriosis and otherwise unexplained infertility. *Clin Endocrinol* 1992; 36: 177–81.
10. Matorras R, Rodriguez F, Perez C et al. Infertile women with and without endometriosis: A case control study of luteal phase and other infertility conditions. *Acta Obstet Gynecol Scand* 1996; 75: 826–31.
11. Pittaway DE, Maxson W, Daniell J, Herbert C, Wentz AC. Luteal phase defects in infertility patients with endometriosis. *Fertil Steril* 1983; 39: 712–3.
12. Hirschowitz JS, Soler NG, Wortsman J. The galactorrhea–endometriosis syndrome. *Lancet* 1978; 1: 896–8.
13. Mio Y, Toda T, Harada T, Terakawa N. Luteinized unruptured follicle in the early stages of endometriosis as a cause of unexplained infertility. *Am J Obstet Gynecol* 1992; 167: 271–3.
14. Wheeler JM, Johnston BM, Malinak LR. The relationship of endometriosis to spontaneous abortion. *Fertil Steril* 1983; 39: 656–60.
15. Burns WN, Schenken RS. Pathophysiology of endometriosis-associated infertility. *Clin Obstet Gynecol* 1999; 42: 586–610.
16. Thomas EJ, Lenton EA, Cooke ID. Follicle growth patterns and endocrinological abnormalities in infertile women with minor degrees of endometriosis. *Br J Obstet Gynaecol* 1986; 93: 852–8.
17. Kusuhara K. Luteal function in infertile patients with endometriosis. *Am J Obstet Gynecol* 1992; 167: 274–7.
18. Matalliotakis I, Panidis D, Vlassis G et al. PRL, TSH and their response to the TRH test in patients with endometriosis before, during, and after treatment with danazol. *Gynecol Obstet Invest* 1996; 42: 183–6.
19. Pittaway DE, Vernon C, Fayez JA. Spontaneous abortions in women with endometriosis. *Fertil Steril* 1988; 50: 711–5.
20. Matorras R, Rodriguez F, Gutierrez de Teran G et al. Endometriosis and spontaneous abortion rate: A cohort study in infertile women. *Eur J Obstet Gynecol Reprod Biol* 1998; 77: 101–5.
21. Senturk LM, Arici A. Immunology of endometriosis. *J Reprod Immunol* 1999; 43: 67–83.

22. De Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: Patho-physiology and management. *Lancet* 2010; 376: 730–8.
23. Yoshida S, Harada T, Iwabe T et al. A combination of interleukin-6 and its soluble receptor impairs sperm motility: Implications in infertility associated with endometriosis. *Hum Reprod* 2004; 19:, 1821–5.
24. Carli C, Leclerc P, Metz CN, Akoum A. Direct effect of macrophage migration inhibitory factor on sperm function: Possible involvement in endometriosis-associated infertility. *Fertil Steril* 2007; 88(4 Suppl): 1240–7.
25. Iborra A, Palacio JR, Martinez P. Oxidative stress and autoimmune response in the infertile woman. *Chem Immunol Allergy* 2005; 88: 150–62.
26. Dun EC, Taylor RN, Wieser F. Advances in the genetics of endometriosis. *Genome Med* 2010; 2: 75.
27. Kunz G, Beil D, Huppert P, Noe M, Kissler S, Leyendecker G. Adenomyosis in endometriosis—Prevalence and impact on fertility. Evidence from magnetic resonance imaging. *Hum Reprod* 2005; 20: 2309–16.
28. Hughes E, Brown J, Collins JJ, Farquhar C, Fedorkow DM, Vanderkerchove P. Ovulation suppression for endometriosis for women with subfertility. *Cochrane Database of Systematic Reviews 2007*, Issue 3. Art. No.: CD000155. DOI: 10.1002/14651858.CD000155.pub2.
29. Marcoux S, Maheux R, Berube S, The Canadian Collaborative Group on Endometriosis. Laparoscopic surgery in infertile women with minimal or mild endometriosis. *N Engl J Med* 1997; 336: 217–22.
30. Gruppo Italiano per lo Studio dell'Endometriosi. Ablation of lesions or no treatment in minimal–mild endometriosis in infertile women: A randomized trial. *Hum Reprod* 1999; 14: 1332–4.
31. Al-Inany HG, Crosignani PG, Vercellini P. Evidence may change with more trials: Concepts to be kept in mind [letters]. *Hum Reprod* 2000; 15: 2447–8.
32. Benschop L, Farquhar C, van der Poel N, Heineman MJ. Interventions for women with endometrioma prior to assisted reproductive technology. *Cochrane Database of Syst Rev* 2010; 11: CD008571.
33. Guzick DS, Carson SA, Coutifaris C et al. Efficacy of superovulation and intrauterine insemination in the treatment of infertility. *N Engl J Med* 1999; 340: 177–83.
34. Deaton JL, Gibson M, Blackmer KM et al. A randomized, controlled trial of clomiphene citrate and intrauterine insemination in couples with unexplained infertility or surgically corrected endometriosis. *Fertil Steril* 1990; 54: 1083–8.
35. Fedele L, Parazzini F, Radici E et al. Buserelin acetate versus expectant management in the treatment of infertility associated with mild endometriosis: A randomized clinical trial. *Fertil Steril* 1992; 58: 28–31.
36. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikainen H. Intrauterine insemination treatment in subfertility: An analysis of factors affecting outcome. *Hum Reprod* 1999; 14: 698–703.
37. Omland A, Tanbo T, Dale PO, Abyholm T. Artificial insemination by husband in unexplained infertility compared with infertility associated with peritoneal endometriosis. *Hum Reprod* 1998; 13: 2602–5.
38. Hughes EG. The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: A meta-analysis. *Hum Reprod* 1997; 12: 1865–72.
39. Tummon IS, Asher LJ, Martin JS, Tulandi T. Randomized controlled trial of superovulation and insemination for infertility associated with minimal or mild endometriosis. *Fertil Steril* 1997; 68: 8–12.
40. Capelo F, Kumar A, Steinkampf M, Azziz R. Laparoscopic evaluation following failure to achieve pregnancy after ovulation induction with clomiphene citrate. *Fertil Steril* 2003; 80: 1450–3.
41. Werbrouck E, Spiessens C, Meuleman C, D'Hooghe T. No difference in cycle pregnancy rate and in cumulative live-birth rate between women with surgically treated minimal to mild endometriosis and women with unexplained infertility after controlled ovarian hyperstimulation and intrauterine insemination. *Fertil Steril* 2005; 86: 566–71.
42. Karaer O, Oruc S, Koyuncu FM. Aromatase inhibitors: Possible future applications. *Acta Obstet Gynecol Scand* 2004; 83: 699–706.
43. Attar E, Bulun S. Aromatase inhibitors: The next generation of therapeutics for endometriosis? *Fertil Steril* 2006; 85: 1307–18.
44. Wu HH, Wang NM, Cheng ML, Hsieh JN. A randomized comparison of ovulation induction and hormone profile between the aromatase inhibitor anastrozole and clomiphene citrate in women with infertility. *Gynecol Endocrinol* 2007; 23: 76–81.
45. Abu Hashim H, El Rakhawy M, Abd Elaal I. Randomized comparison of superovulation with letrozole vs. clomiphene citrate in an IUI program for women with recently surgically treated minimal to mild endometriosis. *Acta Obstet Gynecol Scand* 2012; 91: 338–45.
46. Cantineau AEP, Cohlen BJ, Klip H, Heineman MJ, Dutch IUI Study Group Collaborators. The addition of GnRH antagonists in intrauterine insemination cycles with mild ovarian hyperstimulation does not increase live birth rates—A randomized, double-blinded, placebo-controlled trial. *Hum Reprod* 2011; 26: 1104–11.
47. The Practice Committee of the American Society of Reproductive Medicine. Endometriosis and infertility. *Fertil Steril* 2006; 86: S156–60.
48. Kuivasaari P, Hippelainen M, Anttila M, Heinonen S. Effect of endometriosis on IVF/ICSI outcome: Stage III/IV endometriosis worsens cumulative pregnancy and live-born rates. *Hum Reprod* 2005; 20: 3130–5.
49. Omland AK, Abyholm T, Fedorcsak P et al. Pregnancy outcome after IVF and ICSI in unexplained, endometriosis-associated and tubal factor infertility. *Hum Reprod* 2005; 20: 722–7.

50. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on *in vitro* fertilization. *Fertil Steril* 2002; 77: 1148–55.
51. Hamdan M, Omar SZ, Dunselman G, Cheong Y. Influence of endometriosis on assisted reproductive technology outcomes: A systematic review and meta-analysis. *Obstet Gynecol* 2015; 125: 79–88.
52. Somigliana E, Garcia-Velasco J. Treatment of infertility associated with deep endometriosis: Definition of therapeutic balances. *Fertil Steril* 2015; 104: 764–70.
53. Chillik CF, Acosta AA, Garcia JE et al. The role of *in vitro* fertilization in infertile patients with endometriosis. *Fertil Steril* 1985; 44: 56–61.
54. Oehninger S, Acosta AA, Kreiner D et al. *In vitro* fertilization and embryo transfer IVF/ET: An established and successful therapy for endometriosis. *J In Vitro Fert Embryo Transf* 1988; 5: 249–56.
55. Matson PL, Yovich JL. The treatment of infertility associated with endometriosis by *in vitro* fertilization. *Fertil Steril* 1986; 46: 432–4.
56. Yovich JL, Matson PL, Richardson PA, Hilliard C. Hormonal profiles and embryo quality in women with severe endometriosis treated by *in vitro* fertilization and embryo transfer. *Fertil Steril* 1988; 50: 308–13.
57. Yovich JL, Matson PL. The influence of infertility etiology on the outcome of IVF–ET and GIFT treatments. *Int J Fertil* 1990; 35: 26–33.
58. Chang MY, Chiang CH, Hsieh TT, Soong YK, Hsu KH. The influence of endometriosis on the success of gamete intrafallopian transfer GIFT. *J Assist Reprod Genet* 1997; 14: 76–82.
59. Dlugi AM, Loy RA, Dieterle S, Bayer SR, Seibel MM. The effect of endometriomas on *in vitro* fertilization outcome. *J In Vitro Fert Embryo Transf* 1989; 6: 338–41.
60. Somigliana E, Infantino M, Benedetti F et al. The presence of ovarian endometriomas is associated with a reduced responsiveness to gonadotropins. *Fertil Steril* 2006; 86: 192–6.
61. Sanchez AM, Vigano P, Somigliana E, Panina-Bordignon P, Vercellini P, Candiani M. The distinguishing cellular and molecular features of the endometriotic ovarian cyst: From pathophysiology to the potential endometrioma-mediated damage to the ovary. *Hum Reprod Update* 2014; 20: 217–30.
62. Dmowski WP, Rana N, Michalowska J et al. The effect of endometriosis, its stage and activity, and of autoantibodies on *in vitro* fertilization and embryo transfer success rates. *Fertil Steril* 1995; 63: 555–62.
63. Bergendal A, Naffah S, Nagy C et al. Outcome of IVF in patients with endometriosis in comparison with tubal factor infertility. *J Assist Reprod Genet* 1998; 15: 530–4.
64. Geber S, Paraschos T, Atkinson G, Margara R, Winston RM. Results of IVF in patients with endometriosis: The severity of the disease does not affect outcome, or the incidence of miscarriage. *Hum Reprod* 1995; 10: 1507–11.
65. Inoue M, Kobayashi Y, Honda I, Awaji H, Fujii A. The impact of endometriosis on the reproductive outcome of infertile patients. *Am J Obstet Gynecol* 1992; 167: 278–82.
66. Pal L, Shifren JL, Isaacson KB et al. Impact of varying stages of endometriosis on the outcome of *in vitro* fertilization–embryo transfer. *J Assist Reprod Genet* 1998; 15: 27–31.
67. Olivennes F, Feldberg D, Liu HC et al. Endometriosis: A stage by stage analysis—The role of *in vitro* fertilization. *Fertil Steril* 1995; 64: 392–8.
68. Oehninger S, Brzyski RG, Muasher SJ, Acosta AA, Jones GS. *In vitro* fertilization and embryo transfer in patients with endometriosis: Impact of a gonadotrophin releasing hormone agonist. *Hum Reprod* 1989; 4: 541–4.
69. Dicker D, Goldman JA, Levy T, Feldberg D, Ashkenazi J. The impact of long-term gonadotropin-releasing hormone analogue treatment on preclinical abortions in patients with severe endometriosis undergoing *in vitro* fertilization–embryo transfer. *Fertil Steril* 1992; 57: 597–600.
70. Rickes D, Nickel I, Kropf S, Kleinstein J. Increased pregnancy rates after ultralong postoperative therapy with gonadotropin-releasing hormone analogs in patients with endometriosis. *Fertil Steril* 2002; 78: 757–62.
71. Chedid S, Camus M, Smitz J, Van Steirteghem AC, Devroey P. Comparison among different ovarian stimulation regimens for assisted procreation procedures in patients with endometriosis. *Hum Reprod* 1995; 10: 2406–11.
72. Nakamura K, Oosawa M, Kondou I et al. Metrodin stimulation after prolonged gonadotropin releasing hormone agonist pretreatment for *in vitro* fertilization in patients with endometriosis. *J Assist Reprod Genet* 1992; 9: 113–7.
73. Marcus SF, Edwards RG. High rates of pregnancy after long-term down-regulation of women with severe endometriosis. *Am J Obstet Gynecol* 1994; 171: 812–7.
74. Mahadevan MM, Trounson AO, Leeton JF. The relationship of tubal blockage, infertility of unknown cause, suspected male infertility, and endometriosis to the success of *in vitro* fertilization and embryo transfer. *Fertil Steril* 1983; 40: 755–62.
75. Wardle PG, Mitchell JD, McLaughlin EA et al. Endometriosis and ovulatory disorder: Reduced fertilisation *in vitro* compared with tubal and unexplained infertility. *Lancet* 1985; 2: 236–9.
76. Sharma V, Riddle A, Mason BA, Pampiglione J, Campbell S. An analysis of factors influencing the establishment of a clinical pregnancy in an ultrasound-based ambulatory *in vitro* fertilization program. *Fertil Steril* 1988; 49: 468–78.
77. Arici A, Oral E, Bukulmez O et al. The effect of endometriosis on implantation: Results from the Yale University *in vitro* fertilization and embryo transfer program. *Fertil Steril* 1996; 65: 603–7.

78. Surrey ES, Silverberg KM, Surrey MW, Schoolcraft WB. Effect of prolonged gonadotropin-releasing hormone agonist therapy on the outcome of *in vitro* fertilization–embryo transfer in patients with endometriosis. *Fertil Steril* 2002; 78: 699–704.
79. de Ziegler D, Gayet V, Aubriot PF et al. Use of oral contraceptives in women with endometriosis before assisted reproduction treatment improves outcomes. *Fertil Steril* 2010; 94: 2796–9.
80. Recai P, Onalan G, Kaya C. GnRH agonist and antagonist protocols for stage I–II endometriosis and endometrioma in *in vitro* fertilization/intracytoplasmic sperm injection cycles. *Fertil Steril* 2007; 88: 832–8.
81. Yanushpolsky EH, Best CL, Jackson KV et al. Effects of endometriomas on oocyte quality, embryo quality, and pregnancy rates in *in vitro* fertilization cycles: A prospective, case–controlled study. *J Assist Reprod Genet* 1998; 15: 193–7.
82. Pellicer A, Oliveira N, Ruiz A, Remohi J, Simon C. Exploring the mechanisms of endometriosis-related infertility: An analysis of embryo development and implantation in assisted reproduction. *Hum Reprod* 1995; 10(Suppl 2): 91–7.
83. Tanbo T, Omland A, Dale PO, Abyholm T. Transfer i*n vitro* fertilization/embryo transfer in unexplained infertility and minimal peritoneal endometriosis. *Acta Obstet Gynecol Scand* 1995; 74: 539–43.
84. Brizek CL, Schlaff S, Pellegrini VA, Frank JB, Worrilow KC. Increased incidence of aberrant morphological phenotypes in human embryogenesis—An association with endometriosis. *J Assist Reprod Genet* 1995; 12: 106–12.
85. Suzuki T, Izumi SI, Matsubayashi H et al. Impact of ovarian endometrioma on oocyte and pregnancy out-come in *in vitro* fertilization. *Fertil Steril* 2005; 83: 908–13.
86. Niu Z, Chen Q, Sun Y, Feng Y. Long-term pituitary downregulation before frozen embryo transfer could improve pregnancy outcomes in women with adenomyosis. *Gynecol Endocrinol* 2013; 29: 1026–30.
87. Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before *in vitro* fertilization (IVF) for women with endometriosis. *Cochrane Database Syst Rev* 2006; 1: CD004635.
88. Simon C, Gutierrez A, Vidal A et al. Outcome of patients with endometriosis in assisted reproduction: Results from *in vitro* fertilization and oocyte donation. *Hum Reprod* 1994; 9: 725–9.
89. Sung L, Mukherjee T, Takeshige T, Bustillo M, Copperman AB. Endometriosis is not detrimental to embryo implantation in oocyte recipients. *J Assist Reprod Genet* 1997; 14: 152–6.
90. Díaz I, Navarro J, Blasco L, Simón C, Pellicer A, Remohí J. Impact of stage III–IV endometriosis on recipients of sibling oocytes: Matched case-control study. *Fertil Steril* 2000; 74: 31–4.
91. Garcia-Velasco JA, Mahutte NG, Corona J et al. Removal of endometriomas before *in vitro* fertilization does not improve fertility outcomes: A matched, case–control study. *Fertil Steril* 2004; 81: 1194–7.
92. Isaacs JD Jr, Hines RS, Sopelak VM, Cowan BD. Ovarian endometriomas do not adversely affect pregnancy success following treatment with *in vitro* fertilization. *J Assist Reprod Genet* 1997; 14: 551–3.
93. Stephansson O, Kieler H, Granath F, Falconer H. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. *Hum Reprod* 2009; 24: 2341–7.
94. Fernando S, Breheny VS, Jaques AM et al. Preterm birth, ovarian endometriomata, and assisted reproduction technologies. *Fertil Steril* 2009; 91: 325–30.
95. Pagidas K, Falcone T, Hemmings R, Miron P. Comparison of reoperation for moderate stage III and severe stage IV endometriosis-related infertility with *in vitro* fertilization–embryo transfer. *Fertil Steril* 1996; 65: 791–5.
96. Jacobson T, Barlow D, Koninckx P, Olive D, Farquhar C. Laparoscopic surgery for subfertility associated with endometriosis. *Cochrane Database Syst Rev* 2002; 4: CD001398.
97. Tsoumpou I, Kyrgiou M, Gelbaya TA, Nardo LG. The effect of surgical treatment for endometrioma on *in vitro* fertilization outcomes: A systematic review and meta-analysis. *Fertil Steril* 2009; 92: 75–87.
98. Minguez Y, Rubio C, Bernal A et al. The impact of endometriosis in couples undergoing intracytoplasmic sperm injection because of male infertility. *Hum Reprod* 1997; 12: 2282–5.
99. Bianchi PH, Pereira RM, Zanatta A, Alegretti JR, Motta EL, Serafini PC. Extensive excision of deep infiltrative endometriosis before *in vitro* fertilization significantly improves pregnancy rates. *J Minim Invasive Gynecol* 2009; 16: 174–80.
100. Littman E, Giudice L, Lathi R et al. Role of laparoscopic treatment of endometriosis in patients with failed *in vitro* fertilization cycles. *Fertil Steril* 2005; 84: 1574–8.
101. Garcia-Velasco JA, Fassbender A, Ruiz-Alonso M, Blesa D, D'Hooghe T, Simon C. Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study. *Reprod Biomed Online* 2015; 31: 647–54.

Polycystic ovary syndrome and assisted reproduction

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common polygenic multifactorial condition affecting a wide population. Historically, the classic combination of symptoms was first described in 1935 by Stein and Leventhal (1), who recognized that enlarged ovaries, amenorrhea, infertility, and hirsutism could be collated together. This collection of symptoms has been widely researched and many conclusions made, yet it continues to prove to be a difficult condition to treat. PCOS is now recognized as a spectrum disorder ranging from ultrasound features of polycystic ovarian morphology (2) to anovulatory infertility. Obesity, hyperandrogenemia, and insulin resistance are all key factors that influence the expression and symptoms of the condition (3,4).

PCOS poses an interesting problem for assisted reproduction. Anovulation is common among women with PCOS and accounts for 80%–90% of World Health Organization (WHO) group II anovulatory subfertility. Treatment has centered on weight management and lifestyle modification followed by ovulation induction with clomiphene citrate (5). The cumulative pregnancy rate with clomiphene after six months of treatment is between 40% and 50% (6). Women who remain anovulatory can be stimulated with low-dose gonadotropins. Ovarian diathermy has been suggested as an effective alternative in ovulation induction (7). For those who remain refractory to these treatments or with coexisting pathologies, assisted reproductive techniques can be employed within a closely supervised setting to produce the desired outcome of pregnancy with the challengingly sensitive polycystic ovary (PCO) (8).

DIAGNOSIS AND PREVALENCE

The classification of PCOS has been accepted and simplified through the Rotterdam consensus workshop in 2003 (9). No single diagnostic criterion is sufficient for the clinical diagnosis of PCOS. Two of the following are required:

1. Oligo and/or anovulation
2. Clinical and/or biochemical signs of hyperandrogenism
3. PCO morphology on ultrasound

Exclusion of other etiologies (such as congenital adrenal hyperplasia, androgen-secreting tumor, or Cushing's syndrome) must be elucidated by appropriate investigation as indicated.

With the advancing technology and imaging of transvaginal ultrasound, the consensus went further in defining PCO morphology (10). Twelve or more follicles measuring 2–9 mm or increased ovarian volume over 10 cm^3 offers the best specificity (99%) and sensitivity (75%) for the diagnosis of PCOS (11). The distribution of follicles and description of the stroma are not required. Jonard et al. reported a significant increase in 2–5 mm follicles with no change in 6–9 mm follicle number compared with controls. This prompted a hypothesis that the hyperandrogenic microenvironment resulted from an increased recruitment of growing follicles followed by their arrest at 6–9 mm (11). Since the Rotterdam consensus, there has been further debate about the definitions of both the syndrome and the morphology of the PCO. More recently, it has been suggested that the definition of PCO should be increased to 25 follicles per ovary (12).

The prevalence of PCOS is determined by the diagnostic criteria used within the study, ethnic origin, and population of women studied. Michelmore et al. (13), who included ultrasound morphology and menstrual disturbance, revealed a prevalence of 26% in 230 volunteers. Further population studies have confirmed a common finding of PCO in 21%–23%, although a significant proportion (25%) were without any clinical features of PCOS (2). The highest reported prevalence of PCO is 52% in South Asians living within the U.K. (14), with menstrual irregularities in 49.1%. South Asians with anovulatory PCOS have greater insulin resistance and severity of symptoms compared with their Caucasian counterparts (15). However, Knochenhauer et al., who did not use ultrasound features, did not find a significant difference between black and white women within a U.S. population (3.4% vs. 4.7%), and the lower prevalence was related to the use of the older National Institutes of Health (NIH) definition of the syndrome (16).

REPRODUCTIVE HEALTH IN PCOS

In addition to anovulation, there may be other factors that contribute to subfertility in women with PCOS, including the effects of obesity and metabolic, inflammatory, and endocrine abnormalities on oocyte quality and fetal development. Oocytes from PCOs may exhibit reduced developmental competence, with a reduced ability to complete meiosis, achieve fertilization, and develop into a normal embryo. Ovarian hyperandrogenism and hyperinsulinemia may promote premature granulosa cell luteinization; furthermore, paracrine dysregulation of growth factors may disrupt the intrafollicular environment, alter granulosa cell–oocyte interactions, and impair cytoplasmic

and/or nuclear maturation of oocytes (17). There is variability, however, and oocyte quality, fertilization, and implantation rates in women with PCOS may be normal (18).

PCOS is associated with metabolic disturbances that include impaired insulin signaling and glucose metabolism in ovarian follicles (19). It is likely that the metabolic lesion in the follicle precipitates an altered metabolic milieu throughout oogenesis, which may have downstream consequences for oocyte energy generation. This may lead to reduced expression of genes encoding oxidative phosphorylation (20). Altered expression of key genes associated with chromosome alignment and segregation has also been attributed to hyperandrogenemia (21). Indeed, it has been shown that differences in metabolism exist in oocytes derived from women with PCOS, and this is associated with chromosomal pre-division; that is, premature separation of sister chromatids (22). During early pregnancy, the embryo may be exposed to androgen excess *in utero*, which may have long-term effects, particularly on female offspring. Fetal hyperandrogenism may disturb epigenetic programming, particularly those genes regulating reproduction and metabolism (23,24). It is also possible that transgenerational effects may be related to the potential influences of hyperinsulinemia and its effect on the intrauterine environment.

In a meta-analysis in which pregnancy outcomes in women with PCOS were compared with controls, women with PCOS demonstrated a significantly higher risk of developing gestational diabetes mellitus (GDM; odds ratio [OR] 2.94; 95% confidence interval [CI]: 1.70–5.08), pregnancy-induced hypertension (OR 3.67; 95% CI: 1.98–6.81), pre-eclampsia (OR 3.47; 95% CI: 1.95–6.17), and preterm birth (OR 1.75; 95% CI: 1.16–2.62). Their babies had a significantly higher risk of admission to a neonatal intensive care unit (OR 2.31; 95% CI: 1.25–4.26) and a higher perinatal mortality (OR 3.07; 95% CI: 1.03–9.21), unrelated to multiple births (25). In addition, GDM may also result in fetal macrosomia. Obesity in its own right is associated with several adverse pregnancy outcomes, including spontaneous miscarriage, pre-eclampsia, GDM, congenital anomalies (e.g., cardiac and spina bifida), and fetal macrosomia (26).

HYPERANDROGENEMIA

Hyperandrogenism, in conjunction with hyperinsulinemia, is a cardinal feature of PCOS. It is plausible that the follicular microenvironment is related to oocyte quality. Teissier et al. showed that follicular testosterone levels were significantly elevated in PCOS, especially in meiotically incompetent oocytes (27). High androgen levels may therefore contribute to the lower fertilization rate among the oocytes retrieved from a PCO. Conversely, a positive correlation exists between testosterone concentration and the number of antral follicles (2–5 mm in diameter). Pretreatment with an aromatase inhibitor increases the ovarian androgen level, improving ovarian response in the low responder within an *in vitro* fertilization (IVF) treatment (28). Increased androgen levels therefore result in an increased recruitment of primordial follicles form the resting pool.

On the day of human chorionic gonadotropin (hCG) administration, the PCOS subject will have a higher estradiol concentration. This may, in part, be explained by an increase in androgen substrates and aromatase activity (29). Under *in vitro* conditions, granulosa cells from a PCO exhibit an increased response to follicle-stimulating hormone (FSH) stimulation compared to size-matched controls (30,31), potentially due to the higher number of FSH receptors attributed to the stimulatory effects of androgen on FSH receptor synthesis (32). Androgen enhances the production of ovarian steroids in response to gonadotropin stimulation whilst promoting the expression of IGF-1 and IGF-1 receptor genes in the growing follicles up to the small antral stage (33).

Serum androgen levels rise during ovarian stimulation and are higher in PCOS patients. The higher levels are suggested to negatively impact on pregnancy outcome (34). A negative correlation exists between androgen concentration and uterine placental protein (PP14, also known as glycodelin) (35) in women with PCOS and recurrent miscarriage. Glycodelin is an important secretory protein from the endometrium and is a marker of endometrial receptivity (36). It is increased in successful conception IVF cycles. Androstenedione causes a dose-dependent reduction in glycodelin and endometrial cell proliferation (37), which are inhibited by administration of an anti-androgen, cyproterone. A reduction in sex hormone-binding globulin in the endometrial stroma in PCOS increases the bioavailability of androgens (38). With an overall increase in expression of endometrial androgen receptors, high levels of estrogen and androgen up-regulate this expression, whilst progesterone has the opposite effect (39). $\alpha V\beta 3$ integrin, a cell adhesion molecule, is suppressed at the time of implantation by high androgen levels, further reflecting endometrial reduced receptivity (40).

OBESITY

Central obesity is a major factor influencing outcomes of both treatment of symptoms and infertility in women with PCOS. Obesity is seen in 38%–66% of those with PCOS, with body mass index (BMI) correlating with severity of the phenotypical features. Clinical pregnancy rates are significantly lower in the obese in both natural and assisted conception cycles (41,42). Jungheim et al. have shown in a retrospective cohort that a BMI >40 kg/m^2 in women with PCOS who undergo IVF significantly reduced the clinical pregnancy rate (32% vs. 72%, relative risk 0.44) (43). This also translated to a reduced live birth rate, although this failed to reach significance (32% vs. 60%) (43). A reduced fertilization rate, fewer oocytes, and reduced peak estradiol level highlight the impaired follicular and oocyte response in the morbidly obese. Other problems related to obesity include miscarriage and cancellation of assisted reproductive cycles (44). Fedorcsak et al. (45) concluded that obesity, independent of insulin resistance, is associated with an increase in miscarriage and gonadotropin resistance and a reduction in oocyte

number. Dokras et al. found a 25.3% IVF cycle cancellation rate (BMI >40 kg/m^2) compared with 10.9% in those with a normal BMI (OR 2.73, 95% CI: 1.49–5.00) (46). Increased FSH is needed in the obese to stimulate the ovary, but once the threshold has been reached, the subsequent response can be dramatic, leading to an uncontrolled response and a risk of ovarian hyperstimulation syndrome (OHSS) (47,48). This may be related in part to insulin resistance.

We should not purely focus on conception as the main problem of the obese. As the epidemic of obesity gains velocity, the importance of maternal health should remain paramount. The triennial confidential enquiry into maternal death—"Saving lives, improving mothers' care"—is sobering reading, with obesity implicated in 27.1% of maternal deaths in the U.K. (49) Both obesity and PCOS increase the risk of developing GDM, pre-eclampsia, and preterm birth. Obesity also increases the need for operative delivery, with the ensuing problems of wound infection and venous thromboembolism. Targeted preconceptional counseling is paramount to reducing the spiraling problems related to obesity, fertility, and childbirth. The national guidance of needing to obtain a BMI of less than 30 kg/m^2 in order to be eligible for National Health Service (NHS) fertility treatment within the U.K. goes a small way to promoting these salient facts.

OVARIAN STIMULATION RESPONSE IN PCOS

Ovulation induction for women with PCOs requires a different approach to that for women with normal ovaries. The response is often initially slow, but then may spiral rapidly to a picture of over-response, with a significant risk of OHSS and cyst formation (50,51). Theoretically, one would expect an altered response in the multifollicular recruitment required for conventional IVF, and indeed this has been illustrated in a number of studies (51). Dor et al. showed a significant increase in oocyte number recovered per cycle when compared with tubal factor infertility (19.4 vs. 5.4, $p < 0.005$), but a lower fertilization rate (40.4% vs. 67.6%, $p < 0.001$) (52). MacDougall et al. compared ultrasonographic PCO patients with matched controls (53). PCO patients needed significantly less human menopausal gonadotropin (hMG), but still reached a significantly higher estradiol level on the ovulation trigger day with hCG (5940 vs. 4370 pmol/L, $p < 0.001$). More oocytes were retrieved (9.3 vs. 6.8, $p = 0.003$), but fertilization rates were reduced (52.8% vs. 66.1%, $p = 0.007$). The pregnancy rate per transfer was comparable (25.4% PCO group vs. 23% controls [nonsignificant]). Kodama et al. demonstrated a higher incidence of embryo transfer cancellation due to failed fertilization and OHSS (54). A recent meta-analysis confirmed these findings despite a wide range of demographics and regimens being included (55). Despite comparable pregnancy rates, the miscarriage rate in women with PCOS following IVF remains high compared with women with normal ovaries (35.8% vs. 23.6%, $p = 0.0038$) (56,57). This unwanted outcome is proportional to BMI, increased waist–hip ratio, and insulin resistance. Fedorcsak et al. (58) report a relative risk of 1.77 (95% CI: 1.05–2.97) of miscarriage for women with a BMI over 25 kg/m^2 before 6 weeks of gestation.

Several explanations exist for the excessive response of the PCO to ovarian stimulation. Women with PCOS have an increased number of antral follicles (59). Contrary to earlier theories, these follicles are not atretic, but rather there is an increased cohort of selectable antral follicles sensitive to exogenous gonadotropins. Anti-Mullerian hormone (AMH), a dimeric glycoprotein produced from the granulosa cells of the pre-antral and antral follicles, is elevated in PCOS (60). AMH has been implicated in two stages of the follicle dysfunction that leads to the development of PCOS (61). Excessive follicle recruitment from the primordial pool is followed by defective selection of the lead follicle, culminating in anovulation. The usual inhibition of follicle recruitment (62) is lost, perhaps due to limited paracrine control from reduced expression of AMH in the primordial follicle pool (63). There is now significant evidence supporting the correlation between AMH, oligo-anovulation, and hyperandrogenemia (61,64–66). Circulating insulin, insulin-like growth factor, and androgen concentrations are all implicated in the higher rate of recruitment (33,67). Elevated AMH can predict the occurrence of OHSS and can help to tailor treatment protocols for those at high risk of over-response (68).

OVARIAN HYPERSTIMULATION SYNDROME

OHSS is the most serious iatrogenic complication of IVF treatment (69). In the most severe cases, hypovolemia, thromboembolism, hemoconcentration, ascites, hydrothorax, pericardial effusion, or adult respiratory distress syndrome can occur. Patients with PCOS undergoing IVF treatment are at a higher risk (70), but the incidence is hard to quantify, ranging from 10% to 18% (71). The documentation of OHSS as a study endpoint has historically been neglected in this high-risk population, as a meta-analysis has shown (46). Higher estradiol concentrations and oocyte numbers occur in those who develop OHSS (72). The cardinal feature of the pathogenesis of OHSS is an increased capillary permeability (73).

Vascular endothelial growth factor (VEGF) is a potent angiogenic endothelial cell mitogen and a key mediator of OHSS (74). Serum and follicular levels are higher in those women with PCOS and those who develop OHSS on the day of egg collection (75). Estradiol and VEGF levels positively correlate on the day of hCG administration, with VEGF being the best predictor of OHSS (76). Furthermore, OHSS is more common in a successful pregnancy cycle or IVF cycle using hCG for luteal support (77). Herr et al. demonstrated the direct effect of hCG on the expression of VEGF mRNA within human luteinized granulosa cells (78). The expression is higher in OHSS subjects compared with controls (79). Miele et al. demonstrated that insulin and IGF-1 increase VEGF mRNA expression (80). The synergistic effect of insulin with gonadotropin and hCG was confirmed by Agrawal et al. (81). More recently, Stanek et al. confirmed the effects of insulin and IGF on VEGF production in PCOS and non-PCOS women (82).

SUPEROVULATION STRATEGIES

Pituitary desensitization with a gonadotropin-releasing hormone (GnRH) agonist has become a universal concept within assisted conception regimens. Reversible hypogonadotropic hypogonadism allows enhanced control of follicular development and improved pregnancy rates in IVF cycles (83). Suppression of endogenous luteinizing hormone (LH) by GnRH agonists may be advantageous for the sensitive PCO, allowing follicular development to occur without the adverse effects of high LH concentrations (84). These oocytes appear to fertilize better than those obtained in cycles without pituitary desensitization.

Few studies exist comparing duration of treatment with GnRH analogs in women with and without PCOS in IVF cycles (85). A prolonged pituitary desensitization (30 days rather than 15) avoids the initial surge of gonadotropins and the resultant ovarian steroid release seen with shorter treatments. Androgen levels may be reduced and an increase in exogenous gonadotropin dosage is not required. Although pregnancy rates are not improved, ovarian hyperstimulation is reduced (86). Unfortunately, there are too few specific randomized controlled comparisons for women with PCOS.

The pros and cons of different gonadotropin preparations have been debated for years. After the initial use of hMG and then highly purified urinary FSH came the recombinant preparations of FSH (rFSH), LH, and hCG. Overall, there appears to be little difference in outcomes when all studies are combined. Teissier et al. demonstrated that women with PCOS undergoing IVF using hMG compared with rFSH have a higher testosterone and estradiol level due to higher LH (87). A meta-analysis has shown no difference in outcome between hMG or rFSH when used in conjunction with a long GnRH agonist protocol (88). There was no significant difference in number of oocytes retrieved, live birth rates, miscarriage, multiple pregnancy, or OHSS. This was confirmed by Andersen et al. with respect to pregnancy outcomes (89).

The more recent introduction of regimens using a GnRH antagonist for pituitary suppression holds promise for PCOS patients. GnRH antagonists do not activate the GnRH receptors and produce a rapid suppression of gonadotropin secretion within hours (90). This offers the potential for shorter treatments compared with the long protocol using a GnRH agonist. Previously, it was suggested that the antagonist protocol led to a reduction in clinical and live birth rates (91). In contrast, a Cochrane review showed no evidence of a statistically significant difference in live birth rate (nine randomized controlled trials [RCTs]; OR 0.86, 95% CI: 0.69–1.08). There was a statistically significant lower incidence of OHSS in the GnRH antagonist group (29 RCTs; OR 0.43, 95% CI: 0.33–0.57) (92). Consideration has recently been given to use of a GnRH antagonist within an ovulation induction protocol to reduce the risk of premature luteinization. Although a small improvement was seen in live birth rate, this was not significant (93).

In another study, patients with PCOS undergoing IVF in a GnRH antagonist protocol were found to have earlier follicular growth and higher estradiol concentration during rFSH stimulation compared with those on a long GnRH agonist regimen (94). Despite a shorter stimulation phase, the number of oocytes and the fertilization and clinical pregnancy rates were not different, but the risk of OHSS was significantly lower. This is consistent with results of early trials (95) and the meta-analysis conducted by Griesinger et al., who concluded for PCOS that the clinical outcome is the same irrespective of the protocols, apart from a reduction in OHSS (96). Lainas et al. again revisited this concept in an RCT confirming similar ongoing pregnancy rates between the two protocols (97). Despite the reduction in those experiencing OHSS, overall there remains a significant majority with OHSS who have PCOS, even when using an antagonist protocol (65.2% vs. 8.1%; $p < 0.05$) (98).

Native GnRH or a GnRH agonist can displace the antagonist from the pituitary GnRH receptors. This realizes the potential to use a GnRH agonist as the final trigger for the LH surge and subsequent oocyte maturation (99). Kol and Itskovitz-Eldor demonstrated a rapid rise in LH after administration of a GnRH agonist, with a peak in levels four hours after injection (100). This trigger is potentially more physiological, with a lower risk of OHSS, due to the shorter half-life of LH (60 minutes vs. 32–34 hours). Fauser et al. have produced comparable data between an agonist trigger and hCG with respect to number of oocytes retrieved, quality of embryos, and implantation and pregnancy rates (101). This is supported by more recent studies (102,103). Unfortunately, this is not supported in the most recent Cochrane review by Al-Inany et al. (92), who demonstrated an inferior live birth rate (OR 0.47, 95% CI: 0.31–0.70; five RCTs, 532 women), but the reduction in OHSS (OR 0.15, 95% CI: 0.05–0.47; eight RCTs, 989 women) was certainly an advantage . The compromised live birth rate is believed to be due to deficient luteal-phase support. LH's shorter half-life is less able to support the corpus luteum in a developing pregnancy. Optimized luteal-phase support with a combination of low-dose hCG, estradiol, and progesterone has been shown to improve the clinical outcome and equal the rates achieved with hCG (104). Two recent retrospective reviews (105,106) support this approach. Iliodromiti et al. administered a bolus of hCG 1500 IU on the day of oocyte retrieval, followed by daily progesterone and estradiol. Using this combination, the authors achieved a clinical pregnancy rate of 41.8% per cycle started, with a severe OHSS rate of 0.72% (105). Datta et al. used a similar luteal-phase support program and made a comparison with their hCG-triggered cycles. These authors achieved a comparable pregnancy rate per embryo transfer (GnRH agonist 40.7% vs. hCG 35.0%), with reductions in freeze-all cycles and incidence of mild to moderate OHSS (GnRH agonist 16.2% vs. hCG 31.0%) in favor of the GnRH agonist trigger (106).

Further suggested mechanisms to induce final follicular maturation include recombinant LH and kisspeptin. A multicenter, double-blind study revealed that recombinant human LH can be as effective as hCG at inducing the final follicular maturation in IVF treatment with a lower incidence of OHSS (107). Kisspeptin, an upstream modulator of GnRH release, has been used in a proof-of-concept study to trigger oocyte maturation in 53 patients and achieve a clinical pregnancy rate of 23% (108). Kisspeptin has yet to be used in clinical high-response group such as those with PCOS. For both of these agents, the clinical application within assisted reproduction technology has yet to be fully elucidated.

Currently, we recommend an antagonist protocols for women with PCOS or PCO who require IVF with consideration of the agonist trigger and modified luteal-phase support.

INSULIN RESISTANCE AND METFORMIN

Insulin resistance is a key factor coupled with hyperandrogenemia in the pathophysiology of PCOS. Insulin resistance is thought to arise from aberrant phosphorylation of tyrosine and serine residues on the insulin receptor, resulting in increasing insulin resistance and compensatory hyperinsulinemia. As insulin binds IGF-1 receptors, it augments the response of theca cells to LH, resulting in disordered steroidogenesis and excess androgen production. Hyperinsulinemia results in reduced hepatic synthesis of sex hormone binding globulin and insulin-like growth factor binding protein-1. In turn, this increases the bioavailability of both androgens and IGF-1 and -2, which are important regulators of ovarian follicular maturation and steroidogenesis (109–111). Insulin resistance has been stipulated as a risk factor for cardiovascular disease in high-risk populations such as those with PCOS (112). Measurement of insulin resistance in this population is best screened for using a traditional oral glucose tolerance test or hemoglobin A1c (113).

With hyperinsulinemia being well recognized in women with PCOS, it is reasonable to assume that the use of insulin-sensitizing drugs should improve many aspects of the syndrome, with respect to both metabolic and reproductive function. Metformin, an oral biguanide, is the most widely researched agent in this category. Metformin reduces hepatic gluconeogenesis, increasing peripheral glucose utilization and mediating receptor kinase activity within numerous cells, including the theca and granulosa cells. A recent systematic review of insulin-sensitizing agents concluded that there was no evidence to suggest metformin used alone improved live birth rates (114). Furthermore, there was no improvement in live birth rate when used in combination with clomiphene versus clomiphene only (seven RCTs, 907 participants; OR 1.16, 95% CI: 0.85–1.56). These authors surmise that metformin's role in improving reproductive outcomes in women with PCOS is limited (114).

The use of metformin as an adjunct within the context of IVF has also been explored. The most recent Cochrane review cites nine RCTs to answer this question (110). All but one of these used a GnRH agonist for down-regulation (115). The total dose and duration of metformin use is not standardized, ranging from 500 mg twice a day to 850 mg three times a day taken for up to 16 weeks, usually up to hCG trigger. Fleming et al. (116) demonstrated that a protracted treatment of metformin over four months may decrease the antral follicle count and AMH levels; however, this was not shown to improve the number of oocytes retrieved or fertilization rates. Tang et al. reported a significant improvement in live birth rates for those taking metformin over a much shorter period of time (from the commencement of GnRH agonist to the day of hCG in a long protocol), with rates of 32.7% versus 12.2% in the placebo arm (117). The lower-than-expected birth rate in the placebo group is difficult to explain, and may be secondary to subtle effects on oocyte/embryo quality or endometrial development. Kjotrod et al. corroborated the findings of Tang et al. by suggesting that the live birth rate may be improved in lean women with PCOS (118,119). Furthermore, a study of 112 women with a BMI <28 kg/m^2 showed that the live birth rate was also higher when metformin was given over 12 weeks (48.6% vs. 32.0%; 95% CI: 1.1–32.2, $p = 0.0383$) (119). No advantage has been shown for women with isolated PCOs without the syndrome (120).

The consistent advantage of using metformin appears to be a reduction in OHSS (OR 0.29, 95% CI: 0.18–0.49; 798 women), as shown in a recent Cochrane review (110). The use of GnRH antagonists in IVF protocols also reduces the risk of OHSS. Doldi et al. presented their findings of a reduction in OHSS from 15% with placebo to 5% with metformin (115). Although promising, this study was inadequately powered to show a significant improvement. A prospective RCT is awaiting publication from our own unit, which concluded that there was no benefit in the addition of metformin to an antagonist IVF cycle with respect to OHSS or live birth rates (ISRCTN 21199799).

Metformin has been observed to reduce serum testosterone concentration (1.96 vs. 2.52 nmol/L, $p = 0.269$) and free androgen index (FAI) (2.43 vs. 3.34) on the day of hCG administration (117). A negative correlation exists between day-12 post-embryo transfer hCG levels and FAI. Through speculation, alleviation of hyperandrogenism and insulin resistance at the ovarian level may improve folliculogenesis and therefore the developmental potential of the embryo. Tang et al. showed no difference in average embryo score of the transferred embryos despite an improvement in clinical pregnancy rate (CPR) and live birth rate (LBR); hence, the changes may be on a metabolomic level that has not yet been classified (117). Serum VEGF and estradiol concentrations on the day of hCG administration are also greatly reduced in those on metformin. By ameliorating the expression of VEGF, the risk of OHSS can be reduced.

Thus, whilst there is variable data on whether metformin improves the "take home baby rate" after IVF, it does

reduce the risk of moderate to severe OHSS in these high-risk patients with PCOS undergoing a GnRH agonist cycle.

IN VITRO MATURATION

In vitro maturation (IVM) has attracted a lot of attention as a new assisted reproductive technique (121). Immature oocytes are retrieved transvaginally from antral follicles from either unstimulated or minimally stimulated ovaries (122). The oocyte matures *in vitro* in a specially formulated medium for 24–48 hours. The oocyte is then fertilized, usually with intracytoplasmic sperm injection (ICSI), and the selected embryo(s) are transferred two to three days later. Although more labor intensive, the potential clinical advantage is that patients generally require less monitoring and, most importantly, avoid the risk of OHSS. For those with PCOS, IVM offers a promising alternative to conventional IVF (123).

The maturation rate of oocytes retrieved from patients with PCOS has been lower than those with normal ovaries (122). However, priming with hCG or gonadotropin before the retrieval has been shown to improve maturation rates from unstimulated PCOs (124,125). In a prospective observational study, Child et al. (121) showed that significantly more immature oocytes are retrieved from the PCO than the normal ovary (10 ± 5.1 vs. 5.1 ± 3.7). The overall maturation and fertilization rates were comparable, but the pregnancy and live birth rates were significantly higher in the PCO/PCOS groups. This is explained in part by the greater number of embryos available to select from, but also the patients with PCOS were considerably younger within the study. Research continues into identifying prognostic indicators of the developmental potential of the embryos. Evaluating the cumulus layer is a potentially useful and simple procedure that may provide a scoring system in this evolving subject (126).

IVM compared with conventional IVF yields significantly fewer mature oocytes (7.8 vs. 12.0, $p < 0.01$), with significantly lower implantation rates (127). The lower implantation rates may be due to a reduced oocyte potential, a higher frequency of abnormal meiotic spindles and chromosomal alignment, or reduced endometrial receptivity (128). It is important to ensure that infants born through such treatment remain healthy in the long term. A prospective observational study on 41 pregnancies showed no increase in preterm birth, birthweight, or major structural malformation as compared with pregnancies achieved through conventional IVF (129). However, much larger studies are required to provide robust safety data on this new technology.

In a Cochrane systematic review, Siristatidis et al. concluded that no RCTs exist upon which to base practice recommendations regarding IVM before IVF or ICSI in women with PCOS (130). Whilst continued improvement in culture medium and synchrony between endometrial and embryonic development may result in improved success rates, IVM as a treatment has yet to be adopted as a routine clinical entity, and may remain purely a research interest.

CONCLUSION

Women with PCOS undergoing IVF cycles respond differently to women with normal ovaries. For the obese, weight loss and lifestyle modification should remain pivotal in the management of infertility, improving success rates and reducing potentially serious outcomes both in the short and longer term. The GnRH antagonist protocol provides a safe alternative to the traditional GnRH agonist, culminating in similar live birth rates but much reduced OHSS severity. The GnRH antagonist protocol allows use of the GnRH agonist trigger and modified luteal-phase support to further reduce the incidence of OHSS. Metformin remains a useful adjunct in the potential reduction of OHSS. IVM is a promising treatment that may ameliorate many of these issues for the notoriously difficult to manage PCO.

REFERENCES

1. Stein I, Leventhal M. Amenorrhoea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol* 1935; 29(2): 181–91.
2. Polson DW, Adams J, Wadsworth J, Franks S. Polycystic ovaries—A common finding in normal women. *Lancet* 1988; 1(8590): 870–2.
3. Tsilchorozidou T, Overton C, Conway GS. The pathophysiology of polycystic ovary syndrome. *Clin Endocrinol* 2004; 60(1): 1–17.
4. Diamanti-Kandarakis E, Piperi C, Spina J et al. Polycystic ovary syndrome: The influence of environmental and genetic factors. *Hormones* 2006; 5(1): 17–34.
5. Clark AM, Thornley B, Tomlinson L, Galletley C, Norman RJ. Weight loss in obese infertile women results in improvement in reproductive outcome for all forms of fertility treatment. *Hum Reprod* 1998; 13(6): 1502–5.
6. Amer SA, Li TC, Metwally M, Emarh M, Ledger WL. Randomized controlled trial comparing laparoscopic ovarian diathermy with clomiphene citrate as a first-line method of ovulation induction in women with polycystic ovary syndrome. *Hum Reprod* 2009; 24(1): 219–25.
7. Balen AH, Jacobs HS. A prospective study comparing unilateral and bilateral laparoscopic ovarian diathermy in women with the polycystic ovary syndrome. *Fertil Steril* 1994; 62(5): 921–5.
8. Buyalos RP, Lee CT. Polycystic ovary syndrome: Pathophysiology and outcome with *in vitro* fertilization. *Fertil Steril* 1996; 65(1): 1–10.
9. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; 19(1): 41–7.
10. Balen AH, Laven JSE, Tan S-L, Dewailly D. Ultrasound assessment of the polycystic ovary: International consensus definitions. *Hum Reprod Update* 2003; 9(6): 505–14.

11. Jonard S, Robert Y, Cortet-Rudelli C, Pigny P, Decanter C, Dewailly D. Ultrasound examination of polycystic ovaries: Is it worth counting the follicles? *Hum Reprod* 2003; 18(3): 598–603.
12. Dewailly D, Lujan ME, Carmina E et al. Definition and significance of polycystic ovarian morphology: A task force report from the androgen excess and polycystic ovary syndrome society. *Hum Reprod Update* 2014; 20(3): 334–52.
13. Michelmore KF, Balen AH, Dunger DB, Vessey MP. Polycystic ovaries and associated clinical and biochemical features in young women. *Clin Endocrinol* 1999; 51(6): 779–86.
14. Rodin DA, Bano G, Bland JM, Taylor K, Nussey SS. Polycystic ovaries and associated metabolic abnormalities in Indian subcontinent Asian women. *Clin Endocrinol* 1998; 49(1): 91–9.
15. Wijeyaratne CN, Balen AH, Barth JH, Belchetz PE. Clinical manifestations and insulin resistance (IR) in polycystic ovary syndrome (PCOS) among South Asians and Caucasians: Is there a difference? *Clin Endocrinol* 2002; 57(3): 343–50.
16. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: A prospective study. *J Clin Endocrinol Metab* 1998; 83(9): 3078–82.
17. Dumesic DA, Padmanabhan V, Abbott DH. Polycystic ovary syndrome and oocyte developmental competence. *Obstet Gynecol Surv* 2008; 63(1): 39–48.
18. Weghofer A, Munne S, Chen S, Barad D, Gleicher N. Lack of association between polycystic ovary syndrome and embryonic aneuploidy. *Fertil Steril* 2007; 88(4): 900–5.
19. Rice S, Christoforidis N, Gadd C et al. Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. *Hum Reprod* 2005; 20(2): 373–81.
20. Skov V, Glintborg D, Knudsen S et al. Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of insulin-resistant women with polycystic ovary syndrome. *Diabetes* 2007; 56(9): 2349–55.
21. Wood JR, Dumesic DA, Abbott DH, Strauss JF, 3rd. Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis. *J Clin Endocrinol Metab* 2007; 92(2): 705–13.
22. Harris SE, Maruthini D, Tang T, Balen AH, Picton HM. Metabolism and karyotype analysis of oocytes from patients with polycystic ovary syndrome. *Hum Reprod* 2010; 25(9): 2305–15.
23. Hickey TE, Legro RS, Norman RJ. Epigenetic modification of the X chromosome influences susceptibility to polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; 91(7): 2789–91.
24. Li Z, Huang H. Epigenetic abnormality: A possible mechanism underlying the fetal origin of polycystic ovary syndrome. *Med Hypotheses* 2008; 70(3): 638–42.
25. Boomsma CM, Eijkemans MJC, Hughes EG, Visser GHA, Fauser BCJM, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. *Hum Reprod Update* 2006; 12(6): 673–83.
26. Wax JR. Risks and management of obesity in pregnancy: Current controversies. *Curr Opin Obstet Gynecol* 2009; 21(2): 117–23.
27. Teissier MP, Chable H, Paulhac S, Aubard Y. Comparison of follicle steroidogenesis from normal and polycystic ovaries in women undergoing IVF: Relationship between steroid concentrations, follicle size, oocyte quality and fecundability. *Hum Reprod* 2000; 15(12): 2471–7.
28. Garcia-Velasco JA, Moreno L, Pacheco A et al. The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves *in vitro* fertilization outcome in low responder patients: A pilot study. *Fertil Steril* 2005; 84(1): 82–7.
29. la Marca A, Morgante G, Palumbo M, Cianci A, Petraglia F, De Leo V. Insulin-lowering treatment reduces aromatase activity in response to follicle-stimulating hormone in women with polycystic ovary syndrome. *Fertil Steril* 2002; 78(6): 1234–9.
30. Misajon A, Hutchinson P, Lolatgis N, Trounson AO, Almahbobi G. The mechanism of action of epidermal growth factor and transforming growth factor alpha on aromatase activity in granulosa cells from polycystic ovaries. *Mol Hum Reprod* 1999; 5(2): 96–103.
31. Willis DS, Watson H, Mason HD, Galea R, Brincat M, Franks S. Premature response to luteinizing hormone of granulosa cells from anovulatory women with polycystic ovary syndrome: Relevance to mechanism of anovulation. *J Clin Endocrinol Metab* 1998; 83(11): 3984–91.
32. Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab* 1999; 84(8): 2951–6.
33. Vendola K, Zhou J, Wang J, Bondy CA. Androgens promote insulin-like growth factor-I and insulin-like growth factor-I receptor gene expression in the primate ovary. *Hum Reprod* 1999; 14(9): 2328–32.
34. Check JH, Nazari A, Dietterich C. Comparison of androgen levels in conception vs. non-conception cycles following controlled ovarian stimulation using the luteal phase gonadotropin-releasing hormone agonist protocol. *Gynecol Endocrinol* 1995; 9(3): 209–14.
35. Okon MA, Laird SM, Tuckerman EM, Li TC. Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. *Fertil Steril* 1998; 69(4): 682–90.

36. Westergaard LG, Yding Andersen C, Erb K et al. Placental protein 14 concentrations in circulation related to hormonal parameters and reproductive outcome in women undergoing IVF/ICSI. *Reprod Biomed Online* 2004; 8(1): 91–8.
37. Tuckerman EM, Okon MA, Li T, Laird SM. Do androgens have a direct effect on endometrial function? An *in vitro* study. *Fertil Steril* 2000; 74(4): 771–9.
38. Maliqueo M, Bacallao K, Quezada S et al. Sex hormone-binding globulin expression in the endometria of women with polycystic ovary syndrome. *Fertil Steril* 2007; 87(2): 321–8.
39. Giudice LC. Endometrium in PCOS: Implantation and predisposition to endocrine CA. *Best Pract Res Clin Endocrinol Metab* 2006; 20(2): 235–44.
40. Rose GL, Dowsett M, Mudge JE, White JO, Jeffcoate SL. The inhibitory effects of danazol, danazol metabolites, gestrinone, and testosterone on the growth of human endometrial cells *in vitro*. *Fertil Steril* 1988; 49(2): 224–8.
41. Wass P, Waldenstrom U, Rossner S, Hellberg D. An android body fat distribution in females impairs the pregnancy rate of *in-vitro* fertilization-embryo transfer. *Hum Reprod* 1997; 12(9): 2057–60.
42. Fedorcsak P, Dale PO, Storeng R, Tanbo T, Abyholm T. The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome. *Hum Reprod* 2001; 16(6): 1086–91.
43. Jungheim ES, Lanzendorf SE, Odem RR, Moley KH, Chang AS, Ratts VS. Morbid obesity is associated with lower clinical pregnancy rates after *in vitro* fertilization in women with polycystic ovary syndrome. *Fertil Steril* 2009; 92(1): 256–61.
44. Brewer CJ, Balen AH. The adverse effects of obesity on conception and implantation. *Reproduction* 2010; 140(3): 347–64.
45. Fedorcsak P, Dale PO, Storeng R et al. Impact of overweight and underweight on assisted reproduction treatment. *Hum Reprod* 2004; 19(11): 2523–8.
46. Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH. Screening women with polycystic ovary syndrome for metabolic syndrome. *Obstet Gynecol* 2005; 106(1): 131–7.
47. Dale PO, Tanbo T, Haug E, Abyholm T. The impact of insulin resistance on the outcome of ovulation induction with low-dose follicle stimulating hormone in women with polycystic ovary syndrome. *Hum Reprod* 1998; 13(3): 567–70.
48. Loveland JB, McClamrock HD, Malinow AM, Sharara FI. Increased body mass index has a deleterious effect on *in vitro* fertilization outcome. *J Assist Reprod Genet* 2001; 18(7): 382–6.
49. MBRRACE-UK. Available from: www.npeu.ox.ac.uk/mbrrace-uk/reports
50. Balen A. The effects of ovulation induction with gonadotrophins on the ovary and uterus and implications for assisted reproduction. *Hum Reprod* 1995; 10(9): 2233–7.
51. Shoham Z, Conway GS, Patel A, Jacobs HS. Polycystic ovaries in patients with hypogonadotropic hypogonadism: Similarity of ovarian response to gonadotropin stimulation in patients with polycystic ovarian syndrome. *Fertil Steril* 1992; 58(1): 37–45.
52. Dor J, Shulman A, Levran D, Ben-Rafael Z, Rudak E, Mashiach S. The treatment of patients with polycystic ovarian syndrome by *in-vitro* fertilization and embryo transfer: A comparison of results with those of patients with tubal infertility. *Hum Reprod* 1990; 5(7): 816–8.
53. MacDougall MJ, Tan SL, Balen A, Jacobs HS. A controlled study comparing patients with and without polycystic ovaries undergoing *in-vitro* fertilization. *Hum Reprod* 1993; 8(2): 233–7.
54. Kodama H, Fukuda J, Karube H, Matsui T, Shimizu Y, Tanaka T. High incidence of embryo transfer cancellations in patients with polycystic ovarian syndrome. *Hum Reprod* 1995; 10(8): 1962–7.
55. Heijnen EMEW, Eijkemans MJC, Hughes EG, Laven JSE, Macklon NS, Fauser BCJM. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. *Hum Reprod Update* 2006; 12(1): 13–21.
56. Balen AH, Tan SL, MacDougall J, Jacobs HS. Miscarriage rates following *in-vitro* fertilization are increased in women with polycystic ovaries and reduced by pituitary desensitization with buserelin. *Hum Reprod* 1993; 8(6): 959–64.
57. Ludwig M, Finas DF, al-Hasani S, Diedrich K, Ortmann O. Oocyte quality and treatment outcome in intracytoplasmic sperm injection cycles of polycystic ovarian syndrome patients. *Hum Reprod* 1999; 14(2): 354–8.
58. Fedorcsak P, Storeng R, Dale PO, Tanbo T, Abyholm T. Obesity is a risk factor for early pregnancy loss after IVF or ICSI. *Acta Obstet Gynecol Scand* 2000; 79(1): 43–8.
59. Dewailly D, Catteau-Jonard S, Reyss AC, Maunoury-Lefebvre C, Poncelet E, Pigny P. The excess in 2-5 mm follicles seen at ovarian ultrasonography is tightly associated to the follicular arrest of the polycystic ovary syndrome. *Hum Reprod* 2007; 22(6): 1562–6.
60. Pellat L, Rice S, Mason HD. Anti-Mullerian hormone and polycystic ovary syndrome: A mountain too high? *Reproduction* 2010; 139(5): 825–33.
61. Piouka A, Farmakiotis D, Katsikis I, Macut D, Gerou S, Panidis D. Anti-Mullerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: Relationship with increased luteinizing hormone levels. *Am J Physiol Endocrinol Metab* 2009; 296(2): E238–43.
62. Durlinger AL, Kramer P, Karels B et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. *Endocrinology* 1999; 140(12): 5789–96.

63. Stubbs SA, Hardy K, Da Silva-Buttkus P et al. Anti-Mullerian hormone protein expression is reduced during the initial stages of follicle development in human polycystic ovaries. *J Clin Endocrinol Metab* 2005; 90(10): 5536–43.
64. Dewailly D, Andersen CY, Balen A et al. The physiology and clinical utility of anti-Mullerian hormone in women. *Hum Reprod Update* 2014.
65. Casadei L, Madrigale A, Puca F et al. The role of serum anti-Mullerian hormone (AMH) in the hormonal diagnosis of polycystic ovary syndrome. *Gynecol Endocrinol* 2013; 29(6): 545–50.
66. Dewailly D, Gronier H, Poncelet E et al. Diagnosis of polycystic ovary syndrome (PCOS): Revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. *Hum Reprod* 2011; 26(11): 3123–9.
67. Kezele PR, Nilsson EE, Skinner MK. Insulin but not insulin-like growth factor-1 promotes the primordial to primary follicle transition. *Mol Cell Endocrinol* 2002; 192(1-2): 37–43.
68. Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJM. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. *Hum Reprod Update* 2011; 17(1): 46–54.
69. Brinsden PR, Wada I, Tan SL, Balen A, Jacobs HS. Diagnosis, prevention and management of ovarian hyperstimulation syndrome. *Br J Obstet Gynaecol* 1995; 102(10): 767–72.
70. Delvigne A, Demoulin A, Smitz J et al. The ovarian hyperstimulation syndrome in *in-vitro* fertilization: A Belgian multicentric study. I. Clinical and biological features. *Hum Reprod* 1993; 8(9): 1353–60.
71. Swanton A, Storey L, McVeigh E, Child T. IVF outcome in women with PCOS, PCO and normal ovarian morphology. *Eur J Obstet Gynecol Reprod Biol* 2010; 149(1): 68–71.
72. Agrawal R, Sladkevicius P, Engmann L et al. Serum vascular endothelial growth factor concentrations and ovarian stromal blood flow are increased in women with polycystic ovaries. *Hum Reprod* 1998; 13(3): 651–5.
73. Whelan JG, 3rd, Vlahos NF. The ovarian hyperstimulation syndrome. *Fertil Steril* 2000; 73(5): 883–96.
74. Pellicer A, Albert C, Mercader A, Bonilla-Musoles F, Remohi J, Simon C. The pathogenesis of ovarian hyperstimulation syndrome: *In vivo* studies investigating the role of interleukin-1beta, interleukin-6, and vascular endothelial growth factor. *Fertil Steril* 1999; 71(3): 482–9.
75. Artini PG, Monti M, Matteucci C, Valentino V, Cristello F, Genazzani AR. Vascular endothelial growth factor and basic fibroblast growth factor in polycystic ovary syndrome during controlled ovarian hyperstimulation. *Gynecol Endocrinol* 2006; 22(8): 465–70.
76. Agrawal R, Tan SL, Wild S et al. Serum vascular endothelial growth factor concentrations in *in vitro* fertilization cycles predict the risk of ovarian hyperstimulation syndrome. *Fertil Steril* 1999; 71(2): 287–93.
77. Jonard S, Dewailly D. The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Hum Reprod Update* 2004; 10(2): 107–17.
78. Herr F, Baal N, Reisinger K et al. HCG in the regulation of placental angiogenesis. Results of an *in vitro* study. *Placenta* 2007; 28(Suppl A): S85–93.
79. Wang TH, Horng SG, Chang CL et al. Human chorionic gonadotropin-induced ovarian hyperstimulation syndrome is associated with up-regulation of vascular endothelial growth factor. *J Clin Endocrinol Metab* 2002; 87(7): 3300–8.
80. Miele C, Rochford JJ, Filippa N, Giorgetti-Peraldi S, Van Obberghen E. Insulin and insulin-like growth factor-I induce vascular endothelial growth factor mRNA expression via different signaling pathways. *J Biol Chem* 2000; 275(28): 21695–702.
81. Agrawal R, Jacobs H, Payne N, Conway G. Concentration of vascular endothelial growth factor released by cultured human luteinized granulosa cells is higher in women with polycystic ovaries than in women with normal ovaries. *Fertil Steril* 2002; 78(6): 1164–9.
82. Stanek MB, Borman SM, Molskness TA, Larson JM, Stouffer RL, Patton PE. Insulin and insulin-like growth factor stimulation of vascular endothelial growth factor production by luteinized granulosa cells: Comparison between polycystic ovarian syndrome (PCOS) and non-PCOS women. *J Clin Endocrinol Metab* 2007; 92(7): 2726–33.
83. Rutherford AJ, Subak-Sharpe RJ, Dawson KJ, Margara RA, Franks S, Winston RM. Improvement of *in vitro* fertilisation after treatment with buserelin, an agonist of luteinising hormone releasing hormone. *Br Med J* 1988; 296(6639): 1765–8.
84. Fleming R, Coutts JR. LHRH analogues for ovulation induction, with particular reference to polycystic ovary syndrome. *Baillieres Clin Obstet Gynaecol* 1988; 2(3): 677–87.
85. Salat-Baroux J, Alvarez S, Antoine JM et al. Comparison between long and short protocols of LHRH agonist in the treatment of polycystic ovary disease by *in-vitro* fertilization. *Hum Reprod* 1988; 3(4): 535–9.
86. Tan SL, Kingsland C, Campbell S et al. The long protocol of administration of gonadotropin-releasing hormone agonist is superior to the short protocol for ovarian stimulation for *in vitro* fertilization. *Fertil Steril* 1992; 57(4): 810–4.
87. Teissier MP, Chable H, Paulhac S, Aubard Y. Recombinant human follicle stimulating hormone versus human menopausal gonadotrophin induction: Effects in mature follicle endocrinology. *Hum Reprod* 1999; 14(9): 2236–41.

88. van Wely M, Kwan I, Burt AL et al. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. *Cochrane Database Syst Rev* 2011; 2: CD005354.
89. Andersen AN, Devroey P, Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: A randomized assessor-blind controlled trial. *Hum Reprod* 2006; 21(12): 3217–27.
90. Al-Inany HG, Youssef MA, Aboulghar M et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011; 5: CD001750.
91. Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. *Cochrane Database Syst Rev* 2006; 3: CD001750.
92. Al-Inany HG, Youssef MAFM, Aboulghar M et al. GnRH antagonists are safer than agonists: An update of a Cochrane review. *Hum Reprod Update* 2011; 17(4): 435.
93. Stadtmauer LA, Sarhan A, Duran EH et al. The impact of a gonadotropin-releasing hormone antagonist on gonadotropin ovulation induction cycles in women with polycystic ovary syndrome: A prospective randomized study. *Fertil Steril* 2011; 95(1): 216–20.
94. Lainas TG, Petsas GK, Zorzovilis IZ et al. Initiation of GnRH antagonist on Day 1 of stimulation as compared to the long agonist protocol in PCOS patients. A randomized controlled trial: Effect on hormonal levels and follicular development. *Hum Reprod* 2007; 22(6): 1540–6.
95. Bahceci M, Ulug U, Ben-Shlomo I, Erden HF, Akman MA. Use of a GnRH antagonist in controlled ovarian hyperstimulation for assisted conception in women with polycystic ovary disease: A randomized, prospective, pilot study. *J Reprod Med* 2005; 50(2): 84–90.
96. Griesinger G, Diedrich K, Tarlatzis BC, Kolibianakis EM. GnRH-antagonists in ovarian stimulation for IVF in patients with poor response to gonadotrophins, polycystic ovary syndrome, and risk of ovarian hyperstimulation: A meta-analysis. *Reprod Biomed Online* 2006; 13(5): 628–38.
97. Lainas TG, Sfontouris IA, Zorzovilis IZ et al. Flexible GnRH antagonist protocol versus GnRH agonist long protocol in patients with polycystic ovary syndrome treated for IVF: A prospective randomised controlled trial (RCT). *Hum Reprod* 2010; 25(3): 683–9.
98. Papanikolaou EG, Pozzobon C, Kolibianakis EM et al. Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist *in vitro* fertilization cycles. *Fertil Steril* 2006; 85(1): 112–20.
99. Engmann L, Siano L, Schmidt D, Nulsen J, Maier D, Benadiva C. GnRH agonist to induce oocyte maturation during IVF in patients at high risk of OHSS. *Reprod Biomed Online* 2006; 13(5): 639–44.
100. Kol S, Itskovitz-Eldor J. Gonadotropin-releasing hormone agonist trigger: The way to eliminate ovarian hyperstimulation syndrome—A 20-year experience. *Semin Reprod Med* 2010; 28(6): 500–5.
101. Fauser BC, de Jong D, Olivennes F et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for *in vitro* fertilization. *J Clin Endocrinol Metab* 2002; 87(2): 709–15.
102. Acevedo B, Gomez-Palomares JL, Ricciarelli E, Hernandez ER. Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates. *Fertil Steril* 2006; 86(6): 1682–7.
103. Babayof R, Margalioth EJ, Huleihel M et al. Serum inhibin A, VEGF and TNFalpha levels after triggering oocyte maturation with GnRH agonist compared with HCG in women with polycystic ovaries undergoing IVF treatment: A prospective randomized trial. *Hum Reprod* 2006; 21(5): 1260–5.
104. Humaidan P, Engmann L, Benadiva C. Luteal phase supplementation after gonadotropin-releasing hormone agonist trigger in fresh embryo transfer: The American versus European approaches. *Fertil Steril* 2015; 103(4): 879–85.
105. Iliodromiti S, Blockeel C, Tremellen KP et al. Consistent high clinical pregnancy rates and low ovarian hyperstimulation syndrome rates in high-risk patients after GnRH agonist triggering and modified luteal support: A retrospective multicentre study. *Hum Reprod* 2013; 28(9): 2529–36.
106. Datta AK, Eapen A, Birch H, Kurinchi-Selvan A, Lockwood G. Retrospective comparison of GnRH agonist trigger with HCG trigger in GnRH antagonist cycles in anticipated high-responders. *Reprod Biomed Online* 2014; 29(5): 552–8.
107. The European Recombinant LH Study Group. Recombinant human luteinizing hormone is as effective as, but safer than, urinary human chorionic gonadotropin in inducing final follicular maturation and ovulation in in vitro fertilization procedures: Results of a multicenter double-blind study. *J Clin Endocrinol Metab* 2001; 86(6): 2607–18.
108. Jayasena CN, Abbara A, Comninos AN et al. Kisspeptin-54 triggers egg maturation in women undergoing *in vitro* fertilization. *J Clin Invest* 2014; 124(8): 3667–77.
109. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Freitas V. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2009; 2: CD006105.
110. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2014; 11: CD006105.

111. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* 2010; 1: CD003053.
112. Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Sr., Wilson PW. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. *Diabetes* 2005; 54(11): 3252–7.
113. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: A systematic review and meta-analysis. *Hum Reprod Update* 2010; 16(4): 347–63.
114. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* 2012; 5: CD003053.
115. Doldi N, Persico P, Di Sebastiano F, Marsiglio E, Ferrari A. Gonadotropin-releasing hormone antagonist and metformin for treatment of polycystic ovary syndrome patients undergoing *in vitro* fertilization-embryo transfer. *Gynecol Endocrinol* 2006; 22(5): 235–8.
116. Fleming R, Harborne L, MacLaughlin DT et al. Metformin reduces serum Mullerian-inhibiting substance levels in women with polycystic ovary syndrome after protracted treatment. *Fertil Steril* 2005; 83(1): 130–6.
117. Tang T, Glanville J, Orsi N, Barth JH, Balen AH. The use of metformin for women with PCOS undergoing IVF treatment. *Hum Reprod* 2006; 21(6): 1416–25.
118. Kjotrod SB, von During V, Carlsen SM. Metformin treatment before IVF/ICSI in women with polycystic ovary syndrome; a prospective, randomized, double blind study. *Hum Reprod* 2004; 19(6): 1315–22.
119. Kjotrod SB, Carlsen SM, Rasmussen PE et al. Use of metformin before and during assisted reproductive technology in non-obese young infertile women with polycystic ovary syndrome: A prospective, randomized, double-blind, multi-centre study. *Hum Reprod* 2011; 26(8): 2045–53.
120. Swanton A, Lighten A, Granne I et al. Do women with ovaries of polycystic morphology without any other features of PCOS benefit from short-term metformin co-treatment during IVF? A double-blind, placebo-controlled, randomized trial. *Hum Reprod* 2011; 26(8): 2178–84.
121. Trounson A, Wood C, Kausche A. *In vitro* maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril* 1994; 62(2): 353–62.
122. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. *In vitro* maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. *Fertil Steril* 2001; 76(5): 936–42.
123. Chian R-C. *In-vitro* maturation of immature oocytes for infertile women with PCOS. *Reprod Biomed Online* 2004; 8(5): 547–52.
124. Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum Reprod* 2000; 15(1): 165–70.
125. Wynn P, Picton HM, Krapez JA, Rutherford AJ, Balen AH, Gosden RG. Pretreatment with follicle stimulating hormone promotes the numbers of human oocytes reaching metaphase II by *in-vitro* maturation. *Hum Reprod* 1998; 13(11): 3132–8.
126. Liu S, Jiang J-J, Feng H-L, Ma S-Y, Li M, Li Y. Evaluation of the immature human oocytes from unstimulated cycles in polycystic ovary syndrome patients using a novel scoring system. *Fertil Steril* 2010; 93(7): 2202–9.
127. Le Du A, Kadoch IJ, Bourcigaux N et al. *In vitro* oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: The French experience. *Hum Reprod* 2005; 20(2): 420–4.
128. Li Y, Feng H-L, Cao Y-J et al. Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured *in vitro*. *Fertil Steril* 2006; 85(4): 827–32.
129. Cha KY, Chung HM, Lee DR et al. Obstetric outcome of patients with polycystic ovary syndrome treated by *in vitro* maturation and *in vitro* fertilization-embryo transfer. *Fertil Steril* 2005; 83(5): 1461–5.
130. Siristatidis CS, Vrachnis N, Creatsa M, Maheshwari A, Bhattacharya S. *In vitro* maturation in subfertile women with polycystic ovarian syndrome undergoing assisted reproduction. *Cochrane Database Syst Rev* 2013; 10: CD006606.

Management of hydrosalpinx

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ANNIKA STRANDELL

INTRODUCTION

In the beginning of the *in vitro* fertilization (IVF) era, tubal factor infertility was the sole indication for the treatment. Today, other indications constitute the majority of treatments and tubal disease may account for as little as 20% in some centers. It is notable that tubal factor infertility is often reported to yield worse results than other causes of infertility. We reported tubal factor infertility to be an independently negative predictive factor of pregnancy and birth, as compared with all other indications (1), in the debate on high multiple pregnancy rates in IVF.

Hydrosalpinx is the severe condition that has attained special interest in research and clinical practice. Tubal diseases like salpingitis isthmica nodosa and other types of proximal tubal occlusions have not been studied exclusively in connection with assisted reproduction technology (ART) and will not be further explored here. This chapter will focus on the problems associated with hydrosalpinx and ART, including diagnosis, prognosis, possible mechanisms, and interventions.

DEFINITIONS AND METHODS OF DIAGNOSIS

Hydrosalpinx is a commonly used term to describe a heterogeneous spectrum of pathology of distal tubal occlusion. A strict definition is a collection of watery fluid in the uterine tube, occurring as the end stage of pyosalpinx. However, hydrosalpinx is used for any distal tubal occlusion regardless of the cause, implying that a non-tubal infection such as an adjacent appendicitis can also cause hydrosalpinx. Furthermore, the end stage of a tubal infection has different appearances: the hydrosalpinx simplex is characterized by excessive distension and thinning of the wall of the uterine tube with the plicae being few and widely separated, while the hydrosalpinx follicularis describes a tube without any central cystic cavity with the lumen being broken up into compartments as the result of fusion of the tubal plicae. Thus, the terminology is not consistent with the original translation, since hydrosalpinx is also used in cases without any obvious fluid in the tubes. Sactosalpinx is also used as a synonym, although the definition is slightly different: dilation of the inflamed uterine tube by retained secretions (*saktos* = stuffed).

The diagnosis of hydrosalpinx can be suspected and, in many cases, also confirmed by transvaginal ultrasound, if the tube is fluid filled. Ultrasound has the obvious advantage over hysterosalpingography of detecting the condition without the instillation of fluid, which carries a high risk of subsequent infection (Figures 61.1 and 61.2). Both methods, including instillation of contrast, can be used to diagnose a distally occluded tube without any fluid prior to instillation. Antibiotic prophylaxis is mandatory. Laparoscopy is obviously the ultimate method for diagnosis of hydrosalpinx and associated pathology of pelvic adhesions. However, the method is highly invasive, and the opportunity should be taken to perform all diagnostic and therapeutic procedures at the same time.

It has been proposed to establish cutoff values for the size of a hydrosalpinx in order to decide when there is a need for intervention prior to IVF. However, the size of a hydrosalpinx, as measured by ultrasound, may vary during a cycle, and it has not been possible to correlate IVF outcome to the precise size. Only two indices of size have been established—detection at ultrasound examination and bilateral affection—and these are discussed in the next section.

HYDROSALPINX: A SIGN OF POOR PROGNOSIS

Since 1994, there have been a large number of retrospective studies dedicated to the influence of hydrosalpinges on pregnancy results in IVF, most of them showing an impaired outcome (2). Patients with hydrosalpinges have been identified as having significantly lower implantation and pregnancy rates than patients suffering from other types of tubal damage. The retrospective data have been compiled and summarized in meta-analyses, one of which is shown in Figure 61.3 (3). There is a consistency in the results, showing a reduction by half in clinical pregnancy and delivery rates and a doubled rate of spontaneous abortion in women with hydrosalpinx. In addition, thaw cycles demonstrated a significantly reduced pregnancy rate, which none of the separate studies has been able to show. The rate of ectopic pregnancy was non-significantly increased in hydrosalpinx patients (odds ratio [OR] 1.3, 95% confidence interval [CI] 0.7–2.6). Patients with tubal infertility have an increased risk of ectopic pregnancy after IVF compared with patients with other indications, but it has not been possible to establish that patients with hydrosalpinges have an increased risk of ectopic pregnancy compared with patients suffering from other types of tubal infertility. Although retrospective cohort studies do not provide the best quality of evidence, it is obvious from the overwhelming consistency in results that patients with hydrosalpinges have an impaired pregnancy outcome after IVF.

Some of the retrospective studies have attempted to characterize further and subdivide the different features of hydrosalpinx. The first publication showed that the size was important by demonstrating that only largely distended hydrosalpinges were associated with significantly reduced pregnancy and delivery rates (2). deWit et al. also demonstrated the importance of size by using ultrasound, and allocated hydrosalpinges according to size depending on whether they were visible or not (4). Pregnancy rates were significantly lower (15%) in patients with

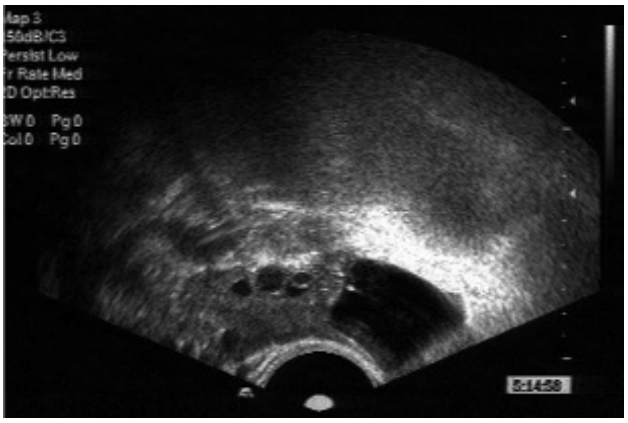

Figure 61.1 Typical appearance of a hydrosalpinx beside the ovary at transvaginal ultrasound investigation.

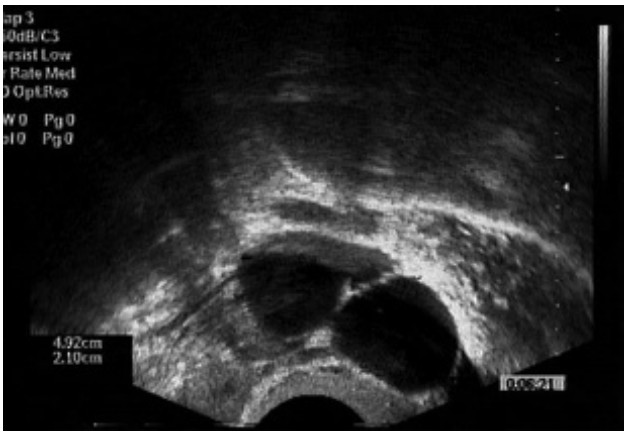

Figure 61.2 The folds of the tubal wall in a distended hydrosalpinx depict as typical spikes into the lumen.

visible hydrosalpinges compared with patients in whom the hydrosalpinges were not visible (31%). Wainer et al. demonstrated that the presence of bilateral as opposed to unilateral hydrosalpinx was associated with significantly lower pregnancy (12% vs. 24%) and implantation rates (5% vs. 11%) (5). These findings suggest that the total amount of fluid in the hydrosalpinges is negatively correlated to the chance of achieving a pregnancy, and these aspects should be considered in the design of a prospective trial.

WHAT IS THE MECHANISM OF HYDROSALPINX IMPAIRING IMPLANTATION?

The hydrosalpinx fluid may act on two different target systems: directly on the transferred embryos or on the endometrium and its receptivity for implantation, or both.

Embryotoxic properties of hydrosalpinx fluid

Potential embryotoxic effects have been evaluated using either mouse or human embryos in human hydrosalpinx fluid. There is a discrepancy in the results of culture systems using human and murine models, but the results from different mouse studies are also diverging. In a review of hydrosalpinx studies, five out of eight studies using a murine model described embryotoxicity at low concentrations of human hydrosalpinx fluid, and three studies demonstrated impaired development, but in undiluted hydrosalpinx fluid only (6). There are only two studies on human embryos, neither of which have been able to demonstrate any obvious toxic effect on embryo development (7,8). The experimental models using mouse and human embryos do not seem to be comparable, and conclusions from studies based on mouse models are not obviously applicable to humans. From studies on embryo development, it may be concluded that hydrosalpinx fluid

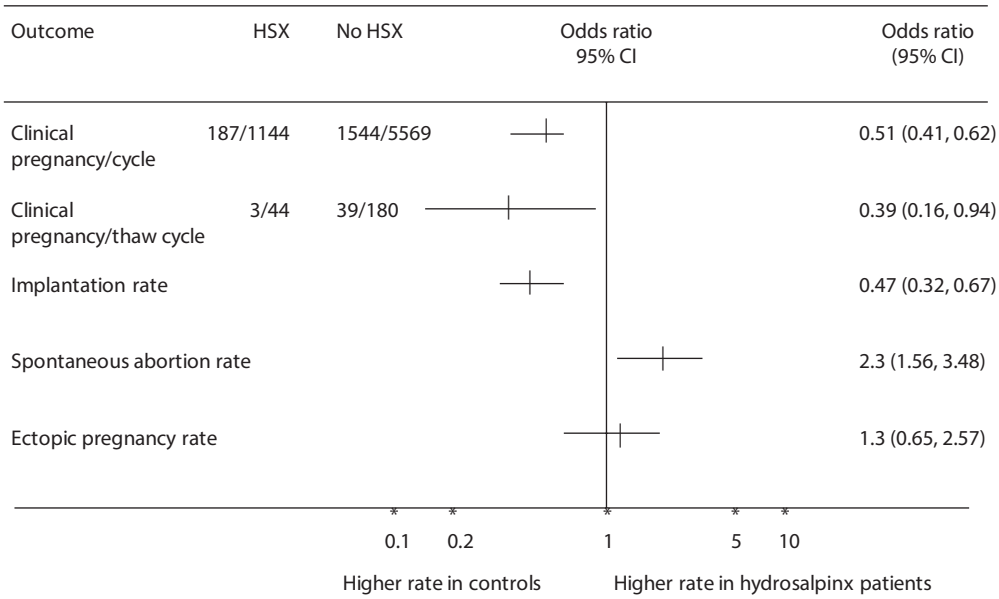

Figure 61.3 Meta-analysis of retrospective studies on *in vitro* fertilization outcomes in HSX patients compared with patients with other forms of tubal infertility. *Abbreviations*: CI, confidence interval; HSX, hydrosalpinx. (Data from Zeyneloglu HB et al. *Fertil Steril* 1998; 70: 492–9.)

does not appear to host a common potent factor that is deleterious to embryo development, and the lack of essential substrates is more likely to be responsible for the impaired development of embryos in undiluted hydrosalpinx fluid.

Is hydrosalpinx fluid toxic in individual cases?

Even though there may not be a common toxic factor in all fluids, the presence of factors inhibitory to embryo development in fluids from certain individuals cannot be excluded. Most experiments are based on small numbers of hydrosalpinx fluids, and individual variations in content may reflect the differences in embryo development. In a study on the effect of hydrosalpinx fluid on gametes and fertilization, one out of four fluids was directly cytotoxic to murine spermatozoa when incubated in 50% hydrosalpinx fluid during capacitation (9).

No pathogenic microorganisms have been detected in any of the published studies, but slightly elevated concentrations of endotoxin have been demonstrated in individual fluids as a sign of previous infection (7). If a toxic substance was responsible for the negative influence, assay of the aspirated hydrosalpingeal fluid before stimulation would be useful in selecting patients for salpingectomy.

An assay of mouse embryo culture in 50% hydrosalpinx fluid has been suggested to predict IVF outcome (10). In a population of 39 patients undergoing transvaginal aspiration of hydrosalpinx fluid, the test had a sensitivity of 64%, a specificity of 86%, and a positive likelihood ratio of 4.5, suggesting the test to be fairly good at detecting toxicity. The diagnostic performance was not improved by including important factors like age and number of good-quality embryos transferred. The use of this technique requires transvaginal puncture, preferably before the start of any stimulation, when the result may be helpful in the decision concerning prophylactic salpingectomy. One case report has described the clinical use of the assay, but the technique still awaits further clinical evaluation (11).

The hydrosalpingeal fluid may also exhibit growth-promoting properties, as seen in a study in which the production of tropho-uteronectin by human cytotrophoblasts was significantly increased by the presence of hydrosalpinx fluid, suggesting promotion of early embryo–integrin interactions (12). Also, a significant increase in trophoblast cell viability and in the production of β-human chorionic gonadotropin in the presence of hydrosalpinx fluid suggested growth-promoting properties of hydrosalpinx fluid.

Oxidative stress

The presence of oxidative and antioxidant systems in various reproductive tissues has evoked interest in the role of oxidative stress in reproductive diseases. Oxidative stress has been defined as an elevation in the steady-state concentration of various reactive oxygen species on a cellular level and has been suggested to be of importance in hydrosalpinx cases. A first report on this issue described a positive effect of low levels of reactive oxygen in relation to blastocyst development, as compared with absence of reactive oxygen species in hydrosalpinx fluid (13). The low levels were suggested to be within a physiological range, and no high levels were detected to demonstrate a negative effect. This hypothesis will need further evaluation.

Endometrial receptivity

The cross-talk between the embryo and the endometrium, which is essential for allowing the embryo to implant, and mediated by the secretion and expression of certain cytokines and other substances during the implantation window, may be disturbed under the presence of hydrosalpinx fluid. Cytokines like interleukin-1 (IL-1), leukemia inhibitory factor (LIF), colony stimulating factor-1 (CSF-1), and the integrin $\alpha_v\beta_3$ are all factors that have been shown to be of importance to implantation. These molecular markers and some of their receptors are secreted or expressed by either the embryo or the endometrium in an increased manner during the implantation window (14,15). By contrast, in hydrosalpinx patients, their expression is reduced.

Chlamydia trachomatis is the most common pathogen, and antibodies to chlamydial heat shock proteins were found to be more prevalent in patients with hydrosalpinx compared with women in couples of male infertility (16). Heat shock proteins elicit intense immune and inflammatory reactions and are thought to be responsible for a local immune response, leading to inflammatory reactions, impaired implantation, and immune rejection after embryo transfer (17).

Ultrasonographic parameters have been investigated in search of effects on endometrial receptivity. Results have been contradictory when measuring the sub-endometrial blood flow among hydrosalpinx patients (18,19). However, the triple-line endometrial pattern was less common among hydrosalpinx patients compared with controls (19), supporting the theory of hydrosalpinx being involved in the regulation of endometrial receptivity and possibly causing simultaneous damage to the endometrium as the original infection.

Mechanical explanations

Leakage of hydrosalpingeal fluid through the uterine cavity, resulting in embryo disposal, has been suggested as a mechanism by several authors (20–22). The clinical feature of hydrorrhea was shown to be a sign of poor prognosis among patients with hydrosalpinx undergoing IVF (21). The existence of a hydrosalpingeal fluid interface on the endometrial surface, sometimes seen during ART cycles, has been suggested to be a hindrance to implantation (21,23). Retrospective studies have demonstrated an association between endometrial cavity fluid and increased cancellation rates and lower clinical pregnancy rates in ART cycles, but without any association with hydrosalpinges visible on ultrasound (24,25). These findings suggest that leakage of hydrosalpingeal fluid through the uterine cavity is only one of several possible explanations of endometrial cavity fluid.

It has also been suggested that hydrosalpinx fluid may cause an increase in endometrial peristalsis. In one report, the uterine dynamics of five patients with hydrosalpinx

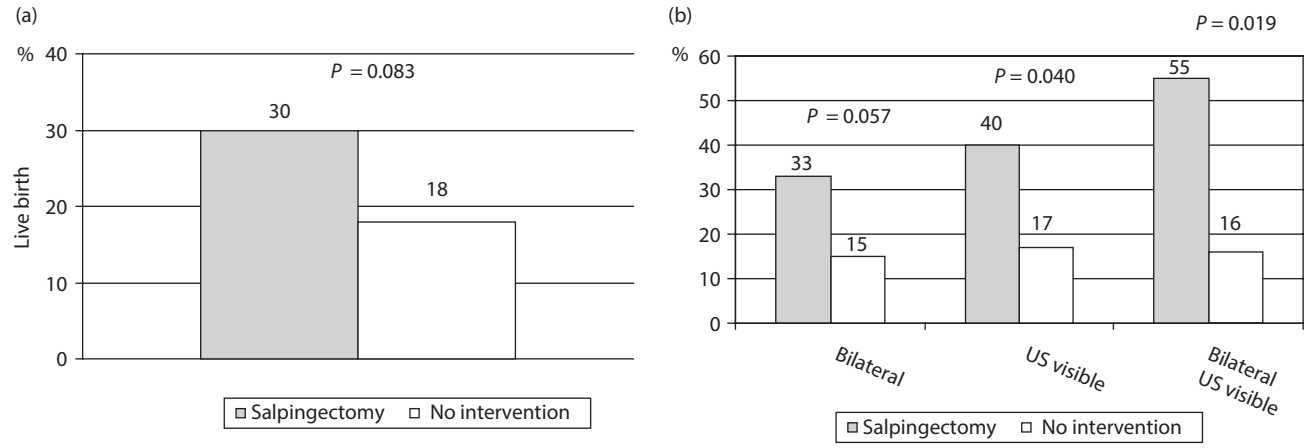

Figure 61.4 Live birth rate in the first transfer cycle of 204 patients in the Scandinavian multicenter trial on salpingectomy prior to *in vitro* fertilization in hydrosalpinx patients. (a) The total study population. (b) The *a priori* decided subgroups of bilateral and/or US-visible hydrosalpinges. *Abbreviation*: US, ultrasound.

were analyzed by image-processing techniques and compared with healthy volunteers (26). The authors described, from a mathematical simulation model, a reflux phenomenon (opposing the cervix-to-fundus intrauterine peristalsis) generated by a pressure gradient from tubal fluid accumulation. It was suggested that this reflux phenomenon could explain the reduced implantation rate associated with hydrosalpinx.

INTERVENTIONS AGAINST HYDROSALPINX IN CONJUNCTION WITH IVF

According to the theory that the hydrosalpingeal fluid plays a causative role in impairing implantation and/or embryo development, any surgical intervention interrupting the communication to the uterus would remove the leakage of the hydrosalpingeal fluid and restore pregnancy rates. Treatment with salpingectomy prior to IVF is the only surgical method that has been evaluated in a sufficiently large randomized controlled trial (RCT), supplying us with a high level of evidence to formulate our recommendation. Other suggested treatments for hydrosalpinx prior to IVF, such as tubal ligation and transvaginal aspiration, may also be considered, but they need further evaluation in large prospective trials.

Salpingectomy

Salpingectomy is the only method of prophylactic surgery in patients with hydrosalpinx that has been properly evaluated in a large randomized trial (27). A multicenter study in Scandinavia compared laparoscopic salpingectomy to no intervention prior to the first IVF cycle. The study demonstrated a significant improvement in pregnancy and birth rates after salpingectomy in patients with hydrosalpinges that were large enough to be visible on ultrasound. Clinical pregnancy rates were 46% versus 22%, and birth rates were 40% versus 17% in salpingectomized patients versus patients without any surgical intervention (Figure 61.4b). The difference in outcome was not statistically significant in the total study population of 204 patients, which included patients with hydrosalpinges that were not visible on ultrasound (Figure 61.4a), demonstrating that the benefit of salpingectomy is only evident if the tube is fluid filled.

Within the group of hydrosalpinges visible on ultrasound, there can still be tubes that are suitable for reconstructive surgery, and the main rule must be that tubes with healthy-looking mucosa should not be removed (Figures 61.5 and 61.6).

The psychological aspect of removing the tubes in an infertile patient is very important and has to be considered. Even if it is obvious that the patient would benefit from salpingectomy, it is crucial that she is psychologically prepared to undergo the procedure. In some cases, it takes one or several failed cycles before the patient is ready to give her consent.

There are four additional RCTs comparing salpingectomy with no surgery prior to IVF (28–31), all of smaller sample sizes as compared to the Scandinavian

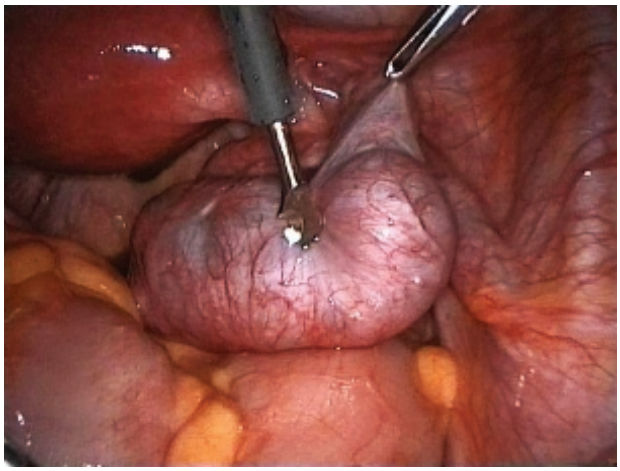

Figure 61.5 A hydrosalpinx without adjacent adhesions is easy to assess at laparoscopy.

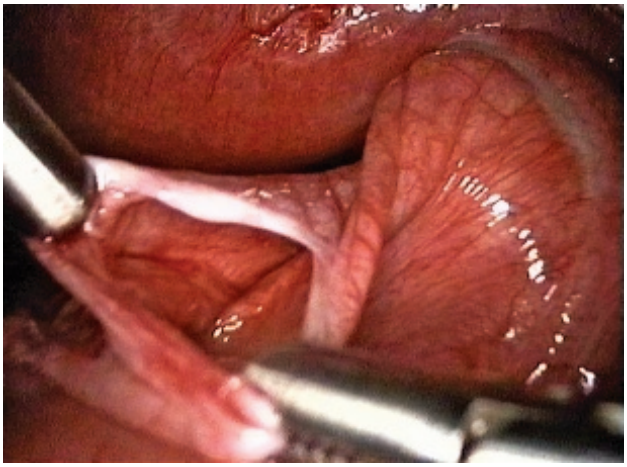

Figure 61.6 Assessment of mucosal status through a distal opening of the hydrosalpinx is recommended before the final decision of salpingectomy or distal tuboplasty is taken.

study. A systematic review from the Cochrane Library (32) included meta-analyses demonstrating a significant improvement in ongoing pregnancy (OR 2.2, 95% CI 1.3–3.8) after IVF if salpingectomy was performed compared with no surgical intervention. If an additional study (only published as an abstract) (31) is also included in a meta-analysis, the common OR for ongoing pregnancy or live birth is 2.7 (95% CI 1.6–4.6), as shown in Figure 61.7.

In the Scandinavian study, the cumulative result, including all subsequent cycles, was evaluated (33). Patients were offered up to three stimulated cycles, and those who were randomized to undergo salpingectomy achieved a cumulative birth rate of 55%. When all subsequent cycles were considered, including all patients regardless of the size of the hydrosalpinx, salpingectomy implied a doubled birth rate as compared to patients with persistent hydrosalpinges (hazard ratio 2.1, 95% CI 1.6–3.6, $p = 0.014$). This result, as well as the compiled data from the Cochrane review, suggests that all patients with hydrosalpinx, regardless of size or fluid accumulation, should undergo salpingectomy. However, the cumulative data from the Scandinavian study revealed that the benefit of salpingectomy mainly affected patients with hydrosalpinges visible on ultrasound, and consequently, those are the only patients to be recommended prophylactic salpingectomy prior to IVF.

A cost-effectiveness analysis based on the Scandinavian RCT showed that the strategy of performing salpingectomy prior to the first IVF cycle was more cost-effective than the strategy of suggesting surgery after one or two cycles had failed (34).

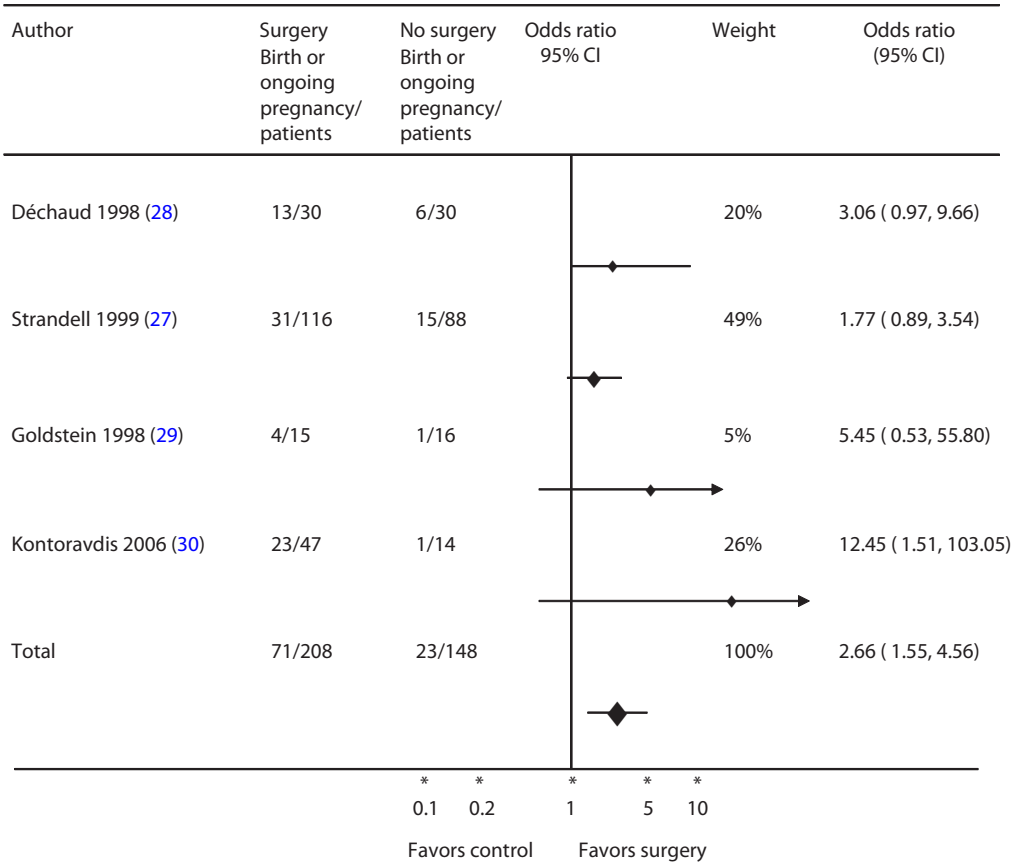

Figure 61.7 Meta-analysis of four randomized trials of laparoscopic salpingectomy versus no surgery in hydrosalpinx patients due to undergo *in vitro* fertilization, with the primary outcome of live birth or ongoing pregnancy. *Abbreviation*: CI, confidence interval.

Effect on ovarian function after salpingectomy

The effect of salpingectomy on ovarian function has been debated, and the results of hitherto published studies are not entirely in consensus (27,30,35–42). A summary of studies is presented in Table 61.1. The close anatomical association of the vascular and nervous supply to the tube and ovary constitute the theoretical rationale for the risk of impaired ovarian function after surgery. Several studies have analyzed the ovarian performance in IVF cycles subsequent to salpingectomy due to ectopic pregnancy. None of them demonstrate an effect on the overall performance, although one study has shown a decreased response in the ovary, ipsilateral to the salpingectomy (36). In the Scandinavian RCT on salpingectomy prior to IVF, there was no difference in the number of retrieved oocytes (Table 61.1) (27). In a subsequent analysis of a subset of patients who underwent a stimulated cycle both before and after the salpingectomy, the effect of salpingectomy on the ovarian performance was examined by measuring the need for follicle-stimulating hormone (FSH) and the number of retrieved oocytes (38). There were no significant differences in either the amount of FSH used or the number of retrieved oocytes. In the cycle after salpingectomy, a mean of 0.7 fewer oocytes were retrieved compared with the cycle before surgery (Table 61.1). In two studies (30,40), different surgical methods for hydrosalpinx were compared. The finding from the retrospective study (40) of significantly fewer retrieved oocytes after salpingectomy in comparison with tubal ligation was not confirmed in the randomized trial (30). One study demonstrated fewer developed follicles on the ipsilateral side when comparing the same ovary before and after surgery, but did not report on the number of retrieved oocytes from separate ovaries (41).

From the results, we cannot conclude that patients with a low ovarian reserve are at greater risk of suffering from poor response after salpingectomy. However, theoretically, it seems important to be very careful not to damage the vascular and nervous supply when performing a salpingectomy. A laparoscopic salpingectomy should be performed with cautious use of electrocautery, with no unnecessary excision of the mesosalpinx, but resection very close to the actual tube to avoid damage to the medial tubal artery. It is preferable to leave a portion of an adherent tube on the ovary rather than to perform an excessively radical salpingectomy. The risk of dehiscence in the uterine wall and subsequent protrusion of the fetus has been described, suggesting that resection not too close to the uterus is to be recommended (43).

Tubal occlusion by laparoscopy

Surgical treatment requiring laparoscopy also includes proximal ligation and salpingostomy. There is one randomized trial in which 115 patients with hydrosalpinx were allocated to proximal tubal occlusion, salpingectomy, or no surgery prior to IVF (30). Salpingectomy but not tubal ligation demonstrated significantly higher ongoing pregnancy rates (49% and 38%, respectively) compared to women having no surgery (7%), analyzed on an intention-to-treat basis. The Cochrane review (32) included an additional small RCT (published as an abstract) (31) and a meta-analysis of these two studies, demonstrating an OR for clinical pregnancy of 4.8 (95% CI 2.2–10.0).

Table 61.1 Summary of studies examining the effect of salpingectomy on ovarian function by measuring the number of retrieved oocytes after controlled ovarian hyperstimulation

First author, year	No. of patients	Reason for surgery	No. of oocytes		Study design
			Ipsilateral vs. contralateral	Overall (two ovaries)	
Verhulst, 1994 (35)	26 vs. 134	Ectopic pregnancy hydrosalpinx sterilization	Not studied	11.2 vs. 1.2, NS	Retrospective comparison with controls
Lass, 1998 (36)	29 vs. 73	Ectopic pregnancy	3.8 vs. 6.0, $p < 0.01$	9.9 vs. 9.1, NS	Prospective comparison with controls
Strandell, 1999 (27)	110 vs. 82	Hydrosalpinx	Not studied	10.6 vs. 10.6, NS	Randomized trial
Dar, 2000 (37)	26	Ectopic pregnancy after IVF	6.1 vs. 5.3, NS	11.1 vs. 9.7, NS	Analysis before and after surgery
Tal, 2002 (39)	26 vs. 52	Ectopic pregnancy	6.3 vs. 6.2, NS	8.6 vs. 8.4, NS	Comparison with matched controls
Gelbaya, 2006 (40)	40 vs. 103	Hydrosalpinx	Not studied	10.2 vs. 12.9, NS	Retrospective cohort
Kontoravdis, 2006 (30)		Hydrosalpinx	Not studied	12.1 vs. 10.9, NS	Randomized trial
Orvieto, 2011 (41)	15	Hydrosalpinx	Not studied	11.6 vs. 10.2, NS	Analysis before and after surgery
Strandell, 2001 (33)	26	Hydrosalpinx	Not studied	9.4 vs. 8.7, NS	Analysis before and after surgery

Note: Controls are the same patients before surgery, the contralateral ovary, or patients without previous tubal surgery.
Abbreviations: IVF, *in vitro* fertilization; NS, non-significant.

Subsequent studies have compared proximal tubal occlusion to salpingectomy, since it is no longer ethically justified to conduct a study with a control group of no surgery. Two RCTs are available and summarized in the Cochrane review (32). There was no significant difference in clinical pregnancy rate when salpingectomy was compared with proximal tubal occlusion (OR 1.3, 95% CI 0.8–2.1).

According to the theory of the hydrosalpingeal fluid affecting the endometrium negatively, the procedure of tubal ligation is likely to be effective at improving pregnancy results. The procedure is currently recommended when pelvic adhesions are too extensive to perform a salpingectomy.

Tubal occlusion by hysteroscopy

Tubal occlusion through hysteroscopy has been suggested when laparoscopy is contraindicated, like in cases with severe obesity or frozen pelvis. The first case report (44) describing the use of the microinsert sterilization device (Essure™) has been followed by several case series (45,46). A systematic review including 11 case series with 115 patients reported successful placement in 96.5% (95% CI 91.1%–98.9%) of women and tubal occlusion in 98.1% (95% CI 93.1%–99.9%) (47). Subsequent IVF resulted in a 38.6% pregnancy rate (95% CI 30.9%–46.8%) and a 27.9% live birth rate (95% CI 21.1%–35.8%) per embryo transfer. There are no controlled studies published yet, although the Dutch Essure versus Salpingectomy for Hydrosalpinx (DESH) trial has been completed (48). The obvious advantage is that the method can be performed under local anesthesia and thus avoids complications related to laparoscopy and general anesthesia.

The use of electrocoagulation with a monopolar roller ball electrode for closing the internal tubal orifice has been reported as an alternative method in a small case series (49).

Salpingostomy

There are no separate studies on salpingostomy prior to IVF, although it has been performed in a few cases and reported as part of a control group for hydrosalpinx patients in retrospective studies. Salpingostomy is naturally the method of choice if the tube is suitable for reconstructive surgery.

A systematic review of 22 salpingostomy case series including 2810 patients reported a pooled spontaneous clinical pregnancy rate of 27% (95% CI 25%–29%) (50). The selection of patients suitable for surgical repair has to be based on the evaluation of the tubal mucosa through an endoscopic technique, and tubes with more than half of the mucosa in a good condition may have a fair chance of spontaneous conception (51). These patients should be given sufficient time to await spontaneous conception, although the woman's age may hasten the need for IVF.

Transvaginal aspiration

Whatever the exact mechanism of the negative influence of hydrosalpinx fluid, the treatment options concern the disposal of the fluid. Since laparoscopic salpingectomy has been shown to be an effective pre-IVF method to improve pregnancy rates, the search for less invasive methods has continued. The simplest way—vaginal aspiration of fluid—has been evaluated in an RCT comparing transvaginal aspiration with no aspiration (52). Unfortunately, the study was stopped in advance due to recruitment difficulties. The study was thus underpowered, including only 66 patients, and the difference in clinical pregnancy rate (31% vs. 18%) did not reach statistical significance.

Subsequently, a randomized trial of 160 patients compared the efficacy of ultrasound-guided transvaginal aspiration with salpingectomy (53). The clinical pregnancy rate was non-significantly higher in the salpingectomy group (40% vs. 27%). The sample size was also insufficient for a firm conclusion in this study.

There is a rapid re-occurrence of fluid that is already noticeable at the time of transfer in many cases, which most likely compromises any beneficial effect of drainage (54).

The majority of studies have examined the effect of aspiration if conducted at the time of oocyte retrieval. It has been clearly shown that aspiration before ovarian stimulation has started is not effective, possibly due to high recurrence rate (55). The RCT comparing aspiration at the time of oocyte retrieval with salpingectomy clearly demonstrated the importance of re-accumulation of fluid (53). In the aspiration group, 34% had rapid re-accumulation of hydrosalpinx fluid, and the clinical pregnancy rate reached 19% compared with 34% in the group without re-accumulation of fluid after aspiration ($p = 0.28$).

To overcome the problem of the high recurrence rate after transvaginal aspiration of hydrosalpinx fluid, ethanol sclerotherapy has been introduced (56). The proposed mechanism of ethanol is to coagulate the endothelial cells lining the hydrosalpinx to harden the salpingeal wall and reduce secretion. There are, however, reported risks of possible harm to the ovarian reserve mediated by the development of fibrosis and severe adhesions (57). These findings are only discussed in a letter to the editor, but the severity of the possible harm calls for caution. The method cannot be recommended in a clinical setting, but only within a clinical trial.

The occurrence of infections in association with puncture of a hydrosalpinx seems to be rare when antibiotics have been given, according to the published reports. The method has the obvious advantage of being less invasive than the other available surgical methods.

It can be concluded that transvaginal aspiration of hydrosalpingeal fluid at the time of oocyte collection is a treatment option, particularly if there is a contraindication to or non-acceptance of surgery, or if a hydrosalpinx develops during ovarian stimulation. In case of rapid re-accumulation of fluid after aspiration, the chance to conceive with a fresh embryo transfer is further reduced. Cryopreservation of embryos and surgical correction of the hydrosalpinx before a frozen–thawed transfer is a better option.

Repeated implantation failure in patients with tubal factor infertility

The question of whether salpingectomy is beneficial in patients without evident hydrosalpinx but with tubal factor infertility often arises if there is a repeated implantation failure after IVF. There are presently no data to support salpingectomy in this group. Indeed, the Scandinavian multicenter study (27,33) demonstrated that salpingectomy in comparison with no surgery did not increase pregnancy rates among patients with distally occluded tubes without fluid accumulation (OR 1.6, 95% CI 0.6–4.8).

INTERVENTIONS AGAINST HYDROSALPINX WITHOUT IVF

As IVF developed and the results improved, the importance of using surgical methods for treating tubal infertility declined. It is well known that the success rate was closely related to the status of the tubal mucosa; the less damage to the tubes, the better chance of a subsequent intrauterine pregnancy. Today, IVF is often also offered as a first-line treatment to patients with mild tubal damage. Whether surgery is discussed or not is mainly a question of surgical competence, availability of IVF and the patient's financial situation, and is not primarily a medical issue.

The work-up of the subfertile couple has also changed over time, so that laparoscopy is no longer a compulsory investigation, due to limited resources and the fact that laparoscopy is a very invasive procedure. This new mode implies that fewer patients will be evaluated laparoscopically during the work-up, unless a hydrosalpinx is detected.

The result from the Scandinavian multicenter study to recommend salpingectomy prior to IVF has raised a number of concerns, such as those of Puttemans et al. (58), who fear that tubes that are suitable for functional surgery could be sacrificed. In the scenario where laparoscopy is not routinely used, this fear might be justified if the tubes are not properly evaluated before a salpingectomy is performed. Even if a patient is scheduled for a laparoscopic salpingectomy, it is necessary to open the distally occluded tube for evaluation of the mucosa before a final decision of salpingectomy is taken. If it is appropriate to perform a salpingostomy, time for spontaneous conception should be given instead of immediate IVF.

Salpingectomy of a unilateral hydrosalpinx may imply an increased chance of spontaneous conception. Two women in the Scandinavian study (27) conceived spontaneously after long-lasting infertility followed by a unilateral salpingectomy and achieved a full term pregnancy. After several case reports on the same theme, one case series on 25 patients has been published (59). All patients had surgery, salpingectomy, or proximal tubal occlusion of the unilateral hydrosalpinx and 18 (88%) conceived spontaneously after a mean of 5.6 months (range 1–21 months) and had intrauterine pregnancies.

The negative effect of a unilateral hydrosalpinx has also been recognized among patients with recurrent abortion. A small RCT compared laparoscopic unilateral proximal tubal occlusion by electrocautery (n = 7) with no surgery (n = 6) (60). All but one patient in each group conceived, and five out of six who had undergone surgery carried their pregnancies to term, while all five who did not have surgery had early miscarriages ($p = 0.02$). Obviously, it is also important to diagnose and treat a unilateral hydrosalpinx in patients who suffer from recurrent miscarriages.

SUMMARY AND CONCLUSIONS

In patients with severe tubal disease presented as a hydrosalpinx on ultrasound and with a destroyed mucosa upon endoscopic inspection, IVF is the method of choice. However, this should be preceded by a discussion of laparoscopic salpingectomy, which will double the patient's chance of a subsequent birth after IVF. In cases of extensive adhesions, rendering the salpingectomy difficult and bearing a risk of complications, proximal tubal occlusion is the preferred method. If the laparoscopic route is contraindicated, hysteroscopic tubal occlusion can be achieved by the placement of sterilization devices. The psychological aspects of removing or interrupting the tubes are very important and always have to be considered.

If no surgical intervention is performed prior to IVF, transvaginal aspiration of the fluid can be performed in conjunction with oocyte retrieval under antibiotic cover. If there is a re-accumulation of hydrosalpinx fluid before the time of transfer, cryopreservation of embryos would allow time for a surgical intervention to improve the chance of conception at a subsequent freeze transfer.

Patients with a preserved mucosa in the hydrosalpinx may have a good chance of spontaneous conception if salpingostomy is performed.

In the presence of a unilateral hydrosalpinx and a contralateral healthy tube, a unilateral salpingectomy can be recommended, followed by sufficient time to await spontaneous conception, before proceeding to IVF. Also, patients with recurrent abortion and a unilateral hydrosalpinx may benefit from unilateral salpingectomy or proximal tubal occlusion.

IMPLICATIONS FOR RESEARCH

The underlying mechanisms of impaired implantation and/or development of embryos in the presence of hydrosalpinx need further exploration. The fields of basic research on endometrial receptivity and implantation are very intense, and as more general knowledge is gained, more specific hypotheses may be directed to the negative role of hydrosalpinx. In addition, the formation of hydrosalpinx following pelvic infection has not yet been completely elucidated. A better understanding of the mechanisms would provide prerequisites for a more rational therapy.

As of today, we recommend very robust surgical methods, but it is possible that the treatment could be more individualized. Salpingectomy, proximal tubal occlusion, and transvaginal aspiration compared to no intervention have been evaluated in randomized trials, but only salpingectomy has been evaluated in a sufficiently large trial. It can no longer be ethically justified to use control groups without intervention,

since salpingectomy has shown its efficacy. To date, comparisons between the methods have been underpowered. A trial designed to evaluate whether hysteroscopic tubal occlusion or transvaginal aspiration are non-inferior to laparoscopic salpingectomy in terms of pregnancy and live birth rates after IVF would be of great clinical value.

REFERENCES

1. Strandell A, Bergh B, Lundin K. Selection of patients suitable for one-embryo transfer may reduce the rate of multiple births by half without impairment of overall birth rates. *Hum Reprod* 2000; 15: 2520–25.
2. Strandell A, Waldenström U, Nilsson L, Hamberger L. Hydrosalpinx reduces *in-vitro* fertilization/embryo transfer rates. *Hum Reprod* 1994; 9: 861–3.
3. Zeyneloglu HB, Arici A, Olive DL. Adverse effects of hydrosalpinx on pregnancy rates after *in vitro* fertilization-embryo transfer. *Fertil Steril* 1998; 70: 492–9.
4. deWit W, Gowrising CJ, Kuik DJ et al. Only hydrosalpinges visible on ultrasound are associated with reduced implantation and pregnancy rates after *in-vitro* fertilization. *Hum Reprod* 1998; 13: 1696–701.
5. Wainer R, Camus E, Camier B, Martin C, Vasseur C, Merlet F. Does hydrosalpinx reduce the pregnancy rate following *in vitro* fertilization. *Fertil Steril* 1997; 68: 1022–6.
6. Strandell A. The influence of hydrosalpinx on *in-vitro* fertilisation and embryo transfer – a review. *Hum Reprod Update* 2000; 6: 387–95.
7. Strandell A, Sjögren A, Bentin-Ley U, Thorburn J, Hamberger L, Brännström M. Hydrosalpinx fluid does not adversely affect the normal development of human embryos and implantation *in vitro*. *Hum Reprod* 1998; 13: 2921–5.
8. Granot I, Dekel N, Segal I, Fieldust S, Shoham Z, Barash A. Is hydrosalpinx fluid cytotoxic? *Hum Reprod* 1998; 13: 1620–4.
9. de Vantéry Arrighi C, Lucas H, El-Mowafi D, Campana A, Chardonnens D. Effects of human hydrosalpinx fluid on *in-vitro* murine fertilization. *Hum Reprod* 2001; 16: 676–82.
10. Chen CD, Yang JH, Lin KC, Chao KH, Ho HN, Yang YS. The significance of cytokines, chemical composition, and murine embryo development in hydrosalpinx fluid for predicting the *in-vitro* fertilization outcome in women with hydrosalpinx. *Hum Reprod* 2002; 17: 128–33.
11. Chen CD, Chao KH, Wu MY, Chen SU, Ho HN, Yang YS. Ultrasound-guided hydrosalpinx aspiration during oocyte retrieval and a mouse embryo assay of hydrosalpinx fluid in a woman with hydrosalpinx and hydrometra during *in vitro* fertilization treatment. *Taiwan J Obstet Gynecol* 2012; 51: 106–8.
12. Sawin SW, Loret de Mola JR, Monzon-Bordonaba F, Wang CL, Feinberg RF. Hydrosalpinx fluid enhances human trophoblast viability and function *in vitro*: implications for embryonic implantation in assisted reproduction. *Fertil Steril* 1997; 68: 65–71.
13. Bedaiwy MA, Goldberg JM, Falcone T, Singh M, Nelson D, Azab H, Wang X, Sharma R. Relationship between oxidative stress and embryotoxicity of hydrosalpingeal fluid. *Hum Reprod* 2002; 17: 601–4.
14. Lessey BA, Castelbaum AJ, Buck CA, Lei Y, Yowell CW, Sun J. Further characterization of endometrial integrins during the menstrual cycle and in pregnancy. *Fertil Steril* 1994; 62: 497–506.
15. Sjöblom C, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor promotes human blastocyst development *in vitro*. *Hum Reprod* 1999; 14: 3069–76.
16. Spandorfer SD, Neuer A, LaVerda D, Byrne G, Liu HC, Rosenwaks Z, Witkin SS. Previously undetected Chlamydia trachomatis infection, immunity to heat shock proteins and tubal occlusion in women undergoing *in-vitro* fertilization. *Hum Reprod* 1999; 14: 60–4.
17. Ajonuma LC, Ng EH, Chan HC. New insights into the mechanisms underlying hydrosalpinx fluid formation and its adverse effect on IVF outcome. *Hum Reprod Update* 2002; 8: 255–64.
18. Ng EH, Chan CC, Tang OS, Chung PC. Comparison of endometrial and subendometrial blood flows among patients with and without hydrosalpinx shown on scanning during *in vitro* fertilization treatment. *Fertil Steril* 2006; 85: 333–8.
19. Cheng F, Li T, Wang QL, Zhou HL, Duan L, Cai X. Effects of hydrosalpinx on ultrasonographic parameters for endometrial receptivity during the window of implantation measured by power color Doppler ultrasound. *Int J Clin Exp Med*. 2015; 8: 6103–8.
20. Mansour RT, Aboulghar MA, Serrour GI, Riad R. Fluid accumulation of the uterine cavity before embryo transfer: a possible hindrance for implantation. *J Vitro Fertil Embryo Transfer* 1991; 8: 157–9.
21. Andersen AN, Lindhard A, Loft A, Ziebe S, Andersen CY. The infertile patient with hydrosalpinges-IVF with or without salpingectomy. *Hum Reprod* 1996; 11: 2081–4.
22. Bloeche M, Schreiner T, Lisse K. Recurrence of hydrosalpinges after transvaginal aspiration of tubal fluid in an IVF cycle with development of serometra. *Hum Reprod* 1997; 12: 703–5.
23. Sharara FI. The role of hydrosalpinx in IVF: simply mechanical? *Hum Reprod* 1999; 14: 577–8.
24. Levi AJ, Segars JH, Miller BT, Leondires MP. Endometrial cavity fluid is associated with poor ovarian response and increased cancellation rates in ART cycles. *Hum Reprod* 2001; 16: 2610–5.
25. He RH, Gao HJ, Li YQ, Zhu XM. The associated factors to endometrial cavity fluid and the relevant impact on the IVF-ET outcome. *Reprod Biol Endocrinol* 2010; 8: 46e51.
26. Eytan O, Azem F, Gull I et al. The mechanism of hydrosalpinx in embryo implantation. *Hum Reprod* 2001; 16: 2662–7.

27. Strandell A, Lindhard A, Waldenström U, Thorburn J, Janson PO, Hamberger L. Hydrosalpinx and IVF outcome: A prospective, randomized multicentre trial in Scandinavia on salpingectomy prior to IVF. *Hum Reprod* 1999; 14: 2762–9.
28. Déchaud H, Daures JP, Arnal F, Humeau C, Hédon B. Does previous salpingectomy improve implantation and pregnancy rates in patients with severe tubal factor infertility who are undergoing *in vitro* fertilization? A pilot prospective randomized study. *Fertil Steril* 1998; 69: 1020–5.
29. Goldstein DB, Sasaran LH, Stadtmauer L, Popa R. Selective salpingostomy-salpingectomy (SSS) and medical treatment prior to IVF in patients with hydrosalpinx. *(Abstracts) Fertil Steril* 1998; 70(3, Suppl 1): S320.
30. Kontoravdis A, Makrakis E, Pantos K, Botsis D, Deligeoroglou E, Creatsas G. Proximal tubal occlusion and salpingectomy results in similar improvement in *in vitro* fertilization outcome in patients with hydrosalpinx. *Fertil Steril* 2006; 86: 1642–9.
31. Moshin V, Hotineanu A. Reproductive outcome of the proximal tubal occlusion prior to IVF in patients with hydrosalpinx. *(Abstracts) Hum Reprod* 2006; 21: i193–4.
32. Johnson N, van Voorst S, Sowter MC, Strandell A, Mol BWJ. Surgical treatment for tubal disease in womendue to undergo in vitro fertilisation. *Cochrane Database of Systematic Reviews* 2010, Issue 1. Art. No.: CD002125. doi: 10.1002/14651858.CD002125.pub3.
33. Strandell A, Lindhard A, Waldenstrom U, Thorburn J. Hydrosalpinx and IVF outcome: Cumulative results after salpingectomy in a randomized controlled trial. *Hum Reprod* 2001; 16: 2403–10.
34. Strandell A, Lindhard A, Eckerlund I. Cost-effectiveness analysis of salpingectomy prior to IVF, based on a randomized controlled trial. *Hum Reprod* 2005; 20: 3284–92.
35. Verhulst G, Vandersteen N, van Steirteghem AC, Devroey P. Bilateral salpingectomy does not compromise ovarian stimulation in an *in-vitro* fertilization/embryo transfer programme. *Hum Reprod* 1994; 9: 624–8.
36. Lass A, Ellenbogen A, Croucher C, Trew G, Margara R, Becattini C, Winston RM. Effect of salpingectomy on ovarian response to superovulation in an *in vitro* fertilization-embryo transfer program. *Fertil Steril* 1998; 70: 1035–8.
37. Dar P, Sachs GS, Strassburger D, Bukovsky I, Arieli S. Ovarian function before and after salpingectomy in artificial reproductive technology patients. *Hum Reprod* 2000; 15: 142–4.
38. Strandell A, Lindhard A, Waldenstrom U, Thorburn J. Salpingectomy prior to IVF does not impair the ovarian response. *Hum Reprod* 2001; 16: 1135–9.
39. Tal J, Paltieli Y, Korobotchka R, Ziskind G, Eibschitz I, Ohel G. Ovarian response to gonadotropin stimulation in repeated IVF cycles after unilateral salpingectomy. *J Assist Reprod Genet* 2002; 19: 451–5.
40. Gelbaya TA, Nardo LG, Fitzgerald CT, Horne G, Brison DR, Lieberman BA. Ovarian response to gonadotrophins after laparoscopic salpingectomy or the division of fallopian tubes for hydrosalpinges. *Fertil Steril* 2006; 85: 1464–8.
41. Orvieto R, Saar-Ryss B, Morgante G, Gemer O, Anteby EY, Meltcer S. Does salpingectomy affect the ipsilateral ovarian response to gonadotropin during *in vitro* fertilization-embryo transfer cycles? *Fertil Steril* 2011; 95: 1842–4.
42. Ödesjö E, Bergh C, Strandell A. Surgical methods for tubal pregnancy - effects on ovarian response to controlled stimulation during IVF. *Acta Obstet Gynecol Scand* 2015. 2015; 94: 1322–6.
43. Inovay J, Marton T, Urbancsek J, Kádár Z, Altdorfer K, Papp Z. Spontaneous bilateral cornual uterine dehiscence early in the second trimester after bilateral laparoscopic salpingectomy and *in-vitro* fertilization. *Hum Reprod* 1999; 14: 2471–3.
44. Rosenfield RB, Stones RE, Coates A, Matteri RK, Hesla JS. Proximal occlusion of hydrosalpinx bt hysteroscopic placement of microinsert before *in vitro* fertilization- embryo transfer. *Fertil Steril* 2005; 83: 1547–50.
45. Mijatovic V, Veersema S, Emanuel MH, Schats R. Essure hysteroscopic tubal occlusion device for the treatment of hydrosalpinx prior to *in vitro* fertilization- embryo transfer in patients with a contraindication for laparoscopy. *Fertil Steril* 2010; 93: 1338–42.
46. Galen DI, Khan NM, Richter KS. Essure multicenter off-label treatment for hydrosalpinx before *in vitro* fertilization. *J Minim Invasive Gynecol* 2011; 18: 338–42.
47. Arora P, Arora RS, Cahill D. Essure(®) for management of hydrosalpinx prior to *in vitro* fertilisation-a systematic review and pooled analysis. *BJOG.* 2014; 12: 527–36.
48. Dutch Consortium for Healthcare Evaluation and Research in Obstetrics and Gynecology - NVOG Consortium 2.0 http://www.studies-obsgyn.nl/home/page.asp?page_id=826
49. Bao HC, Wang MM, Wang XR, Wang WJ, Hao CF. Clinical application of operative hysteroscopy in treatment of complex hydrosalpinx prior to IVF. *Iran J Reprod Med.* 2015; 13: 311–6.
50. Chu J, Harb HM, Gallos ID, Dhillon R, Al-Rshoud FM, Robinson L, Coomarasamy A. Salpingostomy in the treatment of hydrosalpinx: a systematic review and meta-analysis. *Hum Reprod* 2015; 30: 1882–95.
51. Vasquez G, Boeckx W, Brosens I. Prospective study of tubal mucosal lesions and fertility in hydrosalpinges. *Hum Reprod* 1995; 10: 1075–8.
52. Hammadieh N, Coomarasamy A, Ola B, Papaioannou S, Afnan M, Sharif K. Ultrasound-guided hydrosalpinx aspiration during oocyte collection improves pregnancy outcome in IVF: a randomized controlled trial. *Hum Reprod* 2008; 23: 1113–7.

53. Fouda UM, Sayed AM, Abdelmoty HI, Elsetohy KA. Ultrasound guided aspiration of hydrosalpinx fluid versus salpingectomy in the management of patients with ultrasound visible hydrosalpinx undergoing IVF-ET: a randomized controlled trial. *BMC Womens Health* 2015; 15: 21. doi: 10.1186/s12905-015-0177-2.
54. Sharara FI, McClamrock HD. Endometrial fluid collection in women with hydrosalpinx after human chorionic gonadotrophin administration: a report of two cases and implications for management. *Hum Reprod* 1997; 12: 2816–9.
55. Aboulghar MA, Mansour RT, Serour GI, Sattar MA, Awad MM, Amin Y. Transvaginal ultrasonic needle guided aspiration of pelvic inflammatory cystic masses before ovulation induction for *in vitro* fertilization. *Fertil Steril* 1990; 53: 311–4.
56. Na ED, Cha DH, Cho JH, Kim MK. Comparison of IVF-ET outcomes in patients with hydrosalpinx pretreated with either sclerotherapy or laparoscopic salpingectomy. *Clin Exp Reprod Med* 2012; 39: 182–6.
57. Shokeir T. Letter to the Editor: Re: Comparison of IVF-ET outcomes in patients with hydrosalpinx pretreated with either sclerotherapy or laparoscopic salpingectomy. *Clin Exp Reprod Med* 2014; 41: 37–8.
58. Puttemans P, Campo R, Gordts S, Brosens I. Hydrosalpinx and ART: hydrosalpinx--functional surgery or salpingectomy? *Hum Reprod* 2000; 15: 1427–30.
59. Sagoskin AW, Lessey BA, Mottla GL, Richter KS, Chetkowski RJ, Chang AS, Levy MJ, Stillman RJ. Salpingectomy or proximal tubal occlusion of unilateral hydrosalpinx increases the potential for spontaneous pregnancy. *Hum Reprod*. 2003; 18: 2634–7.
60. Zolghadri J, Momtahan M, Alborzi S, Mohammadinejad A, Khosravi D. Pregnancy outcome in patients with early recurrent abortion following laparoscopic tubal corneal interruption of a fallopian tube with hydrosalpinx. *Fertil Steril*. 2006; 86: 149–51.

Fertility preservation strategies

62

STINE GRY KRISTENSEN and CLAUS YDING ANDERSEN

OVERVIEW

Fertility preservation is primarily focused on saving gametes in girls and young women who run the risk of losing the entire pool of ovarian follicles, such as by having a cancer or a genetic disease. Future areas include women who wish to delay childbearing (1) or hormonal effects that focus on the steroid-producing capacity of follicles and include postponing menopause (2).

For many cancers, the chance of surviving is steadily increasing and is now at around 80%. Many women have started to focus on quality of life aspects after treatment, and the possibility of having their own children is of high priority. Around 2% of women in their reproductive age suffer from invasive cancer and are at risk of ovarian failure after receiving sterilizing chemotherapy and radiotherapy (3). In contrast to the testis, the ovary is equipped with a fixed number of oocytes without germ stem cells, leaving no possibility for replenishment of the pool of oocytes. Until recently, cryopreserved oocytes or embryos from *in vitro* fertilization (IVF) treatment were considered the only possible options for women to conceive after recovery from a sterilizing cancer treatment. These methods, however, cannot sustain long-term fertility, including support of functioning ovulatory cycles, and are not applicable to prepubertal girls. Further, they require at least two weeks for stimulation, among other factors, which may be incompatible with an urgent cancer treatment. Cryopreservation and transplantation of ovarian tissue overcome a number of these shortcomings: grafting of cryopreserved ovarian tissue can restore menstrual cyclicity to patients who entered menopause as a consequence of the treatment, and the patient gets the possibility of spontaneous conception (4,5). The technique can be performed from one day to another. Moreover, the method is applicable even in prepubertal girls, and ovarian tissue cryopreservation may be performed even in cases where chemotherapy has already been initiated (6), in contrast to IVF treatment.

Fertility preservation in young women and men who have experienced gonadotoxic treatment is now a central topic for professionals and patients (7), and the different strategies in this area will be discussed in this chapter. There is a special focus on cryopreservation of ovarian tissue, as this method is now gaining ground as a valid method of long-term fertility preservation in girls and women facing gonadotoxic therapy (8), while freezing testicular tissue is still in its infancy.

EFFECTS OF CHEMOTHERAPY AND RADIOTHERAPY ON THE OVARY

Chemotherapy drugs cause a reduction in the number of primordial follicles, diminish ovarian weight, and augment ovarian atrophy. The extent of the damage depends largely on the specific regimen of chemotherapy used and the age of the patient at treatment (9,10). The fact that ovaries of young girls contain a higher number of follicles than ovaries from older women makes them more resistant to chemotherapy and delays the age at which they potentially enter primary ovarian insufficiency (POI) (11).

Alkylating agents such as cyclophosphamide and busulfan are far more gonadotoxic than other chemotherapeutic agents. A study of young cancer patients found that the use of alkylating agents had an odds ratio (OR) of 4.0 for POI, which is significantly higher than when using platinum agents (OR = 1.8), plant alkaloids (OR = 1.2), or antimetabolites (OR <1) (12). The alkylating agents have been shown to cause extensive loss of primordial follicles in cancer patients (9,13), and animal studies indicate that this loss is dose dependent (14). The alkylating agent cyclophosphamide is a cell cycle-non-specific drug, and as such is more cytotoxic to the ovaries than cell cycle-specific drugs, as it may harm both resting and dividing cells. Studies in mice have shown massive apoptosis observed in the granulosa cells of growing, though not resting, follicles of cyclophosphamide-treated mice (10). However, interestingly, the same study demonstrated both a decrease in primordial follicles and an increase in early growing follicles, which suggest that cyclophosphamide actually activated the growth of the resting primordial follicle pool in mice, resulting in loss of the ovarian reserve (10).

Radiotherapy interrupts the normal cellular proliferation cycle and causes extensive cell damage. However, despite postnatal oocytes being mitotically inactive, they are still highly susceptible to the damage caused by radiotherapy. The prepubertal ovary it less vulnerable than in later reproductive life simply because of higher numbers of oocytes, but the risk of POI after abdominal radiotherapy is still considerable (15). It has been estimated that a total radiation exposure of 20 Gy fractionated over six weeks in younger women and children produces sterility with 95% confidence (16). Finally, the high-dose chemotherapies and radiotherapies used prior to bone marrow transplantation (BMT) leave the vast majority of patients without ovarian function and fertility (12).

CANDIDATES FOR FERTILITY-PRESERVING METHODS

Giving the patient an estimation of the risk of POI is very challenging due to a number of factors, including age, disease, stage of disease, and the fact that the planned chemotherapy treatment often changes during the course of treatment (17,18). To help physicians evaluate each patient, selection criteria such as the Edinburgh criteria

can be used for guidance (19). In the Danish fertility preservation program, the criteria are; a more than 50% risk of post-treatment infertility and an estimated greater than 50% chance of surviving five years after diagnosis. There is no strict upper age limit and in clinical practice women with a relatively high number of antral follicles in their mid-30s may be offered the procedure. Collectively, selection criteria should merely be used as guidance and not exact rules, as each woman should have an individual assessment of her ovarian reserve as well as her risk of POI.

Risk assessment

Most antineoplastic treatments in childhood are not hazardous to the immediate ovarian function of the affected girls, although they may reduce the future ovarian function and fertility potential. However, some treatments and cancer diagnoses are associated with a high risk of POI, and in these cases, fertility-preserving methods should be discussed with and offered to the woman or, in case of a young girl, together with her parents (20). The different fertility-preserving techniques' pros and cons and their relevance to girls and young women according to the planned treatment are presented in Table 62.1. Patients with an almost 100% risk of POI are those for whom BMT is planned and those receiving abdominal radiation. In cases where high-dose chemotherapy is planned, the indication for fertility preservation should be evaluated individually in relation to the planned dose and type of drug used. In patients who have already received a relatively mild chemotherapy because of a malignancy and who later experience a relapse, cryopreservation of ovarian tissue may be considered before the second round of chemotherapy, which usually includes more aggressive and gonadotoxic chemotherapeutics (20). For a more detailed description of the risk of treatment-related infertility with the main specific anticancer therapies, see Lambertini et al. (18).

Fertility preservation was initially indicated only for cancer patients receiving sterilizing chemotherapy; however, today indications cover patients receiving gonadotoxic chemotherapy for other systematic illnesses, such as autoimmune diseases, and in some patients undergoing oophorectomy for benign ovarian conditions.

CURRENT OPTIONS FOR THE PRESERVATION OF FEMALE FERTILITY

When a patient faces a substantial risk of POI, the different methods for fertility preservation (Figure 62.1), their advantages and disadvantages, their efficiency, and the possible experimental nature of the treatment need to be taken into consideration.

Hormonal suppression

It has been suggested that co-treatment with gonadotropin-releasing hormone (GnRH) analogs should protect the ovaries from the harmful effects of chemotherapy (21,22). Currently, there is no solid evidence to show a beneficial effect of GnRH analogs (23,24).

Cryopreservation of mature oocytes or embryos

Methods for cryopreservation of mature oocytes and embryos derived from couples undergoing IVF treatment are now standard and represent an effective method for preserving female fertility. In the infertile population, pregnancy rates between fresh and frozen–thawed oocytes or embryos are now almost the same. Among women with cancer, one retrospective study reported a live birth rate of 44.4% (25).

The primary drawbacks to IVF include the time required, cost, and risk of ovarian hyperstimulation syndrome. Moreover, patients should be aware that around 20 vitrified oocytes are required to achieve a live birth, as the live birth rate per vitrified oocyte (oocyte donation) is

Table 62.1 Fertility preserving measures applicable to female patients with a malignant diagnosis—pros and cons

Method	Planned treatment	Age group	Mode of obtaining future pregnancy	Advantages	Disadvantages
Oophoropexy	Abdominal radiation	P– girls P+ girls Adult women	Spontaneous or IVF	Standard procedure	Scatter radiation
Cryopreservation of oocytes and embryos	BMT, abdominal radiation, and high-dose AA	(P+ girls?) Adult women	Fertilization of oocytes and/or embryo transfer	Established technique	May incur delay Requires sperm Fixed fertility potential Not appropriate for P– girls
Cryopreservation of ovarian tissue	BMT, abdominal radiation, and high-dose AA	P– girls P+ girls Adult women	Spontaneous or IVF after transplantation of frozen–thawed tissue	Minimal delay Restores ovarian function → spontaneous and repeated conception No lower age limit	Requires surgery Risk of malignant cell contamination Efficacy unknown

Source: Table modified from Schmidt KT et al. *BJOG* 2010; 117: 163–74.
Abbreviations: AA, alkylating agents; BMT, bone marrow transplantation; IVF, *in vitro* fertilization; P–, prepubertal; P+, postpubertal.

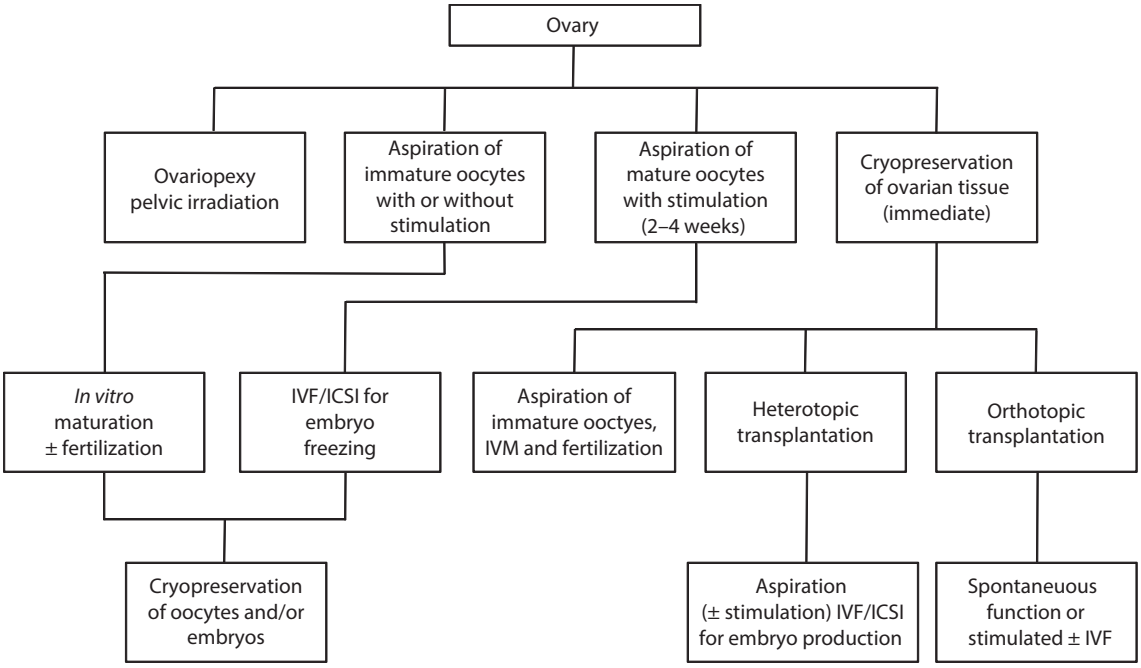

Figure 62.1 Options for fertility preservation in women. *Abbreviations*: IVM, *in vitro* maturation; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

5.7% in the most experienced teams in the world (26). The standard controlled ovarian hyperstimulation protocol starts at the onset of menses, which could result in a delay of two to four weeks. Fortunately, use of GnRH antagonists shortens this interval, and random-start protocols have proven to decrease the total time to starting the IVF cycle and cancer treatment, without compromising oocyte or embryo yield (27,28).

Cryopreservation of ovarian tissue

The success of ovarian cryopreservation is based on the high cryopreservation tolerance of small (resting) primordial follicles in contrast to the vulnerable, larger, growing follicles. The vast majority of primordial follicles are located in the outermost 1 or 2 mm of the ovarian cortex, which is relatively easy to isolate from the rest of the ovarian tissue. When the ovarian cortex has been frozen, it can be stored for years in liquid nitrogen, allowing time for the patient to recover. After the patient is cured, some of the cryopreserved tissue can be re-transplanted to those who entered menopause, and the ovarian grafts are able to re-establish a cyclic endocrine hormone milieu, including appropriate conditions for conception, gestation, and parturition, possibly via IVF–embryo transfer (29–31).

In the future, cryopreservation of ovarian tissue might be associated with the aspiration of small antral follicles followed by *in vitro* maturation (IVM), which could potentially offer the patient an additional chance of becoming pregnant (26,32). Immature oocytes can be collected from antral follicles in the ovarian tissue or found in the dissection medium at the time of the cryopreservation procedure, matured *in vitro*, and then cryopreserved (33,34). The first live birth resulting from a cryopreserved embryo obtained from *in vitro*-matured oocytes collected after oophorectomy was recently reported (35), as was the second clinical pregnancy (36).

TECHNICAL ASPECTS OF OVARIAN TISSUE CRYOPRESERVATION

The Danish Fertility Preservation Program was initiated in 1999. Since then, more than 1000 patients have had ovarian tissue cryopreserved for clinical purposes, and currently, 15–16 ovaries per year per million inhabitants are frozen. Since 2003, 107 autotransplantations have been performed in 85 women, resulting in 33 clinical pregnancies, of which 15 resulted in the birth of healthy babies (37–39), two legal abortions (40), and two second-trimester miscarriages. Thus, the Danish protocol for ovarian tissue cryopreservation has proven quite robust, and is becoming a well-established method of fertility preservation in the Danish healthcare system.

The Danish protocol

In most Danish patients, an entire ovary is removed surgically, often during laparoscopy (Figure 62.2a). The advantages of removing the whole ovary are the minimized risk of postoperational complications and the possibility of prolonging the window of possible fertility by repeated transplantations. Women with a single ovary may have slightly elevated serum follicle-stimulating hormone (FSH) concentrations (41), but appear to have an unreduced fertility potential either through natural conception or via IVF (42). However, the decision to cryopreserve one whole ovary in contrast to parts of it remains a matter of debate (43).

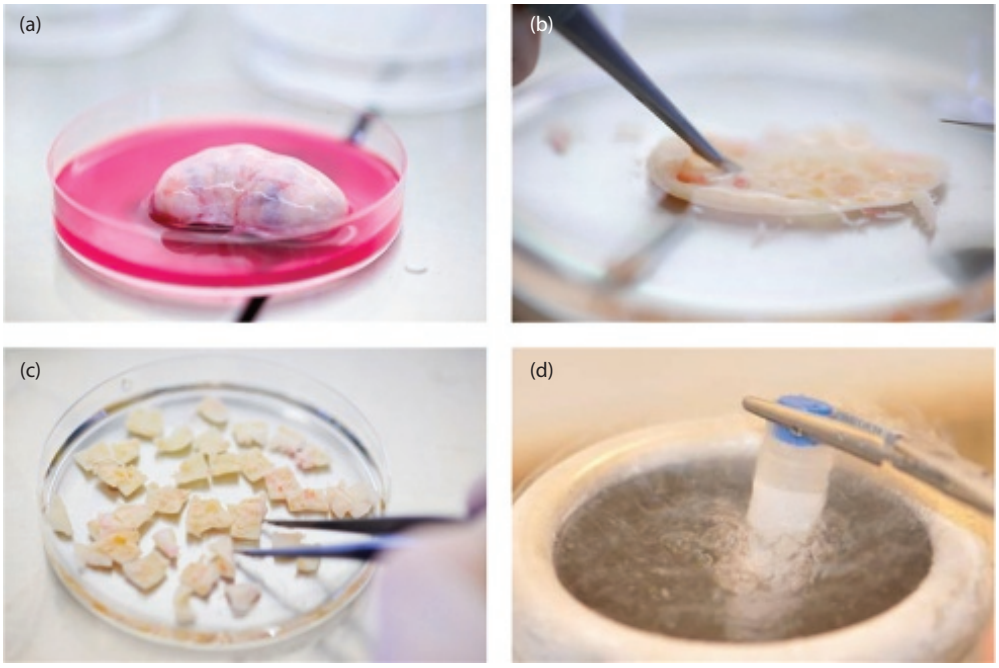

Figure 62.2 Cryopreservation of human ovarian tissue for fertility preservation. (a) One ovary or part of an ovary is surgically removed. (b) The medulla is removed and the cortex is trimmed to a thickness of 1–2 mm. (c) The cortex is then cut into pieces of 5 × 5 mm. The pieces of cortex equilibrate in a cold freezing solution for 25 minutes on ice. (d) The cortex pieces are transferred to individual cryotubes, manually seeded, and slow frozen in liquid nitrogen with a programmable Planer Freezer.

The ovary is transported to the cryopreservation facility and, at a sterile bench, the ovary is placed in a 10-cm Petri dish containing 20 mL isotonic saline solution, and the cortex is isolated using hooked forceps and scalpels (Figure 62.3). When all medullary tissue is removed and the cortex has been trimmed to a thickness of 1–2 mm (Figure 62.2b), it is cut into 5 × 5–15-mm pieces (Figure 62.2c). During the trimming procedure, the tissue is rinsed several times in an isotonic saline solution. The pieces are transferred to a 50-mL plastic tube containing 30 mL freezing solution (0.1 mol/L sucrose, 1.5 mol/L ethylene glycol, and 10 mg/mL human serum albumin [HSA] in phosphate-buffered saline [PBS]), and equilibrated for approximately 25 minutes at 1–2°C on a tilting table. The fragments of cortex are transferred individually to 1.8-mL cryovials (Nunc A/S, Roskilde, Denmark) using sterile forceps, each containing 1 mL fresh freezing solution, and these are cryopreserved using a programmable freezer (Planer 360-1.7, Planer Ltd, Middlesex, U.K.) (Figure 62.2d). The following program is used: starting temperature 1°C, then –2°C/minute to –9°C, five minutes of soaking, followed by manual seeding for ice crystal induction, –0.3°C/minute to –40°C, –10°C/minute to –140°C, and then directly into liquid nitrogen. From the moment the tissue enters the freezing solution and until initiation of the cryoprogram, exactly 30 minutes elapse and the temperature is constantly kept at around 1–2°C. Following freezing, the tubes are sealed in a second plastic holster (double sealing) (Figure 62.4a), and half of the tissue is long-term stored in each of two separate nitrogen tanks (Figure 62.4b). During the processing of the cortical tissue, a small piece of cortex is taken for histology and used to estimate the follicular density.

Figure 62.3 Instruments used for preparation of the ovarian cortex. Hooked forceps ensure a firm hold on the ovarian tissue during dissection, and a scalpel with a long cutting edge enables a smooth trimming of the cortex.

Quality control by xenotransplantation of human ovarian tissue

To qualitatively assess follicle survival following freezing, frozen–thawed ovarian cortical biopsies from 42 women were transplanted under the skin of oophorectomized immunodeficient mice a total of 49 times in our program (30). From these women, 36 had a malignant diagnosis prior to cryopreservation: breast cancer (n = 9);

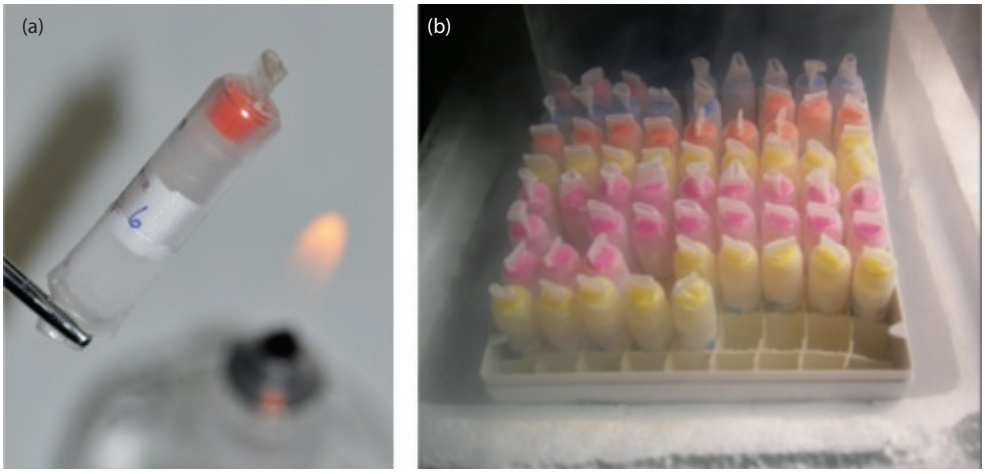

Figure 62.4 Dobbelt sealing and storage of cryotubes. (a) Once frozen, the cryotubes containing the ovarian tissue are double sealed in cryoflex. (b) Before long-term storage. Notice the different color codes for each patient.

Hodgkin's and non-Hodgkin's lymphoma (n = 9); leukemia (acute lymphoblastic, chronic myeloid, and acute myeloid; n = 7); sarcoma (n = 5); and miscellaneous (n = 6). The mice were killed after four weeks (Figure 62.5a). Histological evaluation showed healthy primordial follicles in all of the cortical biopsies and confirmed that follicular viability was maintained after thawing (Figure 62.5b). Transplantation of frozen–thawed ovarian tissue to immunodeficient mice is still considered to be the best way of evaluating the survival of follicles.

Slow freezing versus vitrification

The most widely used protocol for ovarian cryopreservation is the slow-freezing technique (44–46), and up until now, all children born (but two) have resulted from slow-frozen cortical tissue (39,47,48). Two techniques are currently being tested as alternatives to the slow-freezing method. The first is vitrification, in which the tissue is exposed to high concentrations of cryoprotectants for a short time and immediately plunged in liquid nitrogen. Compared with slow freezing, vitrification may be associated with improved maintenance of ovarian follicular and stromal structures, as well as increased follicle survival rates (49–51). However, others find superior results using slow freezing (52,53), and it is currently questionable whether vitrification offers any significant clinical benefit. The second alternative is whole-ovary freezing, in which the cryoprotectants are introduced through the vascular pedicles *in vitro* followed by cryopreservation (54), and this approach may avoid the ischemia-induced follicle loss that occurs in connection with transplantation because

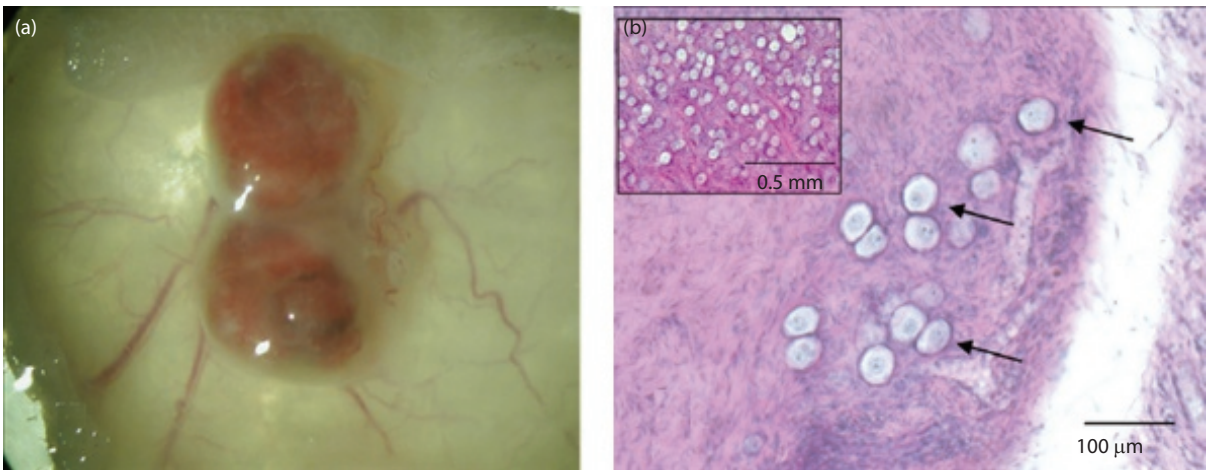

Figure 62.5 (a) Two pieces of frozen–thawed human ovarian cortex (5 × 5 mm) transplanted under the skin of an oophorectomized mouse. After two weeks, macroscopically visible revascularization was established. (b) Human ovarian cortex kept on ice for 20 hours prior to freezing. Thawed tissue was transplanted under the skin of an oophorectomized mouse for four weeks. Histology showed healthy primordial follicles (arrows) surrounded by small blood vessels. Insert shows histology of the fresh sample. (From Rosendahl M et al. *Reprod Biomed Online* 2011; 22: 162–71.)

anastomosis of ovarian vessels ensures a rapid blood supply (55). However, currently, the ovarian vessels seem to become damaged during the freezing process.

Transportation of ovarian tissue prior to cryopreservation

Ovarian tissues remain viable after transportation for up to five hours on ice prior to freezing (30,56). This allows hospitals without cryopreservation expertise to treat women locally for the cancer disease and just send the ovarian tissue to the center that performs cryopreservation. This facilitates quality control, proper equipment, and personnel to fulfill clinical, legal, and scientific standards required for proper conduction of the procedure. The feasibility of centralized cryobanking has been proven by the Danish experience of transporting ovarian tissue prior to freezing, and these principles have now been introduced in Germany and many other countries (5). We have demonstrated good follicle survival after freezing and transplantation of human ovarian cortex to ovariectomized immunodeficient mice for a period of four weeks following a transport period of 20 hours on ice prior to cryopreservation (Figure 62.5b) (30), and recent German results show that overnight transportation of tissue before freezing facilitates live births (57).

AUTOTRANSPLANTATION OF CRYOPRESERVED OVARIAN TISSUE

Although ovarian tissue from thousands of girls and women has been cryopreserved, globally, results from transplantation are accumulating at a slow pace. Usually, the patient needs at least two years for cure before receiving transplantation. Furthermore, fortunately, merely half of the women who had one ovary cryopreserved actually entered menopause immediately or shortly after termination of treatment (58). The number of re-implantations performed worldwide is not known; however, it is estimated to have approached 400–500 by mid-2017.

Thawing of cryopreserved ovarian tissue

Thawing consists of a three-step procedure, each lasting 10 minutes (Figure 62.6). The vials containing the frozen tissue are placed in a 37°C water bath. Immediately after the solution becomes liquid, the cortical tissue is removed and placed in the first thawing medium (0.75 mol/L ethylene glycol, 0.25 mol/L sucrose, and 10 mg/mL HSA in PBS) and then moved to the second medium with sterile forceps (0.25 mol/L sucrose and 10 mg/mL HSA in PBS) on a tilting table at room temperature. For the last 10 minutes of thawing, the tissue is transferred to PBS with 10 mg/mL HSA in a 10-mL plastic tube (Figure 62.6, insert), which is brought to the operating theatre for immediate re-implantation. The period of time between transplantation and revascularization of the tissue appears to be critical to follicle survival, since 60%–70% of follicles have been found to be lost in connection with transplantations in sheep (59), whereas only a small fraction is lost due to the actual cryopreservation procedure.

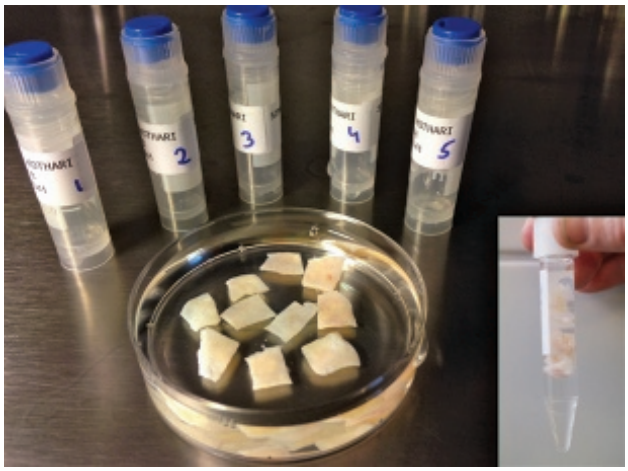

Figure 62.6 Three-step thawing procedure. Pieces of cortex are transferred to thawing solutions with decreasing concentrations of cryoprotectant. Insert shows the final step of thawing, and the tissue is subsequently brought to the operating theater for transplantation.

Orthotopic and heterotopic transplantation

In most Danish patients, transplantation has been performed as a combined laparoscopy/mini-laparotomy to subcortical pockets of the remaining menopausal ovary (60). Under general anesthesia, a 50-mm surgical incision to the lower abdomen is performed, and the remaining ovary is mobilized laparoscopically and made available on the surface. Longitudinal incisions in the ovarian cortex are made, thus creating small pockets just below the cortex on each side of the ovary (Figures 62.7a and 62.7b). The fragments are aligned next to one another in the pockets with the cortex side outward (60). Normally, 6–10 pieces of cortex can be positioned onto the remaining ovary depending on the size of the ovary. Whenever possible, the tissue is transplanted under the cortex of the remaining ovary left *in situ* (orthotopic transplantation; Figures 62.7a and 62.7b); however, in some cases in which the remaining ovary has been removed or the ovarian volume is significantly reduced, it is necessary to transplant the tissue to peritoneal pockets on the anterior abdominal wall or to the lateral pelvic wall (peritoneal orthotopic or heterotopic transplantation; Figures 62.7c and 62.7d). However, only two twin births are products of heterotopic graft sites (61,62), perhaps reflecting a reduced pregnancy potential for such tissue.

RESTORATION OF OVARIAN ACTIVITY AND OUTCOMES

Ovarian activity is normally restored within 3.5–6.5 months post-grafting, which concurs with the period of follicle growth from the primordial to the antral stage (30,31,63). FSH concentrations measured after the first transplantations in 12 Danish patients show that FSH remains high for a short period after transplantation until follicular growth reaches a stage in which estradiol and inhibin B are secreted, and then starts to decline toward premenopausal concentrations (Figure 62.8).

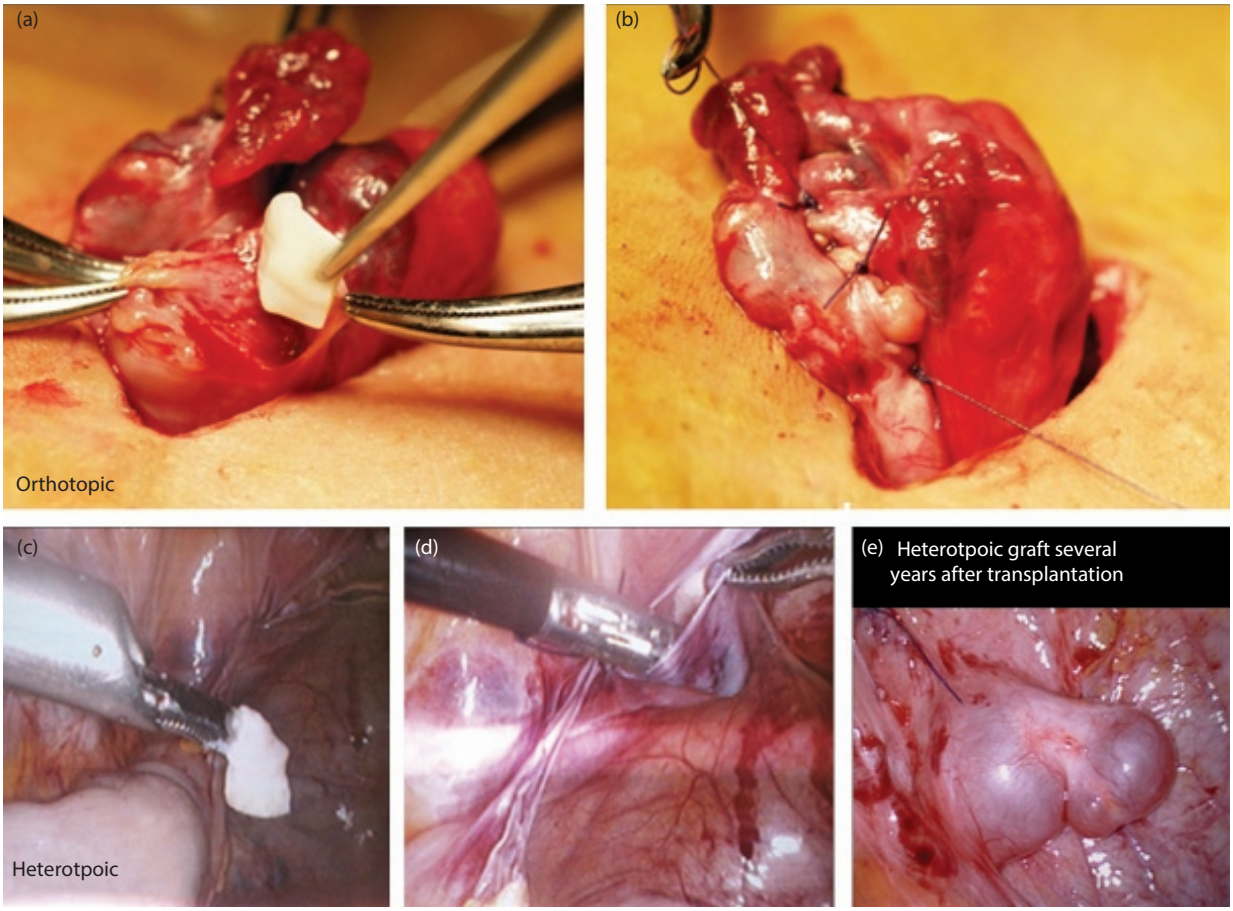

Figure 62.7 Transplantation of cryopreserved ovarian tissue. (a and b) Orthotopic transplantation: pieces of thawed ovarian cortex being transplanted in a subcortical pocket in the *in situ* ovary. (c and d) Heterotopic transplantation: pieces of thawed ovarian cortex being transplanted in a subperitoneal pocket corresponding to the pelvic wall. (e) Two human antral follicles at a heterotopic graft site several years after transplantation of thawed ovarian tissue.

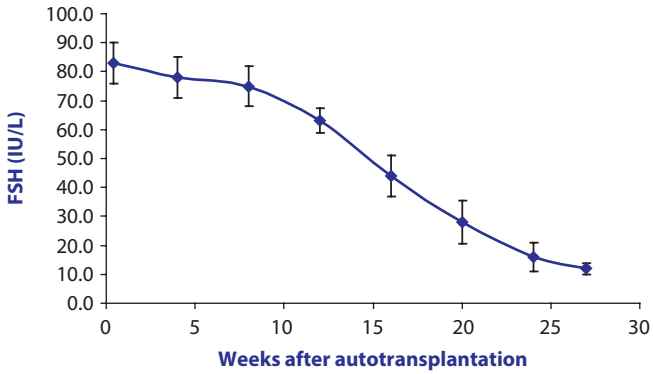

Figure 62.8 Restoration of ovarian function serum levels of FSH (IU/L) in 12 Danish patients after autotransplantation of frozen–thawed ovarian tissue (mean ± standard error of the mean). *Abbreviation*: FSH, follicle-stimulating hormone.

In 2013, three different European centers (from Belgium, Denmark, and Spain) collected and evaluated the results of 60 orthotopic re-implantation cases (31). Fifty-one of the 60 patients were followed up over six months later. The study demonstrated that 93% of the women showed restoration of ovarian activity. Eleven of these 51 patients became pregnant and six had already given birth to 12 healthy children at the time of the follow-up. In addition, >50% of the women who became pregnant were able to conceive naturally, which favors orthotopic transplantation. Moreover, the age of patients at the time of cryopreservation has previously been reported to be a predictive factor for pregnancy (64), and the majority of pregnant women were actually under the age of 30 years.

In addition, two groups have been able to induce puberty by re-implanting frozen–thawed prepubertal ovarian tissue in two young girls (65,66), which demonstrates the wide range of possibilities this method offers.

Pregnancy and live birth rate

Many cases of re-implantation of cryopreserved ovarian tissue have been performed throughout the world and, to date, almost 100 live births have been reported in peer-reviewed journals and abstracts of congresses (39,67). The worldwide expansion of this technique suggests its application in routine clinical practice. In the series of 60 reported live births, the number of re-implantations performed in each center is unknown, as well as most of the intrinsic ovarian activity before grafting, which makes it impossible to estimate a pregnancy rate (67,68). Recently, in 2015, results from five centers were collected to evaluate a series of 111 cases of orthotopic re-implantation (8). In this large series, the proportion of women who conceived was 29% (n = 32). Because the number of transplantations (the denominator) is known, this information is highly relevant and based on evidence. Two women delivered three babies each, proving the efficacy of the technique, as well as the possibility of conceiving naturally several times after only one procedure (31,68).

In connection with IVF, it should be pointed out that in series published by Andersen et al. (29), Dolmans et al. (69), and Meirow et al. (70), empty follicle rates as high as 29%–35% were observed during the IVF procedure, and the percentage of immature or degenerated oocytes is much higher (37%) in patients with frozen–thawed transplanted tissue than in the general population undergoing intracytoplasmic sperm injection (ICSI) (69). In a study by Greve and co-workers, 12 women who had thawed ovarian tissue transplanted received assisted reproductive techniques, and the outcome per cycle was a pregnancy rate of 6.9% and a live birth rate of 3.8% (71). It was suggested that the poor outcome reflected reduced follicular selection, rather than aged or damaged oocytes.

Longevity of the grafts

In the Danish cohort, the longevity of the transplants was found to be between nine months and nine years and still functioning (38,71,72). Figure 62.7e shows two human antral follicles at a heterotopic graft site several years after transplantation of thawed ovarian tissue, demonstrating successful grafting and integration of the tissue. Other studies have shown that the transplanted grafts have a mean longevity of approximately four to five years when the follicular density was well preserved (26). Given that the longevity of the tissue is good and that, in many cases, the women have enough tissue stored for two to three transplantations, the cryopreserved tissue could be enough to restore endocrine function until the natural age of menopause.

EVALUATION OF OVARIAN TISSUE FOR RESIDUAL DISEASE

There may be a risk of reintroducing the original cancer in connection with transplantation of ovarian tissue that is removed before the patients receive chemotherapy. However, ovarian tissue cryopreservation is usually only offered to patients with a high chance of long-term survival, and these patients will typically have low-stage and limited disease, with a minimal risk of dissemination and ovarian involvement (26,30). Nevertheless, one exception is those patients who have hematological malignancies, where ovarian involvement cannot be excluded.

To minimize risk of grafting ovarian tissue to cancer survivors, a variety of different techniques may be used either separately or in combination (73): (i) the surgeon performing excision of the tissue should observe for possible gross pathology near and on the ovaries; (ii) before re-implantation, a piece of the frozen–thawed tissue can be evaluated by histology and immunohistochemistry using the markers that characterized the original tumor; (iii) evaluation by reverse transcription and quantitative polymerase chain reaction can be carried out for specific cancer markers; and (iv) immunodeficient mice can be transplanted with pieces of ovarian tissue and kept for four to six months to detect whether the original human cancer develops. If the mice do not develop cancer, it cannot be excluded that the tissue contains tumor cells, as some human cancers do not grow well in mice. However, even though one ovarian piece has been evaluated to be risk free, it is impossible to completely conclude that other pieces from the same patient might not be harboring malignant cells and thereby lead to a relapse of the oncological disease.

Risk of malignant cell contamination

Numerous studies have been conducted on the risk of cell contamination by re-implanting cryopreserved ovarian tissue. In 2013, three independent groups carried out systematic reviews of the literature. All three reviews concluded that the highest risk of reintroducing cancer cells via autotransplantation of cryopreserved ovarian tissue was for leukemic patients (74–76). Additionally, some of the studies estimated that there was a moderate risk of transplantation for any of the gastrointestinal cancers (74,76). The most reassuring data were found in relation to autotransplantation of patients surviving lymphoma. All three studies concluded that there was a low risk of metastasis in Hodgkin's lymphoma (74–76). Concerning patients with leukemia, two *in vivo* studies from Denmark and Belgium have found that immunodeficient mice with xenotransplanted tissue from patients in complete remission at the time of tissue collection did not develop leukemia. These studies concluded that collection of ovarian tissue from patients with leukemia should be done when they are in complete remission (77,78). Even though these results are encouraging for continued storage of tissue from leukemic patients, there is no established method to regain fertility for patients who suffered from leukemia, but in the future, isolated follicle transplantation or IVM (79,80) may become possible.

Most importantly, no relapses have been reported at any graft sites. However, one case report documented a relapse concerning the recurrence of a granulosa cell tumor, but no evidence of tumor was found at the graft site (81). This could suggest that ovarian diseases may require stricter precautions.

PRESERVATION OF MALE FERTILITY

Subfertility affects approximately 15% of all couples, and a severe male factor is identified in 17% of couples who are affected by subfertility. While the etiology of a severe male factor infertility remains largely unknown, prior gonadotoxic treatment and genomic aberrations have been associated with this type of subfertility (82).

Effects of chemotherapy and radiotherapy on the testis

The testis has been shown to be highly susceptible to the toxic effects of irradiation and chemotherapy at all stages of life (83). The impact of combination chemotherapy on the spermatogenic epithelium is dependent on the type and dosage of the drugs used (83–86). The threshold dose of cyclophosphamide in relation to infertility has been estimated to be between 7.5 and 9 g/m^2 (87,88), and in postpubertal boys to be 10 g/m^2 (89). In the prepubertal testis, germline stem cells are acutely and dose-dependently depleted following radiation exposure (90,91). Doses of more than 6 Gy are able to deplete the spermatogonial stem cell (SSC) pool and lead to permanent infertility (92,93). Recovery of spermatogenesis can occur from the remaining stem cells, and relies on the type, dose, and fractionation of cytotoxic drugs and irradiation (94).

Current options for the preservation of male fertility

Cryopreservation of ejaculated sperm is the routinely used tool for fertility preservation in adult male patients (95). Success rates in achieving a pregnancy using cryopreserved sperm have greatly improved by ICSI (96). All pubertal boys with testis volumes above 10–12 mL are encouraged to donate a semen sample prior to cancer therapy (95,96). Alternatively, electroejaculation, penile vibratory stimulation, search for spermatozoa in urine sample, or testicular sperm extraction from a biopsy can be used as sources for retrieving spermatozoa for boys who are unable to ejaculate (97). Since prepubertal boys cannot benefit from sperm banking and cryopreserved samples are finite resources that do not offer the possibility of restoring natural fertility, a potential alternative strategy for preserving their fertility is cryopreservation of testicular tissue and SSCs prior to cancer treatment (98). This application involves enzymatic isolation of SSCs from the frozen–thawed testicular biopsy, *in vitro* propagation, and transplantation of SSCs into the seminiferous tubules via the efferent duct or rete testis (99,100). Upon SSC transplantation, SSCs migrate to the basement membrane of the seminiferous tubules, colonize the epithelium, and undergo self-renewal and differentiation so that permanent spermatogenesis is established, which should allow natural conception without further fertility treatment.

Several protocols have already been developed for the cryopreservation of cell suspensions and testicular fragments from adult and cryptorchid testes using propanediol, glycerol, ethylene glycol, or dimethyl sulfoxide (90,101–103).

Fertility restoration by testicular grafting and transplantation of SSCs

Prepubertal testicular tissue from different species (mice, hamsters, and monkeys) survives freezing surprisingly well and, after xenografting, is able to support sperm production, which can be retrieved from the tissue for assisted reproductive technique procedures (104). However, no report of successful testicular autografting in men has been published yet.

SSC injection is considered the most promising tool for fertility restoration in prepubertal cancer patients. In mice, germ cell transplantation was successfully performed for the first time in 1994, when microinjection of spermatogonia into the seminiferous tubules prompted germ cell development up to complete spermatogenesis (105). Due to differences in anatomy and consistency and the larger testis size, injection of SSCs via the rete testis has proved to be a better treatment site for species such as cattle, primates, and humans (106). In the context of human fertility restoration, adult and prepubertal human SSCs have been successfully grown *in vitro* without losing their stem cell capacity or ability to colonize the seminiferous tubules upon xenotransplantation (107,108).

Most recently, rhesus monkey SSCs have been injected under slow constant pressure into the rete testis under ultrasound guidance, and sperm cells that were able to fertilize oocytes by ICSI were found in the ejaculate of recipients (109). This study in non-human primates is of course an important milestone towards using SSCs to restore human fertility; however, it remains vitally important to prove that the epigenetic programming and stability of SSCs are not compromised following cryopreservation, culture, and transplantation in humans (110).

CONCLUSIONS

Both established and experimental therapies can now be used to allow young women and men to overcome the infertility that may result from gonadotoxic treatment. Ovarian tissue cryopreservation is becoming a well-established technique for fertility preservation worldwide, and almost 100 healthy children have been born so far using this approach. The number of transplantations is increasing as women surviving their illness return to get their fertility restored. The longevity of the grafts is surprisingly long in some cases, lasting up to nine years and still functioning, thereby showing the strength of this technique. In men, cryopreservation of sperm is the gold standard procedure for preserving fertility, and young boys and men can have their testicular tissue cryopreserved with good results, but strategies for transplantation still need to be established in order to restore fertility.

Most importantly, if there is a risk of gonadal damage and fertility loss, patients should be referred to the infertility specialist by hematologists and oncologists before gonadotoxic treatment is initiated in order to receive the proper counseling on the available fertility preservation strategies.

FUTURE ASPECTS

For the future, new strategies should be optimized and investigated. IVM of early follicle stages from which mature fertilizable oocytes could be retrieved is one way to avoid the risk of transmitting malignant cells when re-implanting frozen–thawed ovarian tissue. This has been achieved in mice (111), and a metaphase II oocyte was retrieved from a primate follicle cultured from a preantral follicle (112). In humans, long-term culture of preantral follicles to early antral stages has been achieved (80,113); however, research is still required to establish this as a possible clinical application. Another approach suggests the transfer of isolated human primordial follicles into an artificial ovary—a specially created scaffold—so as to provide an alternative way of restoring fertility in patients who cannot benefit from transplantation of cryopreserved ovarian tissue (114,115).

REFERENCES

1. Stoop D, Maes E, Polyzos NP et al. Does oocyte banking for anticipated gamete exhaustion influence future relational and reproductive choices? A follow-up of bankers and non-bankers. *Hum Reprod* 2015; 30: 338–44.
2. Andersen CY, Kristensen SG. Novel use of the ovarian follicular pool to postpone menopause and delay osteoporosis. *Reprod Biomed Online* 2015; 31: 128–31.
3. Jemal A, Siegel R, Ward E et al. Cancer statistics. *CA Cancer J Clin* 2007; 57: 43–66.
4. Meirow D, Hardan I, Dor J et al. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. *Hum Reprod* 2008; 23: 1007–13.
5. Von Wolff M, Donnez J, Hovatta O et al. Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy—A technique in its infancy but already successful in fertility preservation. *Eur J Cancer* 2009; 45: 1547–53.
6. El Issaoui M, Giorgione V, Mamsen LS, Rechnitzer C, Birkebæk N, Clausen N, Kelsey TW, Andersen CY. Effect of first line cancer treatment on the ovarian reserve and follicular density in girls under the age of 18 years. *Fertil Steril* 2016; 106: 1757–62.
7. Lamar CA, Decherney AH. Fertility preservation: State of the science and future research directions. *Fertil Steril* 2008; 91: 316–19.
8. Donnez J, Dolmans MM, Diaz C, Pellicer A. Ovarian cortex transplantation: Time to move on from experimental studies to open clinical application. *Fertil Steril* 2015; 104: 1097–8.
9. Meirow D, Biederman H, Anderson RA et al. Toxicity of chemotherapy and radiation on female reproduction. *Clin Obstet Gynecol* 2010; 53: 727–39.
10. Kalich-Philosoph L, Roness H, Carmely A et al. Cyclophosphamide triggers follicle activation and "burnout"; AS101 prevents follicle loss and preserves fertility. *Sci Transl Med* 2013; 5: 185ra62.
11. Nicholson HS, Byrne J. Fertility and pregnancy after treatment for cancer during childhood or adolescence. *Cancer* 1993; 71: 3392–9.
12. Meirow D. Reproduction post-chemotherapy in young cancer patients. *Mol Cell Endocrinol* 2000; 169: 123–31.
13. Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. *Cancer* 2007; 110: 2222–9.
14. Meirow D, Lewis H, Nugent D et al. Subclinical depletion of primordial follicular reserve in mice treated with cyclophosphamide: Clinical importance and proposed accurate investigative tool. *Hum Reprod* 1999; 14: 1903–7.
15. Wallace WHB, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. *Int J Radiat Oncol Biol Phys* 2005; 62: 738–44.
16. Lushbaugh CC, Casarett GW. The effects of gonadal irradiation in clinical radiation therapy: A review. *Cancer* 1976; 37: 1111–20.
17. Donnez J, Dolmans MM. Fertility preservation in women. *Nat Rev Endocrinol* 2013; 9: 735–49.
18. Lambertini M, Del Mastro L, Pescio MC et al. Cancer and fertility preservation: International recommendations from an expert meeting. *BMC Med* 2016; 14: 1.
19. Wallace WH, Smith AG, Kelsey TW et al. Fertility preservation for girls and young women with cancer: Population-based validation of criteria for ovarian tissue cryopreservation. *Lancet Oncol* 2014; 15: 1129–36.
20. Schmidt KT, Larsen EC, Andersen CY, Andersen AN. Risk of ovarian failure and fertility preserving methods in girls and adolescents with a malignant disease. *BJOG* 2010; 117: 163–74.
21. Badawy A, Elnashar A, El-Ashry M, Shahat M. Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: Prospective randomized study. *Fertil Steril* 2009; 91: 694–7.
22. Peeyra Pacheco B, Méndez Ribas JM, Milone G et al. Use of GnRH analogs for functional protection of the ovary and preservation of fertility during cancer treatment in adolescents: A preliminary report. *Gynecol Oncol* 2001; 81: 391–7.
23. Blumenfeld Z, von Wolff M. GnRH-analogues and oral contraceptives for fertility preservation in women during chemotherapy. *Hum Reprod Update* 2008; 14: 543–52.
24. Munhoz RR, Pereira AA, Sasse AD et al. Gonadotropin-releasing hormone agonists for ovarian function preservation in premenopausal women undergoing chemotherapy for early-stage breast cancer: A systematic review and meta-analysis. *JAMA Oncol* 2016; 2: 65–73.
25. Dolmans MM, Hollanders de Ouderaen S, Demylle D et al. Utilization rates and results of long-term embryo cryopreservation before gonadotoxic treatment. *J Assist Reprod Genet* 2015; 32: 1233–7.

26. Donnez J, Dolmans MM. Ovarian tissue freezing: Current status. *Curr Opin Obstet Gynecol* 2015; 27: 222–30.
27. von Wolff M, Thaler CJ, Frambach T et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. *Fertil Steril* 2008; 92: 1360–5.
28. Cakmak H, Katz A, Cedars MI et al. Effective method for emergency fertility preservation: Random-start controlled ovarian stimulation. *Fertil Steril* 2013; 100: 1673–80.
29. Andersen CY, Rosendahl M, Byskov AG et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. *Hum Reprod* 2008; 23: 2266–72.
30. Rosendahl M, Schmidt KT, Ernst E et al. Cryopreservation of ovarian tissue for a decade in Denmark: A view of the technique. *Reprod Biomed Online* 2011; 22: 162–71.
31. Donnez J, Dolmans MM, Pellicer A et al. Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: A review of 60 cases of reimplantation. *Fertil Steril* 2013; 99: 1503–13.
32. Wilken-Jensen HN, Kristensen SG, Jeppesen JV et al. Developmental competence of oocytes isolated from surplus medulla tissue in connection with cryopreservation of ovarian tissue for fertility preservation. *Acta Obstet Gynecol Scand* 2014; 93: 32–7.
33. Yin H, Jiang H, Kristensen SG et al. Vitrification of *in vitro* matured oocytes collected from surplus ovarian medulla tissue resulting from fertility preservation of ovarian cortex tissue. *J Assist Reprod Genet* 2016; 33: 741–6.
34. Fasano G, Moffa F, Dechène J et al. Vitrification of *in vitro* matured oocytes collected from antral follicles at the time of ovarian tissue cryopreservation. *Reprod Biol Endocrinol* 2011; 9: 150.
35. Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, Loh SF, Chia YN. First pregnancy and live birth resulting from cryopreserved embryos obtained from *in vitro* matured oocytes after oophorectomy in an ovarian cancer patient. *Hum Reprod* 2014; 29: 276–8.
36. Segers I, Mateizel I, Van Moer E et al. *In vitro* maturation (IVM) of oocytes recovered from ovariectomy specimens in the laboratory: A promising "*ex vivo*" method of oocyte cryopreservation resulting in the first report of an ongoing pregnancy in Europe. *J Assist Reprod Genet* 2015; 32: 1221–31.
37. Macklon KT, Jensen AK, Loft A et al. Treatment history and outcome of 24 deliveries worldwide after autotransplantation of cryopreserved ovarian tissue, including two new Danish deliveries years after autotransplantation. *J Assist Reprod Genet* 2014; 31: 1557–64.
38. Jensen AK, Kristensen SG, Macklon KT et al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. *Hum Reprod* 2015; 30: 2838–45.
39. Jensen AK, Macklon KT, Fedder J, Ernst E, Humaidan P, Andersen CY. 86 successful births and 9 ongoing pregnancies worldwide in women transplanted with frozen-thawed ovarian tissue: Focus on birth and perinatal outcome in 40 of these children. *J Assist Reprod Genet* 2017; 34: 325–336.
40. Greve T, Ernst E, Markholt S, Schmidt KT, Andersen CY. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue. *Acta Obstet Gynecol Scand* 2010; 89: 1589–91.
41. Cooper GS, Thorp JM, Jr. FSH levels in relation to hysterectomy and to unilateral oophorectomy. *Obstet Gynecol* 1999; 94: 969–72.
42. Lass A. The fertility potential of women with a single ovary. *Hum Reprod Update* 1999; 5: 546–50.
43. Anderson RA, Wallace WH. Fertility preservation in girls and young women. *Clin Endocrinol (Oxf)* 2011; 75: 409–19.
44. Hovatta O, Silye R, Krausz T et al. Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectants. *Hum Reprod* 1996; 11: 1268–72.
45. Fuller B, Paynter S. Fundamentals of cryobiology in reproductive medicine. *Reprod Biomed Online* 2004; 9: 680–91.
46. Donnez J, Dolmans MM, Demylle D et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004; 364: 1405–10.
47. Kawamura K, Cheng Y, Suzuki N et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA* 2013; 110: 17474–9.
48. Suzuki N, Yoshioka N, Takae S et al. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Hum Reprod* 2015; 30: 608–15.
49. Keros V, Xella S, Hultenby K et al. Vitrification versus controlled-rate freezing in cryopreservation of human ovarian tissue. *Hum Reprod* 2009; 24: 1670–83.
50. Amorim CA, Curaba M, Van Langendonckt A, Dolmans MM, Donnez J. Vitrification as an alternative means of cryopreserving ovarian tissue. *Reprod Biomed Online* 2011; 23: 160–86.
51. Ting AY, Yeoman RR, Lawson MS, Zelinski MB. *In vitro* development of secondary follicles from cryopreserved rhesus macaque ovarian tissue after slow-rate freeze or vitrification. *Hum Reprod* 2011; 26: 2461–72.
52. Isachenko V, Isachenko E, Weiss JM, Todorov P, Kreienberg R. Cryobanking of human ovarian tissue for anti-cancer treatment: Comparison of vitrification and conventional freezing. *Cryo Lett* 2009; 30: 449–54.
53. Oktem O, Alper E, Balaban B et al. Vitrified human ovaries have fewer primordial follicles and produce less antimüllerian hormone than slow-frozen ovaries. *Fertil Steril* 2011; 95: 2661–4.e1.
54. Martinez-Madrid B, Camboni A, Dolmans MM et al. Apoptosis and ultrastructural assessment after cryopreservation of whole human ovaries with their vascular pedicle. *Fertil Steril* 2007; 87: 1153–65.

55. Bromer JG, Patrizio P. Fertility preservation: The rationale for cryopreservation of the whole ovary. *Semin Reprod Med* 2009; 27: 465–71.
56. Schmidt KL, Ernst E, Byskov AG, Nyboe Andersen A, Andersen CY. Survival of primordial follicles following prolonged transportation of ovarian tissue prior to cryopreservation. *Hum Reprod* 2003; 18: 2654–9.
57. Dittrich R, Hackl J, Lotz L et al. Pregnancies and live births after 20 transplantations of cryopreserved ovarian tissue in a single center. *Fertil Steril* 2015; 103: 462–8.
58. Rosendahl M, Andersen CY, Ernst E et al. Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: A follow-up study. *Hum Reprod* 2008; 23: 2475–83.
59. Baird DT, Webb R, Campbell BK, Harkness LM, Gosden RG. Long-term ovarian function in sheep after ovariectomy and transplantation of autografts stored at –196 C. *Endocrinology* 1999; 140: 462–71.
60. Schmidt KT, Rosendahl M, Ernst E et al. Autotransplantation of cryopreserved ovarian tissue in 12 women with chemotherapy-induced premature ovarian failure: The Danish experience. *Fertil Steril* 2011; 95: 695–701.
61. Stern CJ, Gook D, Hale LG et al. First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy. *Hum Reprod* 2013; 28: 2996–9.
62. Kristensen SG, Giorgione V, Humaidan P, Alsbjerg B, Bjørn AB, Ernst E, Andersen CY. Fertility preservation and refreezing of transplanted ovarian tissue-a potential new way of managing patients with low risk of malignant cell recurrence. *Fertil Steril*. 2017; 107(5): 1206–1213.
63. Schmidt KL, Andersen CY, Loft A et al. Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation. *Hum Reprod* 2005; 20: 3539–46.
64. Donnez J, Silber S, Andersen CY et al. Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births. *Ann Med* 2011; 43: 437–50.
65. Poirot C, Abirached F, Prades M et al. Induction of puberty by autograft of cryopreserved ovarian tissue. *Lancet* 2012; 379: 588.
66. Ernst E, Kjærsgaard M, Birkebæk NH et al. Case report: Stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. *Eur J Cancer* 2013; 49: 911–4.
67. Donnez J, Dolmans MM. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. *J Assist Reprod Genet* 2015; 32: 1167–70.
68. Andersen CY. Success and challenges in fertility preservation after ovarian tissue grafting. *Lancet* 2015; 385: 1947–8.
69. Dolmans MM, Donnez J, Camboni A et al. IVF outcome in patients with orthotopically transplanted ovarian tissue. *Hum Reprod* 2009; 24: 2778–87.
70. Meirow D, Levron J, Eldar-Geva T et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. *N Engl J Med* 2005; 353: 318–21.
71. Greve T, Schmidt KT, Kristensen SG et al. Evaluation of the ovarian reserve in women transplanted with frozen and thawed ovarian cortical tissue. *Fertil Steril* 2012; 97: 1394–8.e1.
72. Andersen CY, Silber SJ, Bergholdt SH, Jorgensen JS, Ernst E. Long-term duration of function of ovarian tissue transplants: Case reports. *Reprod Biomed Online* 2012; 25: 128–32.
73. Rauff S, Giorgione V, Yding Andersen C. Potential malignant cell contamination in transplanted ovarian tissue. *Expert Opin Biol Ther* 2016; 16: 285–9.
74. Dolmans MM, Luyckx V, Donnez J et al. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. *Fertil Steril* 2013; 99: 1514–22.
75. Bastings L, Beerendonk CC, Westphal JR et al. Autotransplantation of cryopreserved ovarian tissue in cancer survivors and the risk of reintroducing malignancy: A systematic review. *Hum Reprod Updat* 2013; 19: 483–506.
76. Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: A review of the literature. *J Assist Reprod Genet* 2013; 30: 11–24.
77. Dolmans MM, Marinescu C, Saussoy P et al. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. *Blood* 2010; 116: 2908–14.
78. Greve T, Clasen-Linde E, Andersen MT et al. Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells. *Blood* 2012; 120: 4311–6.
79. Dolmans MM, Yuan WY, Camboni A et al. Development of antral follicles after xenografting of isolated small human preantral follicles. *Reprod Biomed Online* 2008; 16: 705–11.
80. Yin H, Kristensen SG, Jiang H et al. Survival and growth of isolated pre-antral follicles from human ovarian medulla tissue during long-term 3D culture. *Hum Reprod* 2016; 31: 1531–9.
81. Stern CJ, Gook D, Hale LG et al. Delivery of twins following heterotopic grafting of frozen–thawed ovarian tissue. *Hum Reprod* 2014; 29(8): 1828.
82. van der Steeg JW, Steures P, Eijkemans MJ et al. Pregnancy is predictable: A large-scale prospective external validation of the prediction of spontaneous pregnancy in subfertile couples. *Hum Reprod* 2007; 22: 536–542.
83. Jahnukainen K, Ehmcke J, Hou M, Schlatt S. Testicular function and fertility preservation in male cancer patients. *Best Pract Res Clin Endocrinol Metab* 2011; 252: 287–302.
84. Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: Who is

at risk and what can be offered? *Lancet Oncol* 2005; 6: 209–18.
85. Aubier F, Flamant F, Brauner R et al. Male gonadal function after chemotherapy for solid tumors in childhood. *J Clin Oncol* 1989; 7: 304–9.
86. Siimes MA, Rautonen J. Small testicles with impaired production of sperm in adult male survivors of childhood malignancies. *Cancer* 1990; 65: 1303–6.
87. Ridola V, Fawaz O, Aubier F et al. Testicular function of survivors of childhood cancer: A comparative study between ifosfamide- and cyclophosphamide-based regimens. *Eur J Cancer* 2009; 45: 814–18.
88. Meistrich ML, Wilson G, Brown BW et al. Impact of cyclophosphamide on longterm reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. *Cancer* 1992; 70: 2703–12.
89. Rivkees SA, Crawford JD. The relationship of gonadal activity and chemotherapy-induced gonadal damage. *JAMA* 1988; 259: 2123–5.
90. Jahnukainen K, Ehmcke J, Schlatt S. Testicular xenografts: A novel approach to study cytotoxic damage in juvenile primate testis. *Cancer Res* 2006; 66: 3813–18.
91. Jahnukainen K, Ehmcke J, Nurmio M et al. Irradiation causes acute and long-term spermatogonial depletion in cultured and xenotransplanted testicular tissue from juvenile nonhuman primates. *Endocrinology* 2007; 148: 5541–8.
92. Rowley MJ, Leach DR, Warner GA, Heller CG. Effect of graded doses of ionizing radiation on the human testis. *Radiat Res* 1974; 59: 665–78.
93. Centola GM, Keller JW, Henzler M, Rubin P. Effect of low-dose testicular irradiation on sperm count and fertility in patients with testicular seminoma. *J Androl* 1994; 15: 608–13.
94. van Alphen MMA, van den Kant HJG, de Rooij DG. Repopulation of the seminiferous epithelium of the rhesus monkey after X irradiation. *Radiat Res* 1988; 113: 487–500.
95. Kamischke A, Jurgens H, Hertle L et al. Cryopreservation of sperm from adolescents and adults with malignancies. *J Androl* 2004; 25: 586–92.
96. Baukloh V, German Society for Human Reproductive Biology. Retrospective multicentre study on mechanical and enzymatic preparation of fresh and cryopreserved testicular biopsies. *Hum Reprod* 2002; 17: 1788–94.
97. Meseguer M, Garrido N, Remohi J et al. Testicular sperm extraction (TESE) and ICSI in patients with permanent azoospermia after chemotherapy. *Hum Reprod* 2003; 18: 1281–5.
98. Picton HM, Wyns C, Anderson RA et al., ESHRE Task Force On Fertility Preservation In Severe Diseases. A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys. *Hum Reprod* 2015; 30: 2463–75.
99. Brinster RL. Male germline stem cells: From mice to men. *Science* 2007; 316: 404–5.
100. Dores C, Alpaugh W, Dobrinski I. From *in vitro* culture to *in vivo* models to study testis development and spermatogenesis. *Cell Tissue Res* 2012; 349: 691–702.
101. Brook PF, Radford JA, Shalet SM et al. Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation. *Fertil Steril* 2001; 75: 269–74.
102. Keros V, Rosenlund B, Hultenby K et al. Optimizing cryopreservation of human testicular tissue: Comparison of protocols with glycerol, propanediol and dimethylsulphoxide as cryoprotectants. *Hum Reprod* 2005; 20: 1676–87.
103. Kvist K, Thorup J, Byskov AG et al. Cryopreservation of intact testicular tissue from boys with cryptorchidism. *Hum Reprod* 2006; 21: 484–91.
104. Schlatt S, Kim SS, Gosden R. Spermatogenesis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopreservation and heterotopic grafting to castrated hosts. *Reproduction* 2002; 124: 339–46.
105. Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. *Proc Natl Acad Sci USA* 1994; 91: 11298–302.
106. Schlatt S, Rosiepen G, Weinbauer GF et al. Germ cell transfer into rat, bovine, monkey and human testes. *Hum Reprod* 1999; 14: 144–50.
107. Sadri-Ardekani H, Mizrak SC, van Daalen SK et al. Propagation of human spermatogonial stem cells *in vitro*. *JAMA* 2009; 302: 2127–34.
108. Sadri-Ardekani H, Akhondi MA, van der Veen F et al. *In vitro* propagation of human prepubertal spermatogonial stem cells. *JAMA* 2011; 305: 2416–8.
109. Hermann BP, Sukhwani M, Winkler F et al. Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. *Cell Stem Cell* 2012; 11: 715–26.
110. Struijk RB, Mulder CL, van der Veen F et al. Restoring fertility in sterile childhood cancer survivors by autotransplanting spermatogonial stem cells: Are we there yet? *Biomed Res Int* 2013; 2013: 903142.
111. Xu M, Kreeger PK, Shea LD, Woodruff TK. Tissue-engineered follicles produce live, fertile offspring. *Tissue Eng* 2006; 12: 2739–46.
112. Xu J, Lawson MS, Yeoman RR et al. Secondary follicle growth and oocyte maturation during encapsulated three-dimensional culture in rhesus monkeys: Effects of gonadotrophins, oxygen and fetuin. *Hum Reprod* 2011; 26: 1061–72.
113. Xiao S, Zhang J, Romero MM, Smith KN, Shea LD, Woodruff TK. In vitro follicle growth supports human oocyte meiotic maturation. *Sci Rep.* 2015 Nov 27; 5: 17323.
114. Luyckx V, Dolmans MM, Vanacker J et al. A new step toward the artificial ovary: Survival and proliferation of isolated murine follicles after autologous transplantation in a fibrin scaffold. *Fertil Steril* 2014; 101: 1149–56.
115. Laronda MM, Rutz AL, Xiao S, Whelan KA, Duncan FE, Roth EW, Woodruff TK, Shah RN. A bioprosthetic ovary created using 3D printed microporous scaffolds restores ovarian function in sterilized mice. *Nat Commun.* 2017 May 16; 8: 15261.

Uterus transplantation

63

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INTRODUCTION

During the last four decades, reproductive medicine has conquered all the major obstacles of infertility, and today most types of both male and female infertility can be treated. Despite these breakthroughs, the medical field has faltered when it comes to one non-negligible subgroup of female infertility: women that suffer infertility due to an absolute uterine factor.

This type of infertility due to an anatomical or functional lack has, up until recently, eluded reproductive medicine. Thousands of women worldwide are affected and the genesis of the condition may either be congenital Mullerian malformations, such as in the Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome, or more common, as is the case for women with Asherman's syndrome, pregnancy-interfering myomas, or hysterectomies.

The anatomical absence of a uterus naturally represents a non-correctable obstacle and is determined as absolute uterine factor infertility. Women that still retain a uterus but where the organ is dysfunctional in terms of bearing a pregnancy are considered to have a relative uterine infertility. Approximately 1 in 500 women (1) suffer from absolute uterine infertility worldwide, and since no treatment has previously been available, the options for them to become mothers have either been to adopt or to go through with gestational surrogacy, with the latter being banned in many countries.

As of 2015, a total of 12 cases of human uterus transplantations had been reported worldwide, conducted in four different countries: Saudi Arabia (2), Turkey (3) Sweden (4), and China (personal communication) (Table 63.1). In 2014, the report of the first live birth following human uterus transplantation was published, showing that uterine factor infertility, even when considered absolute, is treatable (5). This very first birth has later been followed by three more births, proving that the outcome of uterus transplantation at this early stage of clinical implementation exceeds expectations for a novel surgical method (6). Given these results, many more cases of uterus transplantation performed at other centers are to be expected in the near future. This chapter reviews the worldwide experience of uterus transplantation as a treatment for absolute uterine factor infertility and the future prospects of uterus transplantation.

BACKGROUND

During the 1970s, 1980s, and 1990s, when the successful introduction of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) enabled fertility in the majority of infertile couples, little attention was paid to research in the field of uterus transplantation. Yet women with uterine factor infertility represent a substantial portion of the infertile population, and for decades have remained the largest non-treatable fraction. This research field was rediscovered in the early 2000s, and has since been progressing rapidly. Uterus transplantation models have been developed in several animal species including rodents (7–9), large domestic species (10–13), and non-human primates (14–16). The experiments have been focusing on a variety of aspects of uterus transplantation such as optimizing the surgery (17), recognition and monitoring of rejection episodes (8,15,18), and ischemic effects (19,20). Successful pregnancies and live births have been described in different species including mice (19,21), rats (22,23), sheep (11,24) and non-human primates (25). In addition, stable uterine allografts have also been achieved in large animals (14).

HUMAN UTERUS TRANSPLANTATION

Live or deceased donors

Uterus donation is not limited solely to either live or deceased organ donors. The World Health Organization (WHO) guiding principle states that donations from deceased donors should be maximized to their full potential, thereby avoiding risks to live donors (26). Nevertheless, due to a growing demand for organs, live donor donations are essential to meet current patient needs. Consequently, despite involving non-negligible risks for the donor, live donation is practiced in several organ transplantation settings. Out of the 11 published cases and one unpublished case of human uterus transplantations performed worldwide up until 2015, 11 were performed with uteri from live donors (2,4) and only one with a uterus from a deceased donor (Table 63.1) (27). A comparison concerning the different aspects relating to the living and deceased donor surgical concepts in uterine transplantation is stated below and in Table 63.2.

Surgery in the live donor setting

In a live donor setting, it is possible to optimize the planning of the surgery, and this concept yields sufficient time to evaluate the donor and organ prior to transplantation. Unsuitable and inappropriate donor candidates and organs of inferior quality can be excluded and thus enhance the outcome. Presence of cervical or endometrial atypia, cervical dysplasia, human papillomavirus (HPV) infection, donor infertility or subfertility, myomas, adenomyosis, polyps, or intrauterine adhesions may

Table 63.1 Worldwide experience of uterus transplantation in 2015

			Donor				
Case number	**Diagnosis**	**Age (years)**	**Type**	**Age (years)**	**Pregnancies**	**Live births**	**Where**
1	Peripartum hysterectomy	26	Living related	46	0	0	Saudi Arabia
2	MRKH syndrome	21	Deceased non-related	22	2	0	Turkey
3	Cervical cancer	33	Living related	52	1	1	Sweden
4	MRKH syndrome	38	Living related	58	0	0	Sweden
5	MRKH syndrome	28	Living related	54	1	0	Sweden
6	MRKH syndrome	27	Living related	50	2	1	Sweden
7	MRKH syndrome	35	Living related	61	1	1	Sweden
8	MRKH syndrome	27	Living related	53	0	0	Sweden
9	MRKH syndrome	28	Living related	50	1	1	Sweden
10	MRKH syndrome	33	Living related	37	1	1	Sweden
11	MRKH syndrome	35	Living related	62	0	0	Sweden
Total 11 patients	—	Mean 30 years	—	Mean 50 years	9	5	Three centers

Note: Only published cases are included.
Abbreviation: MRKH, Mayer–Rokitansky–Küster–Hauser.

Table 63.2 Comparison of a living donor versus a deceased donor setting in uterus transplantation

	Living donor	**Deceased donor**
Planning of surgery	+	–
Investigation of donor qualities	+	±
Investigation of organ qualities	+	±
Donor autonomy	+	+
Donor pain	±	NA
Donor time commitment	±	NA
Donor complication	±	NA
Long-term organ function	±	±
Complexity of surgery	±	+

Abbreviations: +, adequate; ±, probably adequate; –, not possible; NA, not applicable.

be thoroughly assessed before the uterus transplantation is executed (28). The recipient and donor should have the transplantation at a specific date when both parties are in optimized condition, with fully prepared surgical teams, thus increasing the odds of graft survival.

A major disadvantage in the live donor setting is the surgical risk for the donor and the innate risks associated with the retrieval surgery. The technically most demanding part in the surgical procedure and thus most likely to create complications is the dissection of the uterine veins and ureters. To minimize the risk for the live donor, it has been suggested that a larger alternative vein, such as one of the ovarian veins, would be preferable to use for anastomosis (29). This would probably require removal of the ovary itself, resulting in hormonal dysfunction in a premenopausal donor. Because of the resulting hormonal dysfunction, the live donor setting with the selection of the ovarian veins will only be suitable for use in postmenopausal donors. The uterine branch of the utero-ovarian vein may provide an adequately good substitute for anastomosis, but the lengths may be poorer. In the future, the surgical technique for uterus transplantation will most likely undergo development and the risks of surgical complications are surely likely to decrease. Moreover, new, less invasive methods like robotic-assisted laparoscopy may provide options to minimize the risk for the donor in a live donor setting (30).

In 2000, the first human uterus transplantation was performed in Saudi Arabia (2). A 26-year-old woman who had previously an emergency peripartum hysterectomy received a uterus from a 46-year-old donor. The non-related healthy donor was scheduled for bilateral oophorectomy due to benign bilateral ovarian cysts. A hysterectomy was added to the surgery and the organ donated to the recipient. The vascular pedicles recovered with the uterus were of insufficient lengths for direct anastomosis to the external iliac vessels and elongated by segments of the saphenous veins. Vascular anastomoses were established with the extended vascular pedicles end-to-side to the external iliac vessels of the recipient. In the donor, a perioperative small ureteric laceration was reported and repaired perioperatively by a urologist (2). Three months postoperatively, the uterus was found to be necrotic and removed. The authors advocated that insufficient tissue graft support led to tension and thrombosis of the supplying vessels.

The 2013 Swedish uterus transplantation trial, in which nine recipients received a uterus, also used the live donor concept (4). Eight of the recipients had MRKH

syndrome and one had undergone a radical hysterectomy due to early-stage cervical carcinoma seven years prior to the transplantation. The donors were mothers (aged 50–58 years) in five cases, close relatives in three cases with one mother's sister (aged 54 years), one sister (aged 37 years), and one mother-in-law (aged 62 years), as well as a family friend (aged 61 years) in one case. All of the donors had a minimum of one normal pregnancy and subsequent delivery prior to transplantation. Five of the donors were postmenopausal, and cyclic hormonal pretreatment was given to these women before uterus transplantation to ensure withdrawal bleedings.

The uterus recovery included dissection and separation of the graft with long bilateral vascular pedicles of the uterine arteries from the internal iliac arteries distal to the branching of the gluteal artery. The major uterine veins down to the internal iliac vein were carefully retrieved (Figure 63.1) (4). After surgical isolation, the uterus was brought to the back-table and flushed bilaterally through the arteries with cold histidine-tryptophan-ketoglutarate (HTK) solution (Custodiol-HTK®). Preparation of the recipients included dissection of the external iliac vessels, separation of the vaginal vault from the bladder and rectum, and preparation of uterine fixation sites (rudimentary round ligaments and paravaginal connective tissues). End-to-side vascular anastomoses of the uterine veins of the graft to the external iliac veins of the recipient and of the anterior divisions of the internal iliac arteries to the external iliac arteries bilaterally were performed. The uterus was then revascularized (Figure 63.2). The extensively harvested bladder peritoneum of the uterine graft was sutured as an overlay of the recipient's bladder for extra structural support (Figure 63.2).

Due to the complex and thorough dissection of the ureters with intact uterine veins and arteries, the donor surgeries lasted longer than estimated, with durations of >10 hours. However, hospital stay was only for six days. One postoperative complication occurred in form of a ureteric–vaginal fistula that was diagnosed two weeks after uterine removal and was repaired successfully after three months, and the affected donor had an uneventful recovery (4).

The surgeries of the recipients lasted between four and six hours. After six months, seven uteri remained *in situ* with regular menstruations, starting within one to two months postoperatively, while two uterine grafts had to been removed (4,31). One hysterectomy was performed three days post-transplantation due to bilateral thrombotic occlusion of the uterine artery (31). This recipient had a heterozygote protein C deficiency, a potentially predisposing factor for the thrombosis. The second hysterectomy

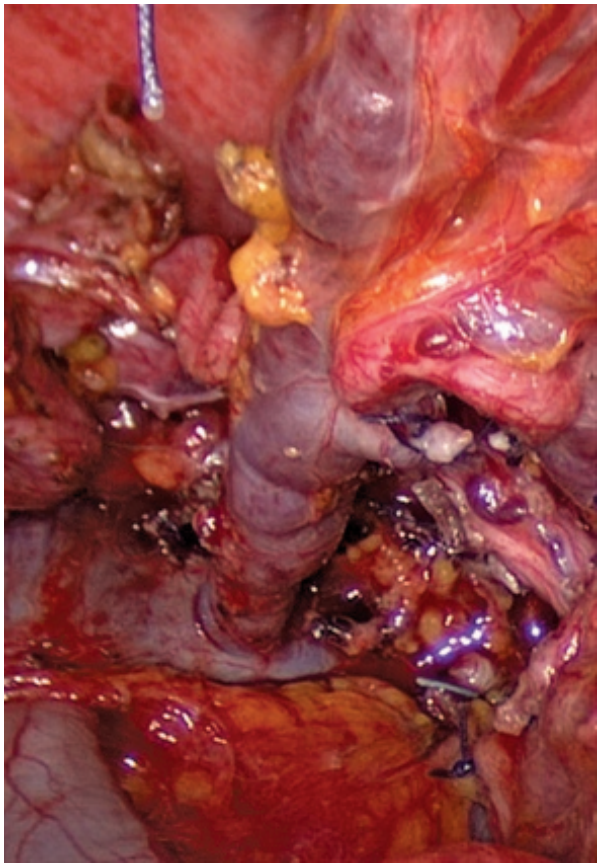

Figure 63.1 Photograph from the Swedish uterus transplantation trial, during which nine uteri were transplanted from live donors. During the retrieval, the major uterine veins down to the internal iliac vein were carefully dissected and included in the graft. (Photograph: Lennart Wiman.)

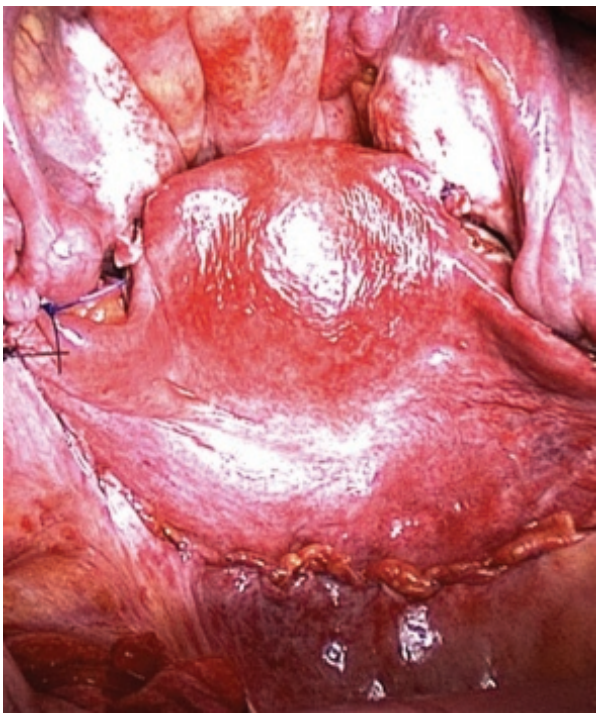

Figure 63.2 Photograph from the Swedish uterus transplantation trial, during which nine uteri were transplanted from live donors. The uterine graft is revascularized and fixed in the pelvis of the recipient. The extensively harvested bladder peritoneum of the uterine graft was sutured as an overlay of the recipient's bladder for extra structural support. (Photograph: Lennart Wiman.)

was performed 3.5 months after uterus transplantation (4). The recipient was readmitted to the hospital at one month post-transplantation due to fever, vaginal discharge, and an intrauterine infection with *Enterococcus faecalis*. Despite intense antibiotic treatments and repeated surgical procedures for drainage, the infection persisted, and after three months, when signs of septicemia were seen, a hysterectomy was performed.

Surgery in the deceased donor concept

The most important advantage of deceased organ donation is the avoidance of surgical donor risk. Furthermore, the surgical retrieval procedure is significantly easier due to not having to thoroughly dissect vital structures, and the retrieval surgery would be considerably shorter in time. The ends of the vascular pedicles would possibly be of a larger diameter, and could thus be used for larger anastomoses, simplifying the transplantation procedure and optimizing graft survival.

Disadvantages of the deceased donor concept would be that the ischemic time is generally longer than in the live donor setting. An extended ischemic time is known to decrease graft function (32) and increase the incidence of both acute and chronic postoperative rejection episodes (33). The tolerable ischemic time differs between organs and is not yet defined in a human uterus. Uterine myometrial and endometrial tissue, based on histological laboratory findings, can endure cold ischemia in Celsior solution for 24 hours and still demonstrate histological integrity (34). In a multiorgan retrieval setting with concurrent uterus retrieval, no histological alterations were found after 12-hour cold ischemia in University of Wisconsin solution (35). Long-term graft viability is shown to be reduced in kidney transplantations when the graft is donated by a deceased donor (36). This may be explained by the brain death-induced systemic inflammation occurring in a deceased donor, which may have an adverse impact on the transplanted organ (37). However, the impact of the systemic inflammation on the uterus is not as significant as in other organ transplantation settings, since the uterus is planned to be a temporary transplant and the long-term viability is of less importance. Nevertheless, the possible lack of available and optimal uterus grafts from deceased donors might make it difficult to meet patient requirements. The donor should not only meet all the donor criteria applied in general organ transplantation but also be female, be preferably premenopausal, have proven fertility, not be previously hysterectomized, and also have a uterus not affected by any dysplasia, HPV, cancer, or myomas, all of which may severely reduce the availability of suitable donors and organs.

The second human uterus transplantation was performed in Turkey in 2011, and this was the first human uterus transplantation performed after multiorgan donation (3). A 21-year-old MRKH patient received a uterus from a 22-year-old nulliparous woman who was confirmed brain dead due to cerebral trauma in a traffic accident. The retrieval surgery was performed 12 hours after brain death confirmation and the uterus retrieval was done as the first procedure in the organ procurement, after which the kidneys, heart, and liver were retrieved. The graft included vascular pedicles with bilateral ovarian and iliac artery and vein tracts, together with wide excision of the broad and round ligaments and the vesico- and sacro-uterine peritoneal sheets. The bilateral uterine arteries were outlined with blunt dissection and freed from the underlying ureters by mobilizing off the medial leaf of the broad ligament. The total organ procurement procedure was reported to last for two hours, but the total ischemic time was not reported (3). The reported transfer time between the two hospitals where the retrieval and the actual transplantation were executed was described to be 30 minutes.

UTERUS TRANSPLANTATION AND IMMUNOLOGY

Since immunological tolerance, precluding the need for maintenance immunosuppression, has proven to be elusive, immunosuppressive drugs are still used to evade graft rejection in transplanted organs. Induction therapy (i.e., perioperative prophylactic immunosuppression) is commonly used to prevent acute rejection in the first months after transplantation. The maintenance therapy is normally given as a combination of drugs with diverse pharmacokinetics to minimize adverse effects. Optimizing the level of immunosuppression requires a balance between preventing episodes of rejection and the adverse effects of the immunosuppressive drugs. The need for immunosuppressive medications is not at a constant level, and the high blood levels of immunosuppression necessary initially can, shortly after the transplantation procedure, be reduced to a lower maintenance level. During pregnancy, physiologic and hemodynamic changes occur, inducing changes in the plasma concentrations of drugs, hence these need to be monitored thoroughly (38).

The first uterus recipient was given induction therapy with corticosteroids pre- and peri-operatively (2). After the transplantation, she was on a triple-immunosuppressive therapy with azathioprine, cyclosporine, and prednisolone.

The second uterus recipient was given induction with thymoglobulin (3). Corticosteroids were given both perioperatively and in the first postoperative week. After the transplantation, she was given mycophenolate mofetil (MMF), tacrolimus from day 7 onwards (aiming for trough levels of 15–20 ng/mL), and corticosteroids.

The nine Swedish recipients received induction with corticosteroids and either thymoglobulin or antithymocyte globulin (ATG) (4). Following the transplantation, tacrolimus, aiming at trough levels of 10–15 ng/mL during the first month and 5–10 ng/mL from the second month onward, was given together with MMF preoperatively and from postoperative day 1, administered twice daily. At six months post-transplantation, the potentially teratogenic MMF was discontinued. The aim of immunosuppression was to use single therapy using tacrolimus. However, in case of repeated rejection episodes, addition of azathioprine and/or glucocorticoids was necessary (31).

REJECTION

The incidence of acute rejection following transplantation varies depending on which organ is transplanted. The highest frequency of acute rejection episodes is shown after lung, heart, and bowel transplantation (35%–40%, 30%–45%, and 55%, respectively) (39–41). Renal and liver transplantation generally shows less frequent episodes of acute rejection, with incidences of 12%–14% and 13%–30%, respectively (42,43). Normally, diagnosis of acute rejection relies on clinical signs, but also on laboratory data such as blood markers (creatinine in renal transplantation, lipase/amylase in pancreas transplantation, and liver enzymes in liver transplantation). Since no uterus-specific blood marker yet exists and subclinical episodes of rejection may occur, noninvasive graft monitoring is desired. One way to detect rejection can be through acute or protocol biopsies. The uterine graft is, unlike other transplanted organs, easily accessible from the vagina. Cervical tissue biopsies are, therefore, if not noninvasive, at least minimally invasive, and provide a good surveillance option of rejection. Unlike an endometrial biopsy, the cervical biopsy does not interfere with the cavity of the uterus and can also function as a form of surveillance of rejection during pregnancy.

In the first case of uterus transplantation, the transplant was monitored by magnetic resonance imaging, Doppler ultrasound, and measurements of the CD4/CD8 ratio in peripheral blood (2). At nine days after surgery, the recipient expressed malaise, fatigue, and low abdominal and back pain. She had subclinical fever, tachycardia, and a vaginal discharge, and these symptoms were anticipated to be signs of acute rejection. She was treated with short-term increased immunosuppression and intravenous (i.v.) corticosteroids. The episode of rejection was not resolved until ATG was given.

The graft of the second uterus recipient was examined by biopsies from the transplanted vaginal tissue fortnightly for the first three months. Thereafter, endometrial biopsies were taken every three months (27). No reports of rejection episodes have been published.

The recipients and their grafts in the Swedish trial were frequently examined clinically, including ultrasound evaluation of the endometrium and the uterus, Doppler ultrasound to evaluate the blood flow in the uterine arteries, and visual inspection of the cervix, as well as cervical cultures and cervical biopsies (31). Occasional subclinical episodes of mild rejection were detected on cervical biopsies during the first postoperative year, and the episodes were effectively reversed by short courses of increased immunosuppression. Rejection was diagnosed based on the histopathologic interpretation of the cervical biopsies and graded according to a grading system for uterine rejection that was originally developed for rejection in non-human primates without immunosuppression and later adopted for humans (14). The classification was according to a four-grade scale running from no rejection to low-, medium-, and high-grade rejection (our unpublished data).

ASSISTED REPRODUCTION TECHNOLOGY IN UTERUS TRANSPLANTATION

Ovarian stimulation, oocyte retrieval, and embryo transfer

To perform IVF treatment prior to uterus transplantation rather than after is a multifaceted decision. Starting with IVF, the recipient is known to have a proven fertility potential, the possibly difficult oocyte pickup in a patient with intra-abdominal adhesions and a uterus with vascular supply not in the exact position of a normal uterus could be avoided, and the duration of immunosuppressive treatment could be minimized. Previous experience from assisted reproduction technology (ART) treatments of MRKH syndrome women aiming for the fertilized oocytes to be transferred into surrogate mothers suggests that a long protocol may be preferable in MRKH syndrome patients to ensure pituitary down-regulation before starting ovarian stimulation (44). Since the patients did not have menstrual bleedings and the unstimulated ovaries, especially the small antral follicles, were not readily visible with ultrasound, the patients went through one or more cyclic assessments of luteinizing hormone (LH; urinary as well as serum), follicle-stimulating hormone (FSH), estradiol, and progesterone, and all showed reasonably regular ovulatory cycles with identifiable LH peaks. To get an estimate of the ovarian reserve, anti-Mullerian hormone was analyzed. In the Swedish case series, a long agonist protocol starting in the mid-to-late luteal phase was used, followed by daily injections of FSH and/or human menopausal gonadotropin. Ovulatory follicle and oocyte maturation was induced with human chorionic gonadotropin (hCG) injection.

Gonadotropin-releasing hormone agonist treatment was commenced eight to nine days after an LH peak, when a luteal progesterone value was demonstrated. Since follicular measurements were uncertain, especially in some of the patients with laterally placed ovaries, monitoring was based mainly on estradiol levels and developmental curves for the first 8–12 days, until follicles could be measured either by abdominal or vaginal ultrasound.

Oocytes were aspirated 36 hours after the injection of hCG, vaginally or abdominally (transcutaneously). Sperm preparation and incubation procedures were standard and fertilization was induced by routine IVF or by ICSI. After normal fertilization was confirmed, the embryos were cultured for ordinary slow freezing of day-2 embryos or vitrification of day-5 blastocysts. The embryos were cryostored until used for frozen–thawed embryo transfer. In most patients, the stimulation–aspiration–freezing procedures had to be repeated one or several times to accumulate a certain number of embryos.

At least one year after the uterus transplantation, patients returned for embryo transfer of a single embryo. Most transfers were performed in the natural cycle, three to six days after the LH peak, but in some patients, endometrial stimulation by sequential estrogen/progesterone medication was performed. All transfers were uneventful,

although a few were technically challenging. Most patients have needed a repeated transfer to get pregnant.

PREGNANCIES AND LIVE BIRTHS

The major issues of uterus transplantation regarding immunosuppression and rejection can be summarized in three different areas of concern: the effect of pregnancy on graft rejection; the effect of the transplanted graft on the pregnancy; and the effect of immunosuppression on both the fertility and the pregnancy outcome.

Throughout pregnancy, intake of immunosuppressive agents is vital to prevent organ rejection. All common medications used to avoid episodes of rejection cross the placenta barrier and subsequently reach the fetal circulation, thus exposing the child to potentially teratogenic agents during important developmental phases (45). Ever since the first reported pregnancy following kidney transplantation with concurrent use of immunosuppression was reported in 1967 (46), data on children born to transplanted women have been reported, and current data as of 2006 exceed 14,000 cases (47). Three large registers offer data about the outcomes of pregnancies in transplant recipients (48–50), and all of these indicate similar trends of an increased incidence of obstetric complications, including ectopic pregnancy, hypertension and pre-eclampsia, miscarriage, premature delivery, low birth weight, stillbirth, and neonatal death. The potential adverse effects of immunosuppressive drugs are of a broad spectrum, ranging from severe malformations to delicate, hardly detectable neurocognitive defects. The U.S. Food and Drug Administration (FDA) has categorized immunosuppressive medications, and based on their recommendation, a ceased intake of some drugs is recommended prior to the attempt of pregnancy (51). Utilizing only the immunosuppressive drugs approved by the FDA during pregnancy, the risk of congenital malformations or anomalies in a pregnancy exposed to immunosuppression is comparable to the risks in a normal pregnancy.

During pregnancy, it is fairly common that the immunosuppressive doses need to be increased, and decreased in the postpartum period, to achieve constant trough levels. Some studies report pregnant recipients requiring an almost two-fold increase in doses compared with pre-pregnancy doses in order to keep the blood levels within a therapeutic window (52).

Eighteen months after the second human uterus transplantation, the Turkish recipient underwent two single-embryo transfers, after which two pregnancies have been reported (53). The first pregnancy was biochemical, but the second was confirmed with visualization by ultrasound of an intrauterine gestational sac. This second pregnancy unfortunately ended in miscarriage prior to gestational week 8.

The first human live birth was reported in 2014 (5). The 35-year-old recipient suffering from MRKH syndrome was transplanted with a live donor uterus from a 62-year-old postmenopausal family friend. Menstruation occurred at 43 days post-surgery and continued with regular intervals (26–36 days). During the first 12 months, two episodes of mild rejection were resolved with short-term i.v. corticosteroids. She had embryo transfer 12 months after the transplantation, which was immediately successful. During the pregnancy, she was on triple immunosuppression (tacrolimus, azathioprine, and corticosteroids). One episode of mild rejection also appeared during the pregnancy (second trimester), but was reversed by i.v. corticosteroids. Fetal growth parameters and blood flows of the uterine arteries and umbilical cord were normal throughout pregnancy. Due to pre-eclampsia, the patient was admitted at week 31 + 5, and because of abnormal cardiotocography tracing, a cesarean section was performed. A healthy baby boy of normal weight (1775 g) in relation to gestational age, showing Apgar scores of 9, 9, and 10, was born. Following this initial birth, another four healthy babies have been born in the Swedish trial (our unpublished data) (Figures 63.3 and 63.4).

PSYCHOLOGY

Well-being of the donors and recipients is of great importance, considering that uterus transplantation is a new type of a major experimental surgery, a quality of life-enhancing transplantation rather than life saving, and a new infertility treatment.

The two initial cases of human uterus transplantation (2,3) did not explore the psychology and well-being of either the recipients or the live donor. It was the case series in Sweden that, in accordance with previous studies of solid organ transplantation, first acknowledged the need for psychological evaluation of not only the recipients and live donors, but also the partners of the recipients in both inclusion and as a follow-up (4). The inclusion of recipients and donors in the Swedish trial was preceded by a thorough psychological evaluation (4,54), including psychometric questionnaires regarding mood (The Hospital Anxiety and Depression Score [HADS] and Dyadic Adjustment Scale [DAS]), quality of life (Psychological General Well-Being Index [PGWB] and Short Form 36 item [SF 36]), relationship and fertility quality of life (only for recipients

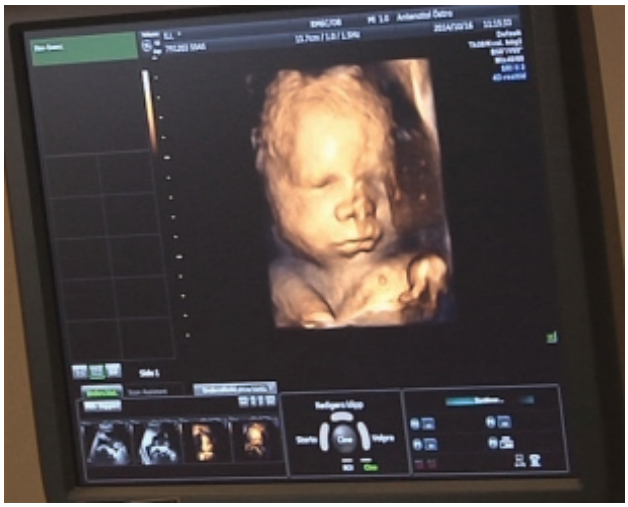

Figure 63.3 Three-dimensional image of a fetal face at organ ultrasound screening in gestational week 18 from the Swedish uterus transplantation trial. (Photograph: Lennart Wiman.)

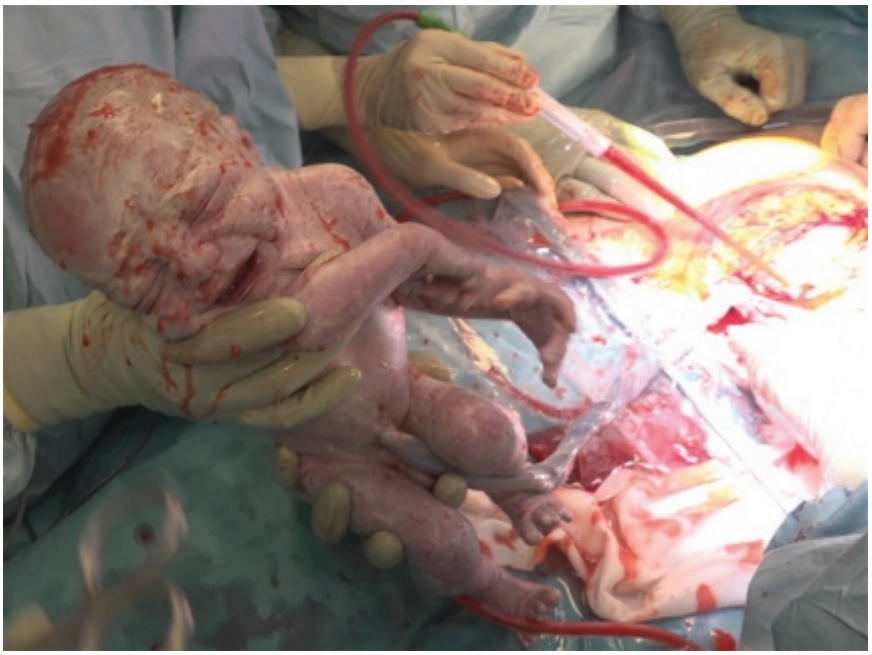

Figure 63.4 The second child born after uterus transplantation just after birth. (Photograph: Lennart Wiman.)

and their partners), and semi-structured interviews targeting psychological well-being, relationship, managing childlessness, knowledge about the project and risk, and relationship with the donor/recipient. The main purpose of this was not to exclude patients, but rather to establish a relationship with the psychologist and to identify those who had inappropriate expectations of the procedure and so might benefit from more psychological support.

The donors, recipients, and their partners were psychologically stable and well prepared prior to transplantation. They all scored as well or better at inclusion when compared to the norm in the population (55,56).

Following the transplantation, the recipients adjusted well to the new life situation, including medications and healthcare controls, and were able to handle stress when needed. In spite of personal outcomes and adverse events, the recipients and their partners were found to be psychologically well adjusted one year after the transplantation.

In a live donor, the certainty and eagerness to donate might to some extent conceal feelings of hesitation, and the fear of being excluded as a donor might lead to a reluctance to report psychological strain. Although the interviews revealed some mixed emotions, all donors were found to be stable and well adjusted to the trial, indicating that live uterus donors, despite their different previous experiences and the strains that in some cases occurred, tolerated the donations well, both medically and psychologically. All donors returned to everyday life without major difficulties. None of the donors expressed regret about their decision to donate and would do it again if required.

CONCLUSION

In summary, uterus transplantation was a breakthrough in the field of reproductive medicine and has so far had a remarkably successful outcome. Bearing this in mind, this procedure is still only a proof of concept for uterus transplantation as a treatment for uterine factor infertility in a live related donor setting by laparotomic technique. The model will surely be expanded and demonstrated in other settings in the near future. New methods to evaluate the recipients, donors, and organs, like angiographic mapping of vessels pre- or even peri-operatively, will possibly simplify the procedure and improve the outcome. Other surgical options and modifications like laparoscopic and robotic-assisted methods, providing the possibility to reduce the surgical duration and concurrent risks for both recipients and live donors, are expected to improve upon the results of the initial trials. Only five years ago, prior to the clinical introduction of uterus transplantation, it was disputed whether it was ethically and morally defensible to perform such a transplantation. Now that it is proven to be successful, the medical society instead faces the issue of whether it will be justifiable not to develop the uterus transplantation procedure further.

REFERENCES

1. Sieunarine K, Cowie AS, Bartlett JD et al. A novel approach in the management of a recurrent adenomatoid tumor of the uterus utilizing a Strassman technique. *Int J Gynecol Cancer* 2005; 15: 671–5.
2. Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. *Int J Gynaecol Obstet* 2002; 76: 245–51.
3. Ozkan O, Akar ME, Ozkan O et al. Preliminary results of the first human uterus transplantation from a multiorgan donor. *Fertil Steril* 2013; 99: 470–6.

4. Brannstrom M, Johannesson L, Dahm-Kahler P et al. First clinical uterus transplantation trial: A six-month report. *Fertil Steril* 2014; 101: 1228–36.
5. Brannstrom M, Johannesson L, Bokstrom H et al. Livebirth after uterus transplantation. *Lancet* 2015; 385: 607–16.
6. Brannstrom M. The Swedish uterus transplantation project: The story behind the Swedish uterus transplantation project. *Acta Obstet Gynecol Scand* 2015; 94: 675–9.
7. Diaz-Garcia C, Johannesson L, Shao R et al. Pregnancy after allogeneic uterus transplantation in the rat: Perinatal outcome and growth trajectory. *Fertil Steril* 2014; 102: 1545–52.e1.
8. El-Akouri RR, Molne J, Groth K et al. Rejection patterns in allogeneic uterus transplantation in the mouse. *Hum Reprod* 2006; 21: 436–42.
9. Wranning CA, Akhi SN, Kurlberg G, Brannstrom M. Uterus transplantation in the rat: Model development, surgical learning and morphological evaluation of healing. *Acta Obstet Gynecol Scand* 2008; 87: 1239–47.
10. Wranning CA, El-Akouri RR, Lundmark C et al. Auto-transplantation of the uterus in the domestic pig (*Sus scrofa*): Surgical technique and early reperfusion events. *J Obstet Gynaecol Res* 2006; 32: 358–67.
11. Dahm-Kahler P, Wranning C, Lundmark C et al. Transplantation of the uterus in sheep: Methodology and early reperfusion events. *J Obstet Gynaecol Res* 2008; 34: 784–93.
12. Gonzalez-Pinto IM, Tryphonopoulos P, Avison DL et al. Uterus transplantation model in sheep with heterotopic whole graft and aorta and cava anastomoses. *Transplant Proc* 2013; 45: 1802–4.
13. Avison DL, DeFaria W, Tryphonopoulos P et al. Heterotopic uterus transplantation in a swine model. *Transplantation* 2009; 88: 465–9.
14. Johannesson L, Enskog A, Molne J et al. Preclinical report on allogeneic uterus transplantation in non-human primates. *Hum Reprod* 2013; 28: 189–98.
15. Tryphonopoulos P, Tzakis AG, Tekin A et al. Allogeneic uterus transplantation in baboons: Surgical technique and challenges to long-term graft survival. *Transplantation* 2014; 98: e51–6.
16. Kisu I, Banno K, Mihara M, Aoki D. Uterus transplantation in nonhuman primates. *Fertil Steril* 2013; 100: e3.
17. Johannesson L, Enskog A, Dahm-Kahler P et al. Uterus transplantation in a non-human primate: Long-term follow-up after autologous transplantation. *Hum Reprod* 2012; 27: 1640–8.
18. Scott JR, Pitkin RM, Yannone ME. Transplantation of the primate uterus. *Surg Gynecol Obstet* 1971; 133: 414–8.
19. Racho El-Akouri R, Wranning CA, Molne J et al. Pregnancy in transplanted mouse uterus after long-term cold ischaemic preservation. *Hum Reprod* 2003; 18: 2024–30.
20. Gauthier T, Piver P, Pichon N et al. Uterus retrieval process from brain dead donors. *Fertil Steril* 2014; 102: 476–82.
21. Racho El-Akouri R, Kurlberg G, Brannstrom M. Successful uterine transplantation in the mouse: Pregnancy and post-natal development of offspring. *Hum Reprod* 2003; 18: 2018–23.
22. Wranning CA, Akhi SN, Diaz-Garcia C, Brannstrom M. Pregnancy after syngeneic uterus transplantation and spontaneous mating in the rat. *Hum Reprod* 2011; 26: 553–8.
23. Diaz-Garcia C, Akhi SN, Wallin A et al. First report on fertility after allogeneic uterus transplantation. *Acta Obstet Gynecol Scand* 2010; 89: 1491–4.
24. Wranning CA, Dahm-Kahler P, Molne J et al. Transplantation of the uterus in the sheep: Oxidative stress and reperfusion injury after short-time cold storage. *Fertil Steril* 2008; 90: 817–26.
25. Mihara M, Kisu I, Hara H et al. Uterine autotransplantation in cynomolgus macaques: The first case of pregnancy and delivery. *Hum Reprod* 2012; 27: 2332–40.
26. WHO Guiding Principles on Human Cell. Tissue and organ transplantation. *Cell Tissue Bank* 2010; 11: 413–9.
27. Ozkan O, Akar ME, Erdogan O et al. Uterus transplantation from a deceased donor. *Fertil Steril* 2013; 100: e41.
28. Brannstrom M, Diaz-Garcia C, Hanafy A et al. Uterus transplantation: Animal research and human possibilities. *Fertil Steril* 2012; 97: 1269–76.
29. Kisu I, Banno K, Mihara M et al. A surgical technique using the ovarian vein in non-human primate models of potential living-donor surgery of uterus transplantation. *Acta Obstet Gynecol Scand* 2015; 94: 942–8.
30. Iavazzo C, Gkegkes ID. Possible role of DaVinci Robot in uterine transplantation. *J Turk Ger Gynecol Assoc* 2015; 16: 179–80.
31. Johannesson L, Kvarnstrom N, Molne J et al. Uterus transplantation trial: 1-year outcome. *Fertil Steril* 2015; 103: 199–204.
32. Quiroga I, McShane P, Koo DD et al. Major effects of delayed graft function and cold ischaemia time on renal allograft survival. *Nephrol Dial Transplant* 2006; 21: 1689–96.
33. Schwarz A, Mengel M, Gwinner W et al. Risk factors for chronic allograft nephropathy after renal transplantation: A protocol biopsy study. *Kidney Int* 2005; 67: 341–8.
34. Sieunarine K, Lindsay I, Ungar L et al. Cold ischaemic preservation of human uterine tissue. *Int Surg* 2008; 93: 366–72.
35. Del Priore G, Stega J, Sieunarine K et al. Human uterus retrieval from a multi-organ donor. *Obstet Gynecol* 2007; 109: 101–4.
36. Segev DL, Gentry SE, Warren DS et al. Kidney paired donation and optimizing the use of live donor organs. *JAMA* 2005; 293: 1883–90.

37. Pratschke J, Wilhelm MJ, Kusaka M et al. Brain death and its influence on donor organ quality and outcome after transplantation. *Transplantation* 1999; 67: 343–8.
38. Tendron A, Gouyon JB, Decramer S. *In utero* exposure to immunosuppressive drugs: Experimental and clinical studies. *Pediatr Nephrol* 2002; 17: 121–30.
39. Smith JM, Skeans MA, Horslen SP et al. OPTN/SRTR 2013 annual data report: Intestine. *Am J Transplant* 2015; 15(Suppl 2): 1–16.
40. Valapour M, Skeans MA, Heubner BM et al. OPTN/SRTR 2013 annual data report: Lung. *Am J Transplant* 2015; 15(Suppl 2): 1–28.
41. Colvin-Adams M, Smith JM, Heubner BM et al. OPTN/SRTR 2013 annual data report: Heart. *Am J Transplant* 2015; 15(Suppl 2): 1–28.
42. Matas AJ, Smith JM, Skeans MA et al. OPTN/SRTR 2013 annual data report: Kidney. *Am J Transplant* 2015; 15(Suppl 2): 1–34.
43. Kim WR, Lake JR, Smith JM et al. OPTN/SRTR 2013 annual data report: Liver. *Am J Transplant* 2015; 15(Suppl 2):1–28.
44. Raziel A, Friedler S, Gidoni Y et al. Surrogate *in vitro* fertilization outcome in typical and atypical forms of Mayer–Rokitansky–Kuster–Hauser syndrome. *Hum Reprod* 2012; 27: 126–30.
45. Ostensen M. Disease specific problems related to drug therapy in pregnancy. *Lupus* 2004; 13: 746–50.
46. Board JA, Lee HM, Draper DA, Hume DM. Pregnancy following kidney homotransplantation from a non-twin. Report of a case with concurrent administration of azathioprine and prednisone. *Obstet Gynecol* 1967; 29: 318–23.
47. Armenti VT, Daller JA, Constantinescu S et al. Report from the National Transplantation Pregnancy Registry: Outcomes of pregnancy after transplantation. *Clin Transpl* 2006: 57–70.
48. Armenti VT, Radomski JS, Moritz MJ et al. Report from the National Transplantation Pregnancy Registry (NTPR): Outcomes of pregnancy after transplantation. *Clin Transpl* 2004: 103–14.
49. Briggs JD, Jager K. The first year of the new ERA–EDTA Registry. *Nephrol Dial Transplant* 2001; 16: 1130–1.
50. Sibanda N, Briggs JD, Davison JM et al. Pregnancy after organ transplantation: A report from the UK Transplant pregnancy registry. *Transplantation* 2007; 83: 1301–7.
51. McKay DB, Josephson MA. Pregnancy in recipients of solid organs—Effects on mother and child. *N Engl J Med* 2006; 354: 1281–93.
52. Akturk S, Celebi ZK, Erdogmus S et al. Pregnancy after kidney transplantation: Outcomes, tacrolimus doses, and trough levels. *Transplant Proc* 2015; 47: 1442–4.
53. Erman Akar M, Ozkan O, Aydinuraz B et al. Clinical pregnancy after uterus transplantation. *Fertil Steril* 2013; 100: 1358–63.
54. Jarvholm S, Johannesson L, Brannstrom M. Psychological aspects in pre-transplantation assessments of patients prior to entering the first uterus transplantation trial. *Acta Obstet Gynecol Scand* 2015; 94: 1035–8.
55. Jarvholm S, Johannesson L, Clarke A, Brannstrom M. Uterus transplantation trial: Psychological evaluation of recipients and partners during the post-transplantation year. *Fertil Steril* 2015; 104: 1010–5.
56. Jarvholm S, Johannesson L, Brannstrom M. Psychological aspects in pre-transplantation assessments of patients prior to entering the first uterus transplantation trial. *Acta Obstet Gynecol Scand* 2015; 94: 1035–8.

Viral disease and assisted reproduction technology

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CAROLE GILLING-SMITH and PIETRO VERNAZZA

INTRODUCTION

In the context of offering assisted reproduction technology (ART), the three most commonly encountered infections are human immunodeficiency virus (HIV) types 1 and 2, hepatitis B virus (HBV), and hepatitis C virus (HCV). Less frequently encountered, but routinely screened for, is human T-lymphocyte virus 1 (HTLV-1) and HTLV-2. More recently, concerns have been raised over Zika virus. A total of 75% of individuals infected with HIV and HCV are in the reproductive age group and wish to both conceive safely and reduce viral transmission risk to their partner and offspring.

Understanding viral transmission risk is paramount to tailoring an effective ART program for those with viral infections. Minimizing viral transmission risk differs according to whether couples are virus serodiscordant or concordant and whether viral load in the blood is detectable or not.

ART centers also have an obligation to screen for viral diseases prior to offering assisted conception treatment and must adapt their laboratory protocols to ensure the safe handling of gametes and embryos in order to reduce the risk of infection to the patients themselves, other patients attending the center, and their staff.

This chapter sets out the evidence base and available guidance for planning and managing a cost-effective, safe, and ethically sensitive fertility service for patients diagnosed with blood-borne viral infections. The management of each viral infection is first set out, followed by general considerations in laboratory practice applicable to all viral infections.

HIV: WHERE ARE WE NOW?

HIV, a retrovirus, uses reverse transcriptase to transcribe RNA into DNA (1). The virus binds to cell surfaces, typically helper $CD4^+$ T-lymphocytes, and leads to their progressive depletion over time (2). Once the $CD4^+$ count falls below 200 per mm^2, development of acquired immunodeficiency syndrome (AIDS) can occur. Disease progression from initial seroconversion to development of symptoms can be slow, with lapses of up to 15 years, allowing for timely intervention. At the point that the lymph nodes become infected with HIV, the virus enters the bloodstream and passes into other body fluids, including semen and breast milk (1). Unprotected intercourse has long been recognized as the predominant form of HIV transmission, with further significant numbers of children becoming infected through vertical perinatal transmission. HIV is measured in blood and other body fluids using polymerase chain reaction (PCR) assays (3).

The introduction in 1995 and further development of combined highly active antiretroviral therapy (HAART) using at least three antiretroviral drugs in combination has fundamentally changed the course of HIV infection (4), leading to its redefinition as a chronic disease (5) and, more recently, the demonstration that individuals that have stable undetectable viral loads are no longer sexually infectious (6,7). Both have had significant implications with respect to the management of infected individuals who wish to conceive.

The HAART regimens comprise reverse transcriptase and protease inhibitors, which interrupt viral replication and slow down and halt $CD4^+$ depletion. In the majority of cases, viral replication in infected individuals is completely stopped, leading to undetectable levels of HIV RNA in blood plasma. As a consequence, $CD4^+$ counts recover to normal levels in the majority of treated individuals. The widespread use of HAART over the last decade has significantly changed the life expectancy of individuals living with HIV in the U.S.A. and Western Europe, to the point that even large insurance companies are now considering offering life insurance to HIV-positive patients (8). In individuals diagnosed soon after initial HIV infection and offered early HAART, their life expectancy approaches that of uninfected individuals (9,10).

By the end of 2015, an estimated 36.7 million people were living with HIV, giving a global HIV prevalence of 0.8% (UN AIDS Fact Sheet 2016). Worldwide, approximately 50% of adults and children have access to HAART, although there are still significant differences in access between developed compared to poorer countries. In 2015, 77% of pregnant women received HAART to minimize HIV vertical transmission. As a direct result of increased implementation of HAART, the incidence of new infections since 2010 has fallen by 6% across all age groups, but with a 50% decline in new infections in children. Currently, there is no vaccine available, so effective early implementation of HAART is still key to minimizing transmission and reducing the number of new infections.

Historically, the identification that unprotected sexual intercourse was the predominant mode of HIV transmission led to widespread public health campaigns promoting the use of condoms. HIV-serodiscordant couples wishing to conceive therefore found themselves in a state of "voluntary infertility." Furthermore, in most countries, the transmission of HIV (even in the situation of mutual consent) was considered legally an offence against public health (and still is in some countries, such as the U.S.A.). Finally, the risk of mother-to-child HIV transmission was

deemed unacceptably high prior to HAART (>30%), and therefore women infected with HIV of childbearing age were advised to avoid pregnancy (11).

HIV SEXUAL TRANSMISSION RISK

The widespread use of HAART has had a clear impact on HIV sexual transmission risk and, in turn, guidance for HIV-infected individuals wishing to reproduce. In 2008, the Swiss Commission on AIDS-related issues published a commentary informing Swiss patients and physicians that, under optimal conditions, the risk of HIV transmission appeared to be negligible (6). Whilst the so-called "Swiss statement" was initially widely disputed by many experts, over the ensuing eight years, it has become evident that the quintessence of the Swiss statement was correct (7). In the only published allegation of a case of transmission during HAART (12), the authors were unable to document that the infection occurred during the HAART of the index case (13). The Swiss statement prompted physicians to seek out and publish cases where transmission from non-viremic HIV-infected individuals could be documented, but no further cases have been published.

A large randomized controlled trial (HPTN 052) investigated the impact of immediate HAART start (instead of delayed start according to treatment guidelines) on the risk of HIV transmission in HIV-discordant couples (14). This study found no case of transmission from any HIV-infected individual where blood viral load was suppressed. Further evidence supporting the Swiss statement came from the European Partner Study. In this observational study, HIV-serodiscordant couples who informed their physicians they were practicing sex without condoms were followed up with six-monthly HIV tests in the uninfected partner (15). After a total follow-up of 1238 couple-years, not a single case of transmission from the infected partner under HAART was documented.

Over the last few years, the premise that HIV-infected individuals with fully suppressed viral load through use of HAART are sexually non-infectious has come to receive widespread support from HIV physicians due to the total lack of any single documented case of transmission under HAART. In fact, the inverse argument has to be raised: is there any evidence to support the use of condoms in non-viremic HIV-infected individuals? Amongst specialists in assisted conception, there remains far more skepticism, largely due to the lack of up-to-date published guidelines on the topic of viral infection and ART.

Sperm washing: An obsolete treatment for HIV-positive men on HAART

Prior to the publication of the Swiss statement, HIV-serodiscordant couples in which the male was HIV positive were advised to consider sperm washing as the first-line treatment to minimize the risk of viral transmission to their partner and future offspring. Sperm washing was first proposed in 1992 by Semprini et al. (16), several years before the introduction and development of HAART. The process requires centrifugation of semen on a density gradient to separate live spermatozoa from seminal plasma and non-germinal cells and, in most cases, this is followed by a swim-up. The technique rests on the fact that HIV is present in seminal fluid and as cell-associated virus in leukocytes and non-spermatozoa cells, but is not capable of attaching to or infecting spermatozoa (17–22). Whilst the technique remains a valid first-line treatment for men unable to achieve undetectable viral levels through HAART, for the vast majority of serodiscordant men with undetectable viral loads through HAART, sperm washing has become obsolete, and natural conception is increasingly considered the first-line option for conceiving a child without increasing the risk of HIV transmission to their partner or future child (23–25). However, not all couples or reproductive specialists understand this or are aware of the evidence cited above. On that basis, some couples still elect to continue to use condoms because of fear of sexual transmission of HIV to their partner, and seek out centers that can offer sperm washing. In practice, when couples are openly and correctly informed about the current evidence on sexual transmission risk, there is growing evidence that the majority will choose to conceive naturally (7).

Natural conception in HIV-serodiscordant couples

Prospective studies of couples attempting to conceive through natural intercourse compared to those conceiving through sperm washing are limited. In a small series of 53 serodiscordant couples in whom the HIV-positive man had been successfully treated with HAART for over six months and had undetectable levels of HIV RNA in the plasma (<50 copies/mL), pregnancy rates of 26% were reported for the first attempt, rising to 66% after five attempts and 75% after 12 attempts (25). Median female age was 33 years and 244 events of unprotected intercourse took place over the study period (see Figure 64.1).

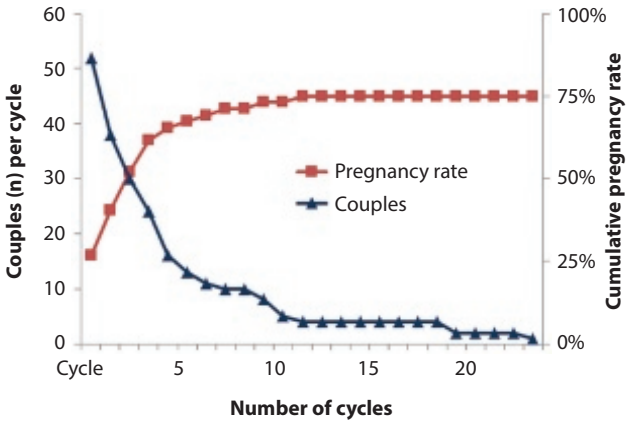

Figure 64.1 Cumulative pregnancy rates per number of cycles. The blue line demonstrates the number of couples who made an attempt to conceive per cycle. The red line demonstrates the cumulative pregnancy rate among those couples who tried to conceive after a certain number of attempts (cycles). (Reproduced with permission from Vernazza PL et al. *AIDS* 2011; 25: 2005–8.)

A Cochrane Database review of natural conception in HIV-serodiscordant couples analyzed seven observational studies and one randomized controlled trial. No transmissions were noted in couples where the HIV-infected partner was on HAART (26). Prior to the availability of the new evidence cited above, pre-exposure prophylaxis (PrEP), in the form of tenofovir at the time of the urine LH surge and 24 hours later, was offered to the HIV-negative female partner, as well as other safeguards to reduce the residual anxiety (25,27). Although the study sizes are small, if the man is under full HIV suppression through HAART, PrEP appears to confer no additional risk reduction in couples attempting to conceive naturally (28). In less developed countries, however, PrEP could have a place in those couples wishing to conceive naturally if the man is not on HAART (27–29).

Whilst published guidance on the issue of natural conception in HIV-serodiscordant couples varies from region to region, as a result of the widespread use of HAART, a clear change in reproductive advice is being offered to HIV-infected individuals in the developed world. In 2013 in the U.K., following careful review of the available published evidence including the Swiss Statement and subsequent debate in the literature, the National Institute for Health and Care Excellence (NICE) published evidence-based guidance for fertility assessment and treatment of patients (30). In the case of HIV-serodiscordant couples where the man is HIV positive, they advised that the risk of HIV transmission to his female partner through unprotected sexual intercourse was negligible, provided the man was compliant with HAART and had a plasma HIV viral load of less than 50 copies/mL maintained for more than six months and that no other infections were present. The guidance recommended that unprotected intercourse should be limited to the time of ovulation, but stated that there was insufficient evidence to recommend that the female partner take PrEP, provided all the criteria cited had been met. The guidance further advised that there was no evidence that sperm washing in these cases could further reduce the risk of infection, but might on the contrary reduce the likelihood of pregnancy due to its effects on semen quantity and quality. Their recommendation was that sperm washing should only be offered to those men who were not compliant with HAART or who failed to achieve a plasma HIV viral load of less than 50 copies/mL, or to those couples who, after a full discussion with their HIV specialist, still perceive the risk of infection to be too great. This appears to be the guidance that is also emerging in many European countries (7,23). In contrast, the U.S.A. maintains a far more conservative approach to the management of HIV-infected men in the assisted conception setting, with sperm washing combined with intracytoplasmic sperm injection (ICSI) still advocated as the first-line treatment for those wishing to conceive with an HIV-negative female partner (31).

HIV VERTICAL TRANSMISSION RISK

The use of selected antiretrovirals during pregnancy and at the time of delivery and elective cesarean section where appropriate and the avoidance of breastfeeding are all measures that have collectively led to a fall in the mother-to-child transmission risk from more than 30% to less than 1% (32–34).

ETHICAL CONSIDERATIONS

The evidence is clear: HIV-infected patients on HAART now have a life expectancy approaching that of HIV-negative patients, and the perinatal vertical transmission risk is approaching zero. These two factors combined have led to widespread acceptance that there are no longer any valid ethical arguments to deny HIV-infected individuals the same reproductive options as HIV-negative individuals (34–42). More importantly, a recent guideline update from the Ethics Committee of the American Society for Reproductive Medicine advised that in third-party reproduction, disclosure of an intended parent's HIV status to gamete donors or gestational carriers should be commensurate with the principles of informed consent (42).

MANAGEMENT OF HIV-POSITIVE PATIENTS IN THE ART SETTING

There has been a continued rise in the desire to conceive amongst HIV-infected individuals (43). It is therefore imperative that reproductive specialists appreciate the true risks of viral transmission for those living with HIV under HAART so that they are able to convey these facts in simple but clear terms to their patients. In this way, patients are empowered with the knowledge they need to make an informed choice about their conception options.

A dedicated multidisciplinary approach should be adopted and should broadly cover three objectives:

1. To offer reproductive counseling in order to enable couples to make an informed choice about their options for conceiving, including the risks, benefits, and costs of any treatment.
2. To offer the same spectrum of fertility treatments to HIV-infected as to HIV-non-infected individuals.
3. To ensure all ART procedures are undertaken using universal precautions and in such a way as to avoid viral transmission risk to healthcare workers and other patients attending the center.

The multidisciplinary team should comprise HIV physicians, reproductive medicine specialists, counselors, and, in the case of HIV-positive females, obstetricians and perinatologists. These principles also apply to patients infected with HBV or HCV, HTLV-1 and -2, and those potentially infected with the Zika virus, as well as those co-infected with more than one virus.

HIV-positive discordant men (female partner HIV negative)

In HIV-serodiscordant couples, natural conception should only be proposed once the blood viral load has been shown to have fallen below 50 copies/mL. Intermittent low-level blips of viral load (in the range of 50–200 copies) have

been reported, but do not appear to increase the risk of HIV transmission. While the Swiss Statement defined the absence of sexually transmitted infections as an additional safeguard, the additional evidence did not reveal an increase of the HIV transmission risk from non-viremic individuals when sexually transmitted infections were present (7). Therefore, the testing for sexually transmitted infections is not a requirement to reduce HIV transmission risk in HIV-serodiscordant couples attempting to conceive naturally, although in the context of offering assisted reproduction to those with fertility issues, it would form part of the normal work-up.

The role of sperm washing in HIV-serodiscordant couples

Sperm washing should still be offered to those couples who, despite reproductive counseling, still perceive the risk of HIV transmission through sexual intercourse to be unacceptable or in the rare cases where the man is unable to achieve stable undetectable viral loads through the use of HAART. It also has a place in poorer countries where access to HAART is still limited.

The laboratory process of sperm washing requires centrifugation of freshly ejaculated semen in a 40%–80% colloidal, silica density gradient to separate progressively motile HIV-free sperm from non-spermatozoa cells and seminal plasma that remain in the supernatant. According to published studies, subsequent processing varies between centers. Swim-up can be carried out, but if the male is on HAART, it does not confer any additional risk reduction and can lead to substantial loss of sperm. For the same reason, routine testing of an aliquot of washed sperm for detectable HIV RNA prior to the sample being used for treatment as previously advocated (44,45) is now largely redundant unless there is a detectable blood plasma viral load. A nucleic acid-based sequence amplification (NASBA, bioMérieux, Basingstoke, U.K.) or similar commercial assay is used to measure viral load (46).

Early studies, carried out at a time when few patients were on HAART, suggested a 3%–6% risk of detectable HIV being present in the washed sample (47–49), and it is for this reason that post-wash testing was advised. A study of 186 seminal samples from men with undetectable viral load (VL) on HAART identified that 18 (9.7%) had demonstrable virus (370–18,000 copies/mL) (50). HIV has been shown to be intermittently excreted in seminal plasma (51–54) and HIV replication in the genital compartment is promoted by the presence of sexually transmitted diseases and other viruses such as cytomegalovirus or herpes simplex virus. For this reason, full sexually transmitted disease screening is still advised prior to offering sperm washing treatment, but would also be routine practice for most assisted conception units in viral-negative patients. Rare cases have been reported where a very high seminal HIV RNA load has been detected despite prolonged use of HAART and undetectable serum viral load (55,56). It is, however, deemed highly improbable that these rare cases could lead to increased sexual viral transmission risk based on the absence of any reported cases of viral transmission through sexual intercourse in serodiscordant couples when the male is virally fully suppressed through use of HAART.

There have been no reported cases of infection of the female partner when sperm washing is carried out following published protocols in over 11,000 published cycles of sperm washing combined with intrauterine insemination (IUI), *in vitro* fertilization (IVF), or ICSI (16,47,48,57–63). ART outcome is reported to compare favorably to that in non-infected patients. The most recent systematic review and meta-analysis of 11,585 cycles of assisted conception with washed sperm in 3994 women reported no single case of HIV transmission (see Table 64.1) (62). There are still, however, no randomized controlled studies or studies from low-income countries, and it is these countries where sperm washing really does have a place and should be offered at low cost.

There are also reports of successful pregnancy outcome with sperm washing in HIV-positive azoospermic men in whom testicular sperm retrieval has been performed (64,65). The limitations to this approach are the quantity and quality of sperm available for washing and subsequent HIV RNA testing. In these cases, the best approach is to ensure full blood viral suppression through the use of HAART prior to embarking on sperm retrieval. In such

Table 64.1 Numbers of human immunodeficiency virus (HIV)-serodiscordant couples and cycles in a meta-analysis and number of HIV seroconversions

Parameter	Number of cycles
ART cycles with semen washing initiated	12,079
ART cycles with semen washing completed	11,915
ART cycles where female HIV status post-treatment known (%)	3994/4257 (93.8%)
Number of HIV seroconversions (95% CI) per:	
Completed ART cycle with semen washing	0/11,585 (0–0.0001)
Woman where HIV status post-treatment known	0/3994 (0–0.0004)
Completed cycle in couples where the man was not virally suppressed through HAART	0/2863 (0–0.0006)

Source: Adapted from Zafer M et al. *Fertil Steril* 2016; 105: 645–55.e2.
Abbreviations: ART, assisted reproduction technology; CI, confidence interval; HAART, highly active antiretroviral therapy.

cases, sperm washing is not indicated and indeed may reduce the chances of obtaining sufficient quantities of viable sperm to use in ICSI.

In the U.S.A., access to sperm washing remains very limited due to the persistence of the recommendation from the Centers for Disease Control and Prevention (CDC) against insemination with semen from HIV-infected men issued in 1990. This was based on a single case of HIV transmission to a female following suboptimal sperm washing techniques (66) at a time when no HAART was available. Despite extensive publications showing the safety of sperm washing, the U.S.A. continues to limit access to sperm washing, which is in contrast to many parts of the developed world, including Europe, Canada, and Australia. There has been a call for the CDC to reverse its stance on sperm washing and indeed on the use of sperm from HIV-infected men in assisted conception (67). A recent guidance document still advises that ICSI with sperm washing should be offered to all HIV-serodiscordant couples seeking to conceive through assisted conception (31).

HIV-positive discordant women (male partner HIV negative)

When the female partner is infected with HIV and has an undetectable viral load through HAART, all the arguments presented above regarding the risk of viral transmission through unprotected intercourse apply. The optimal scenario in these women in order to allow them to conceive naturally and to maximally reduce the risk of mother-to-child transmission is to start HAART prior to conception. In a mother with fully suppressed blood viral load throughout pregnancy, the risk of mother-to-child transmission is virtually non-existent. In the case of HIV-positive women with fertility issues, they should receive the same service as HIV-negative women.

Clinical work-up prior to offering assisted reproduction should include a full medical and social history as well as a sexual health screen and fertility screen in both partners, as would be carried out in viral-negative patients. Genital lesions and/or infections should be treated before any fertility treatment is envisaged and treatment planned according to underlying fertility issues.

Pre-conceptual planning in the HIV-positive female

HIV-positive women need to address a number of issues when planning to conceive. Their HIV physician is best placed to provide the required pre-conceptual advice on choice of HAART and measures that will need to be put in place during pregnancy in order to reduce the mother-to-child transmission risk, as well as advising on any long-term health issues related to HIV, which might be a contraindication to pregnancy. Relatively few antiretroviral medications (e.g., efavirenz) are contraindicated during pregnancy due to potential teratogenic effects on the fetus (32), but the evidence on the safety of some antiretrovirals during pregnancy is under continual review and, in some cases, incomplete. Folic acid should be given antenatally in order to minimize the risk of neural tube defects, as antiretrovirals are known to have a folate antagonist effect.

HIV-concordant couples

Couples who are both HIV positive, whether one, both, or neither are on HAART, can elect to conceive through timed unprotected intercourse, but face the very small risk of superinfection. This is defined as reinfection with a second strain of HIV after the first infection has been established through seroconversion. The true risk is unquantifiable, but documented to be very low in patients who are chronically infected with HIV (68). The risk of superinfection also depends on whether the man and woman are infected with the same HIV strain and whether or not either or both are on HAART. All HIV-concordant couples should be counseled on the risk of superinfection by their HIV physician, and sperm washing should be considered as a means of eliminating this risk wherever possible (69).

EFFECT OF HIV ON FERTILITY

In both men and women, the effect of HIV and HAART on fertility parameters has been extensively studied, and in both, fertility appears to be impaired. This is of importance as couples do need to be made aware of the risks of impaired fertility and offered fertility screening at an earlier stage than would be advised in HIV-negative cases.

HIV-positive men

Studies of HIV-positive men suggest that they have semen parameters below the defined World Health Organization (WHO) normal range. However, in the two largest studies to date of semen parameters in HIV-positive men, Nicopoullos and colleagues (70,71) consistently found all parameters to be significantly impaired compared to HIV-negative controls, with a significant negative correlation between CD4 count as a marker of HIV infection and immune status on sperm count, motility, and morphology. There was a significant decrease in volume, count, motility, morphology, and post-wash parameters when CD4 counts dropped below median levels (450 cells/mm^3), but no effect of viral load on any sperm parameter. Similar findings were reported by Kehl et al. (72), with no significant effect of HAART on semen parameters. Earlier studies (73,74), by contrast, found viral load to correlate with sperm motility and morphology. A recent study specifically looking at the effect of HAART on semen parameters found no significant effect other than in those patients on efavirenz, where a reduction in sperm motility was noted (75). The effect of HIV on semen parameters would suggest that HIV-positive men should consider a semen analysis sooner rather than later when attempting to conceive naturally.

HIV-positive women

There is increasing evidence to suggest that HIV-positive women have reduced fertility (43,76,77). A slight increase in the incidence of cycle irregularity in HIV-positive women has been reported, although this dates to a time when

HAART was not routinely available (78). Cycle irregularity was less marked in cases of higher CD4 counts. IVF outcome data would suggest that ovarian reserve is reduced in HIV-positive women compared to HIV-negative women (see section entitled "ART outcomes for patients with HIV, HBV, and HCV"). HAART may also have a direct effect on oocyte quality by causing mitochondrial toxicity, as mitochondrial depletion has been observed in the oocytes of HIV-positive women on antiretroviral therapy (77). Retrospective data from Sub-Saharan Africa (79,80) and prospective data from the U.K. indicate an increased incidence of tubal infertility in HIV-positive women (43,81) of at least twice that of HIV-negative controls. On the basis of increased risk of low ovarian reserve and increased tubal infertility, HIV-positive women trying to conceive should be referred sooner rather than later for fertility evaluation, and certainly if they have not conceived within 6–12 months. Referral should be earlier if there is a history of pelvic inflammatory disease or in women over 35 years of age in order to assess tubal function and ovarian reserve.

REDUCING HIV TRANSMISSION RISK DURING ART PROGRAMS

Aside from those few individuals who are not on HAART or who elect to have sperm washing despite being informed of the safety of natural conception, the indications for referring an HIV-positive man or woman for ART programs are now identical to those for HIV-negative couples (i.e., where demonstrable or unexplained causes of infertility are identified).

There are no additional specific measures that should be taken during fertility treatment to further reduce viral transmission risk to the partner or future child, which, as previously discussed, is now regarded as non-existent in those with undetectable serum viral load through HAART. The question of whether sperm washing should be considered in HIV-positive men with undetectable viral load is highly questionable. During routine ART use for IUI, IVF, and ICSI, semen is normally processed on a single density gradient. A further wash and swim-up as would be carried out in semen washing may reduce the quantity of motile sperm available for treatment without having any significant role to play in reducing viral transmission risk.

HBV AND HCV

HBV and HCV are major causes of chronic hepatitis, cirrhosis, and hepatocellular cancer.

HBV is a DNA virus and one of the major causes of liver disease worldwide. Vertical transmission accounts for over 40% of cases of chronic infection, and the sexual transmission risk is two-fold higher than for HIV and six-fold higher than for HCV. Overall, as a virus, it is about 100-times more infective than HCV or HIV, and therefore poses a theoretically higher contamination risk in the laboratory. However, unlike HIV and HCV, an effective vaccine exists for HBV, and all healthcare workers and partners of known infected individuals should be vaccinated. Uninfected women with an HBV-infectious partner should only consider conception post-vaccination. The effectiveness of HBV vaccination is measured by the presence of HBV surface antibody. Approximately 5% of vaccinated individuals do not produce HBV surface antibodies ("non-responders"). The first step in such cases is to exclude a pre-existing HBV infection by anti-HBc antibody testing. Several options (including combination with hepatitis A vaccination and intradermal application) are available to improve the response in non-infected non-responders (82). Sperm washing is not required in serodiscordant couples as a means of reducing horizontal transmission risk unless a woman fails to develop adequate immunity through vaccination and the partner has a positive viral load (HBV DNA). In such a case, priority should be given to treating the infected male with antiretrovirals before considering assisted conception. If ART is required in HBV-positive couples for fertility issues, similar clinical protocols to those used in non-infected patients should be followed.

Vertical transmission risk for an HBV-positive woman during pregnancy is <10% if she is only HBsAg positive, and 80%–90% if she is also positive for HBeAg or HBV DNA positive (83). In such situations, infection in the neonate can be minimized if immunoprophylaxis (HBV vaccination and one dose of HBV immunoglobulins) is given within 24 hours of birth, with further doses at one and six months (84). Breastfeeding does not appear to play a role in perinatal transmission (44). However, this approach does not sufficiently prevent vertical transmission if the HBV DNA level in the mother exceeds 200,000 IU/mL. Therefore, current management is to consider antiviral therapy of the pregnant woman if the HBV DNA load exceeds 100,000 IU/mL.

HCV infection is primarily transmitted by parenteral spread (blood products, shared needles, and needle-stick injuries). Although sexual transmission has been observed mostly to HIV-positive men who have sex with men (85), the transmission appears to be limited to specific sexual practices involving exchange of blood (86). Heterosexual transmission among monogamous HCV-serodiscordant partners is essentially non-existent, and the use of condoms is not recommended for these couples. Natural conception is advised and, if fertility issues exist, ART without any additional procedures such as sperm washing can be performed (87,88).

There is currently no vaccine for HCV, but antiviral treatment is recommended for those infected individuals who meet the country-specific requirements for HCV treatment, and this should be offered if possible prior to planning conception, with the aim of clearing the virus. This is normally a decision taken in conjunction with their infectious disease or hepatology expert and must take into consideration the risks of delaying conception against the benefits of reducing viral transmission risks during conception and pregnancy and improving the health of the individual.

Vertical transmission risk in HCV RNA-positive women is around 5%–6% and doubles for HIV-positive mothers (89). For HCV RNA and HIV-negative mothers, vertical

transmission of HCV has not been observed (90–92). In the absence of a vaccine, there are no specific measures available to protect the neonate, and the administration of immunoglobulin offers no protection. There are no data to suggest HCV is transmitted during breastfeeding and there is no indication for cesarean section delivery (91).

ART OUTCOMES FOR PATIENTS WITH HIV, HBV, AND HCV

Overall, studies suggest that the performance of women with HIV is poorer than in non-infected women. Earlier studies suggested reduced ovarian response, implantation, and pregnancy rates in patients with viral infection (81,93,94). The most recent and largest study to date is a retrospective case–control study, which compared ART outcomes in 82 women infected with HIV with outcomes in HIV-negative controls. This study found no statistically significant difference between the two groups with regard to ovarian stimulation, fertilization rate, or numbers of embryos transferred, but statistically significant lower implantation rates (10% vs. 21%), clinical pregnancy rates (12% vs. 32%), and live birth rates (7% vs. 19%) were seen in HIV-positive women (see Table 64.2) (95). The lower rates have been attributed to a premature fall in ovarian reserve (78) and the impact of HAART on oocyte quality in these women (77). IVF outcome does not appear to be affected in HIV-positive women undergoing ovum donation, pointing to an effect of HIV and/or immunosuppression on ovarian response and ovarian reserve rather than on implantation (96). A study of oocytes from both fresh and frozen cycles showed increased mitochondrial DNA depletion in women who had been on HAART for over nine years and had undetectable viral loads (77).

Recent studies on HBV-positive women suggest either no difference in outcome with ART (97) or an improved outcome compared to non-infected controls (98). HCV appears to have a significant negative effect on semen quality (99), but there are no studies specifically addressing assisted reproductive outcomes in HCV-positive women.

In the case of men infected with HIV, HBV, or HCV where sperm washing is used in IVF/ICSI, there are no reported differences in cycle outcomes compared to non-infected cases.

HTLV-1 AND -2

Whilst the majority of scientific publications on viral infection in the ART setting focus on HIV and hepatitis, consideration should also be given to HTLV-1/2 infection. This is an ancient retrovirus that infects CD4 T-cells. Although transmitted vertically, intravenously, and by sexual contact, the modes of transmission are distinct from HIV and hepatitis: vertical transmission occurs primarily through breastfeeding. Sexual transmission is much more efficient from males to females (60% in a 10-year partnership) than in the opposite direction (<1%/10 years) (100). The potential for causing human disease is far lower than for HIV, HBV, and HCV, but 1%–4% of infected patients develop adult T-cell leukemia or spastic paraparesis. Low endemic rates (1%) are reported in North and South America, Africa, and Japan. Studies of prevalence in the assisted reproduction population in Sweden suggest a seroprevalence of 2.3 per 10,000 (101).

Screening of all women seeking ART services for HTLV-1/2 is probably not reasonable, but targeted screening of individuals from endemic areas (the Caribbean, South America, Central Africa, and Japan) should be considered (102). Couples where one partner is found to be HTLV-1/2 positive should be counseled as outlined in a CDC recommendation (103). Given the lack of a recommendation for barrier precautions in HTLV-1/2-serodiscordant couples, natural conception is the preferred mode of conception. ART in infertile couples affected by HTLV-1/2 should be performed with the standard precautions when handling biologic materials. Women infected with HTLV-1/2 should be advised against breastfeeding their infant.

ZIKA VIRUS

Zika virus is a mosquito-transmitted flavivirus that was first identified and isolated from mosquitos from the Zika forest in Uganda. The virus has spread rapidly and

Table 64.2 Assisted reproduction technology (ART) outcomes in human immunodeficiency virus (HIV)-infected women and matched controls

ART outcome	HIV-1-positive (n = 82), % (n)	HIV-negative controls (n = 82), % (n)	OR (95% CI)[a]	p-value[a]
Transfer/oocyte retrieval	85 (70/82)	95 (78/82)	0.23 (0.06–0.84)	0.027
Clinical Pregnancy/oocyte retrieval	12 (10/82)	32 (26/82)	0.30 (0.13–0.70)	0.006
Clinical pregnancy/embryo transfer	14 (10/70)	33 (26/78)	0.35 (0.15–0.83)	0.017
Implantation rate	10 (10/104)	21 (26/122)	0.38 (0.17–0.87)	0.022
Live Birth/embryo transfer	7 (5/70)	19 (15/78)	0.26 (0.08–0.82)	0.022

Source: Adapted from Zafer M et al. *Fertil Steril* 2016; 105: 645–55.e2.

[a] Adjusted for age, ethnicity, ovarian reserve (anti-Mullerian hormone and antral follicle count), rank attempt, and fertilization technique.

Abbreviations: CI, confidence interval; OR, odds ratio.

is now prevalent in South America, Central America, the Caribbean, the Pacific Islands, Singapore, and Thailand. The main form of viral transmission is by mosquitos. The incubation period for the virus is up to two weeks, and symptoms are only seen in 20% of those infected. These include fever, myalgia, conjunctivitis, and rash. From the perspective of those wishing to conceive, Zika infection can lead to congenital Zika syndrome, which consists of multiple developmental issues including microcephaly and fetal loss. It can also be associated with Guillain–Barré syndrome (104). Currently, no vaccine or treatment exists to prevent Zika virus syndrome.

Although the majority of reported cases result from mosquito transmission, sexual transmission is increasingly being reported (105). Zika virus RNA has been detected in both semen and vaginal fluid (106,107). Due to the persistence of the virus in semen (107–109), the CDC in the U.S.A. has published guidance on the risk of sexual transmission (110), as has the WHO. A study of Zika RNA in blood, urine, and semen from a 32-year-old man returning from French Guyana showed persistence of the virus in semen for up to 141 days after onset of symptoms, but no detectable virus in the plasma or urine from 37 days after onset of symptoms (see Figure 64.2) (107). Although the number of reported cases is still limited, the data indicate a wide variation in the persistence of the virus in semen. Guidance is therefore emerging for those working with gametes in the assisted conception setting, with particular reference to gamete donation. In the U.K., such guidance has been published by the Human Fertilisation and Embryology Authority (HFEA) and is consistent with the advice being given to potential blood, organ, and tissue donors by Public Health England. Any individual with a known Zika virus infection should not try to conceive naturally, donate gametes, or proceed with fertility treatment for six months after onset of infection unless the semen tests are negative for Zika virus RNA by nucleic acid testing (NAT). Individuals who have traveled to an area where the Zika virus is present are advised not to try to conceive naturally, donate gametes, or proceed with fertility treatment for eight weeks after their return (http://www.hfea.gov.uk/10258.html). It follows that sperm donors should be asked about recent travel and consideration given to NAT for Zika virus if they wish to proceed with sperm donation before the six-month window is over. Difficulties arise, of course, for those individuals who live in areas where the Zika virus is endemic.

VIRAL SCREENING PRIOR TO OFFERING ASSISTED CONCEPTION

In the U.K. and most IVF centers in Europe and North America, patients presenting to undergo assisted conception are required to be screened for HIV, HBV, and HCV. Certain groups of patients will need to be screened for HTLV-1/2 if they have traveled or work in high-risk areas where the virus is endemic. This has the benefit of identifying high-risk patients from the outset so that they can receive appropriate counseling and, where necessary, antiretroviral therapy before embarking on conception. National guidelines on the matter vary widely, particularly with reference to frequency of screening and timing of screening before assisted conception. All gamete donors are required to be screened, and viral-positive donors are excluded from donating in the majority of countries. In the case of sperm donors, viral and sexual health screening is required both before cryostorage commences as well as after all the samples of sperm have been collected and frozen. Use of RNA PCR testing (NAT) avoids the need for a prolonged quarantine period before the samples can be released for use.

In the case of egg donors, the situation is more complex, and policy on timing of screening varies. Ideally, egg donors should be screened as closely as possible to the timing of vaginal egg collection to minimize any risk. In practice,

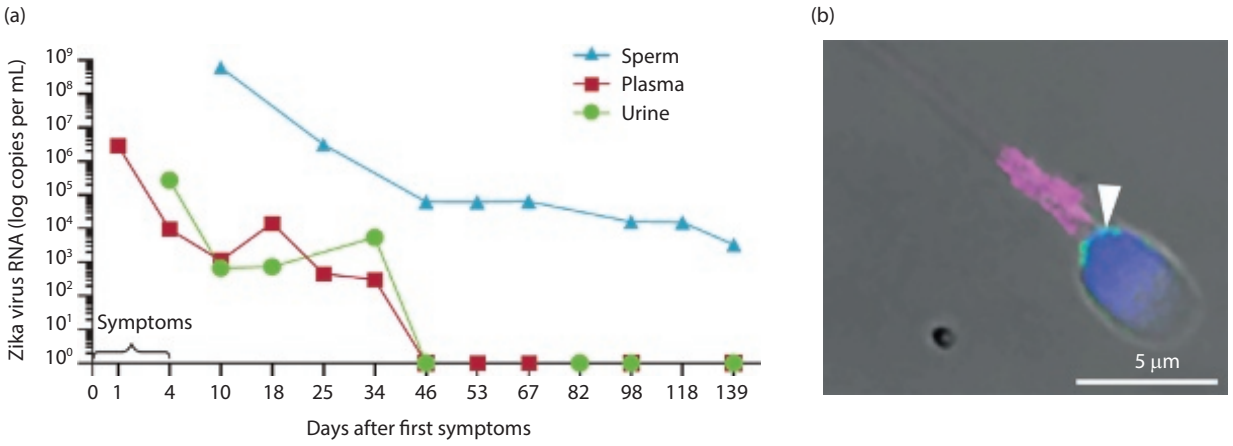

Figure 64.2 Zika virus infects spermatozoa. (a) The kinetics of Zika virus RNA detection in plasma, urine, and semen quantified by real-time polymerase chain reaction (RealStar Zika Virus RT-PCR Kit 1.0, Altona Diagnostics GmbH, Hamburg, Germany). (b) Immunohistochemical detection of Zika virus (green; arrowhead) on bright-field microscopy in the head of spermatozoa obtained from a patient; Tom20 is pink, Zika virus is green, and Hoechst stain is blue. (From Mansuy JM et al. *Lancet Infect Dis* 2016; 16: 405. With permission.)

most centers offer screening within three months of donation, often just before donors start ovarian stimulation.

LABORATORY PROTOCOLS FOR VIRAL-POSITIVE PATIENTS

Despite previously published concerns regarding the handling and freezing of gametes and embryos from patients who carry blood-borne viruses (111–113), there have been no reported cases of cross-contamination in the ART setting. Published guidelines on the matter remain limited (111), and for this reason, many ART centers will not treat patients with a known viral infection. The European Society of Human Reproduction and the Embryology Committee of the Special Interest Group on Embryology published guidelines in 2008 on good practice in laboratories. They recommended that gametes and embryos from patients infected with HIV, HBV, and HCV be handled in a dedicated laboratory space at allocated times and processing of these samples within a biosafety cabinet to minimize the risk of cross-contamination of patient specimens. The Practice Committee of the American Society for Reproductive Medicine issued guidance in 2013 recommending that samples from viral-positive patients be handled separately in time or space and that separate cryostorage facilities be used when freezing samples. In the U.K., the HFEA mandates separate cryostorage for gametes and embryos with different viral infections or infection combinations. What is clear from reviewing the published literature is that there are no universally agreed guidelines. The emergence of new viruses such as the Zika virus exemplifies the importance of treating all samples as potentially infectious and using universal precautions at all times, as is used in operating theaters and emergency rooms. There is no difference between handling a sample from a known virally infected patient and a sample from a patient who screened negative for HIV, HBV, and HCV three months before, but might have seroconverted due to a casual relationship in the interim.

In addition to using universal precautions in the assisted conception laboratory, samples from patients with known or suspected blood-borne viruses should ideally be handled separately in time or space to reassure viral-negative patients that all measures have been taken to minimize any risk of viral contamination. In practice, this should not affect viral transmission risk if appropriate universal precautions are used. The majority of countries currently recommend that gametes and embryos from patients with known viral infections are cryopreserved in separate heat-sealed straws (114) and cryostorage tanks due to a purely theoretical risk of transmission in liquid nitrogen (115,116), but a clear benefit of this recommendation has not been shown. Although it has been suggested that vapor-phase storage would offer more security against the risk of cross-contamination as compared to liquid nitrogen without affecting embryo and sperm survival (117), there are no long-term data to assess the safety and efficacy of this approach.

There is certainly no longer any justification for denying a patient assisted conception on the grounds of inadequate laboratory facilities, as all ART centers should be employing universal precautions.

THIRD-PARTY REPRODUCTION AND VIRAL INFECTION

A challenging situation has emerged as a result of improved care for patients infected with HIV. Should individuals infected with HIV be legally allowed to enlist assistance from gamete donors and gestation carriers? Should the HIV status of the intended parents be disclosed to third parties enlisted to assist the individual or couple to have a child? There are no simple or correct answers to these difficult questions. Each country should seek legal guidance.

Informed consent dictates that physicians disclose any information that might be material to a person's decision to undergo or refuse treatment. In the case of an HIV-infected patient who wishes to use their gametes in third-party reproduction, disclosure of the intended parent's viral status should be disclosed to any gamete donor or gestational surrogate, since this might affect their decision to be involved in treatment. In some countries, like the U.K., it remains illegal for patients with HIV, HBV, or HCV to donate eggs or sperm or to be gamete donors in any surrogate situation. In the U.S.A., disclosure is of relevance to the woman who is to receive the gametes of a virally infected patient. She should be fully informed of the potential risks to herself (negligible based on current evidence, provided the male is viral negative through antiretrovirals), and in some states she will be asked to sign a written waiver to acknowledge the risks associated with such a transfer (42).

CONCLUSION

Antiretroviral therapy has radically changed the natural course of HIV infection and, in turn, the way professionals should manage reproductive care for these patients. Those individuals who, as a result of treatment, become viral negative can expect to lead near normal lives and have children naturally through unprotected intercourse. Where fertility issues exist, these patients should be offered the same full spectrum of assisted conception treatments offered to non-infected individuals within a safe laboratory environment equipped to deal with both known and unknown viral risk. In the case of HIV, sperm washing is not required if the infected male partner has a fully suppressed viral load through the use of antiretroviral therapy. Natural conception with PrEP should be considered in those parts of the world where there is limited access to HAART, and it is in these patients that sperm washing should be considered as a high priority and developed as a cost-effective risk-reduction option.

Patients infected with HBV and HCV should be offered very similar guidance to those with HIV, and in the case of HBV, vaccination should be offered to the uninfected partner. No ART center should find itself unable to offer a viral-positive patient treatment other than on ethical grounds unrelated to viral infection.

Reproductive counseling should only be offered by appropriately qualified personnel able to discuss viral transmission risk in both natural and assisted conception

settings and the effects of the virus and/or the antiviral treatment on fertility so as to fully inform these patients of their risks and options.

HIV-serodiscordant couples electing to have sperm washing with no fertility issues should be initially offered IUI in a natural cycle using washed sperm (with the exception of those patients being treated in the U.S.A., where sperm washing with ICSI is currently advised). If there are fertility issues, they should be managed as any HIV-negative couple seeking ART (118).

Reproductive specialists and patients should remember that they share the responsibility of preventing viral infection to the uninfected partner and child and to other patients and staff attending the center.

REFERENCES

1. Schnittman SM, Fauci AS. Human immunodeficiency virus and acquired immunodeficiency syndrome: An update. *Adv Intern Med* 1994; 39: 305–55.
2. Doitsh G, Galloway NL, Geng X et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014; 505: 509–14.
3. Hart C, Schochetman G, Spira T et al. Direct detection of HIV RNA expression in seropositive subjects. *Lancet* 1988; 2: 596–9.
4. Hogg RS, Heath KV, Yip B et al. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. *JAMA* 1998; 279: 450–4.
5. Scandlyn J. When AIDS became a chronic disease. *West J Med* 2000; 172: 130–3.
6. Vernazza P HB, Bernasconi E, Flepp M. Les personnes séropositives ne souffrant d'aucune autre MST et suivant un traitement antirétroviral efficace ne transmettent pas le VIH par voie sexuelle. *Bulletin des Médecins Suisses* 2008; 89: 165–9.
7. Vernazza P, Bernard EJ. HIV is not transmitted under fully suppressive therapy: The Swiss Statement—Eight years later. *Swiss Med Wkly* 2016; 146: w14246.
8. von Overbeck J. Update on HIV infection. *J Insur Med* 2005; 37: 201–13.
9. Nakagawa F, May M, Phillips A. Life expectancy living with HIV: Recent estimates and future implications. *Curr Opin Infect Dis* 2013; 26: 17–25.
10. van Sighem AI, Gras LA, Reiss P, Brinkman K, de Wolf F. Life expectancy of recently diagnosed asymptomatic HIV-infected patients approaches that of uninfected individuals. *AIDS* 2010; 24: 1527–35.
11. Centers for Disease Control (CDC). Recommendations for assisting in the prevention of perinatal transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus and acquired immunodeficiency syndrome. *MMWR Morb Mortal Wkly Rep* 1985; 34: 721–6, 731–2.
12. Sturmer M, Doerr HW, Berger A, Gute P. Is transmission of HIV-1 in non-viraemic serodiscordant couples possible? *Antivir Ther* 2008; 13: 729–32.
13. Vernazza PL, Hirschel B. HIV transmission hunting—The chase for low risk events. *Antivir Ther* 2008; 13: 641–2.
14. Cohen MS, Chen YQ, McCauley M et al. Antiretroviral therapy for the prevention of HIV-1 transmission. *N Engl J Med* 2016; 375: 830–9.
15. Cohen MS, Chen YQ, McCauley M et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011; 365: 493–505.
16. Semprini AE, Levi-Setti P, Bozzo M et al. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet* 1992; 340: 1317–9.
17. Bagasra O, Farzadegan H, Seshamma T et al. Detection of HIV-1 proviral DNA in sperm from HIV-1 infected men. *AIDS* 1994; 8: 1669–74.
18. Vernazza PL, Gilliam BL, Dyer J et al. Quantification of HIV in semen: Correlation with antiviral treatment and immune status. *AIDS* 1997; 11: 987–93.
19. Quayle AJ, Xu C, Mayer KH, Anderson DJ. T lymphocytes and macrophages, but not motile spermatozoa, are a significant source of human immunodeficiency virus in semen. *J Infect Dis* 1997; 176: 960–8.
20. Quayle AJ, Xu C, Tucker L, Anderson DJ. The case against an association between HIV-1 and sperm: Molecular evidence. *J Reprod Immunol* 1998; 41: 127–36.
21. Kim LU, Johnson MR, Barton S, Nelson MR, Sontag G, Smith JR et al. Evaluation of sperm washing as a potential method of reducing HIV transmission in HIV-discordant couples wishing to have children. *AIDS* 1999; 13: 645–51.
22. Baccetti B, Benedetto A, Burrini AG et al. HIV particles detected in spermatozoa of patients with AIDS. *J Submicrosc Cytol Pathol* 1991; 23: 339–45.
23. Bujan L, Pasquier C. People living with HIV and procreation: 30 years of progress from prohibition to freedom? *Hum Reprod* 2016; 31: 918–25.
24. Vandermaelen A, Englert Y. Human immunodeficiency virus serodiscordant couples on highly active antiretroviral therapies with undetectable viral load: Conception by unprotected sexual intercourse or by assisted reproduction techniques? *Hum Reprod* 2010; 25: 374–9.
25. Vernazza PL, Graf I, Sonnenberg-Schwan U, Geit M, Meurer A. Preexposure prophylaxis and timed intercourse for HIV-discordant couples willing to conceive a child. *AIDS* 2011; 25: 2005–8.
26. Anglemyer A, Rutherford GW, Baggaley RC, Egger M, Siegfried N. Antiretroviral therapy for prevention of HIV transmission in HIV-discordant couples. *Cochrane Database Syst Rev* 2011; 8: CD009153.
27. Whetham J, Taylor S, Charlwood L et al. Pre-exposure prophylaxis for conception (PrEP-C) as a risk reduction strategy in HIV-positive men and HIV-negative women in the UK. *AIDS Care* 2014; 26: 332–6.

28. Hoffman RM, Jaycocks A, Vardavas R et al. Benefits of PrEP as an adjunctive method of HIV prevention during attempted conception between HIV-uninfected women and HIV-infected male partners. *J Infect Dis* 2015; 212: 1534–43.
29. Sun L, Wang F, Liu A et al. Natural conception may be an acceptable option in HIV-serodiscordant couples in resource limited settings. *PLoS One* 2015; 10: e0142085.
30. National Collaborating Centre for Women's and Children's Health. *National Institute for Health and Clinical Excellence: Guidance. Fertility: Assessment and Treatment for People with Fertility Problems*. London, U.K.: Royal College of Obstetricians & Gynaecologists National Collaborating Centre for Women's and Children's Health, 2013.
31. Jindal SK, Rawlins RG, Muller CH, Drobnis EZ. Guidelines for risk reduction when handling gametes from infectious patients seeking assisted reproductive technologies. *Reprod Biomed Online* 2016; 33: 121–30.
32. de Ruiter A, Mercey D, Anderson J et al. British HIV Association and Children's HIV Association guidelines for the management of HIV infection in pregnant women 2008. *HIV Med* 2008; 9: 452–502.
33. Connor EM, Sperling RS, Gelber R et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N Engl J Med* 1994; 331: 1173–80.
34. de Ruiter A, Taylor GP, Clayden P et al. British HIV Association guidelines for the management of HIV infection in pregnant women 2012 (2014 interim review). *HIV Med* 2014; 15(Suppl 4): 1–77.
35. Gilling-Smith C, Smith JR, Semprini AE et al. HIV and infertility: Time to treat. There's no justification for denying treatment to parents who are HIV positive ART in HIV-infected couples: Has the time come for a change of attitude? *BMJ* 2001; 322: 566–7.
36. Ethics Committee of the American Society for Reproductive Medicine. Human immunodeficiency virus and infertility treatment. *Fertil Steril* 2002; 77: 218–22.
37. Minkoff H, Santoro N. Ethical considerations in the treatment of infertility in women with human immunodeficiency virus infection. *N Engl J Med* 2000; 342: 1748–50.
38. Lyerly AD, Anderson J. Human immunodeficiency virus and assisted reproduction: Reconsidering evidence, reframing ethics. *Fertil Steril* 2001; 75: 843–58.
39. Ethics Committee of the American Society for Reproductive Medicine. Human immunodeficiency virus and infertility treatment. *Fertil Steril* 2010; 94: 11–5.
40. Sauer MV. Providing fertility care to those with HIV: Time to re-examine healthcare policy. *Am J Bioeth* 2003; 3: 33–40.
41. Gilling-Smith C. Risking Parenthood? Serious viral illness, parenting and welfare of the child. In: *Contemporary Ethical Dilemmas in Assisted Reproduction 52006*. SFS C (ed.), Elsevier, Birmingham, Alabama, pp. 57–69.
42. Ethics Committee of American Society for Reproductive Medicine. Human immunodeficiency virus (HIV) and infertility treatment: A committee opinion. *Fertil Steril* 2015; 104: e1–8.
43. Frodsham LCG, Boaq F, Barton S, Gilling-Smith C. Human immunodeficiency virus infection and fertility care in the United Kingdom—Demand and supply. *Fertil Steril* 2006; 85: 285–9.
44. Gilling-Smith C, Almeida P. HIV, hepatitis B and hepatitis C and infertility: Reducing risk. *Hum Fertil (Camb)* 2003; 6: 106–12.
45. Semprini AE, Fiore S. HIV and reproduction. *Curr Opin Obstet Gynecol* 2004; 16: 257–62.
46. Burgisser P, Vernazza P, Flepp M et al. Performances of five different assays for the quantification of viral load in persons infected with various subtypes of HIV-1. Swiss HIV Cohort Study. *J Aquir Immune Defic Syndr* 2000; 23: 138–44.
47. Marina S, Marina F, Alcolea R et al. Pregnancy following intracytoplasmic sperm injection from an HIV-1 seropositive man. *Hum Reprod* 1998; 13: 3247–9.
48. Marina S, Marina F, Alcolea A et al. Human immunodeficiency virus type I-serodiscordant couples can bear healthy children after undergoing intrauterine insemination. *Fertil Steril* 1998; 70: 35–9.
49. Giling-Smith C, Nicopouloos JDM, Semprini AE, Frodsham LCG. HIV and reproductive care—A review of current practice. *BJOG* 2006; 113: 1–10.
50. Vourliotis M, Nicopoullos JDM, Gilling-Smith C et al. A comparison of sperm yield following changes in HIV sperm washing laboratory practice. *Hum Reprod* 2009; 24(Suppl 1): i166.
51. Anderson JA, Ping LH, Dibben O et al. HIV-1 populations in semen arise through multiple mechanisms. *PLoS Pathog* 2010; 6: e1001053.
52. Vernazza PL, Gilliam BL, Flepp M et al. Effect of antiviral treatment on the shedding of HIV-1 in semen. *AIDS* 1997; 11: 1249–54.
53. Vernazza PL, Troiani L, Flepp MJ et al. Potent antiretroviral treatment of HIV-infection results in suppression of the seminal shedding of HIV. The Swiss HIV Cohort Study. *AIDS* 2000; 14: 117–21.
54. Leruez-Ville M, Dulioust E, Costabliola D et al. Decrease in HIV-1 seminal shedding in men receiving highly active antiretroviral therapy: An 18 month longitudinal study (ANRS EP012). *AIDS* 2002; 16: 486–8.
55. Pasquier CJ, Moinard N, Saune K et al. Persistent differences in the antiviral effects of highly active antiretroviral therapy in the blood and male genital tract. *AIDS* 2008; 22: 1894–6.

56. Pasquier C, Moinard N, Saune K et al. Antiviral effect of maraviroc in semen: A case report. *Antivir Ther* 2012; 17: 933–6.
57. Ohl J, Partisani M, Wittemer C et al. Assisted reproduction techniques for HIV serodiscordant couples: 18 months of experience. *Hum Reprod* 2003; 18: 1244–9.
58. Garrido N, Meseguer M, Simon C, Pellicer A, Remohi J. Assisted reproduction in HIV and HCV infected men of serodiscordant couples. *Arch Androl* 2004; 50: 105–11.
59. Bujan L, Hollander L, Coudert M et al. Safety and efficacy of sperm washing in HIV-1-serodiscordant couples where the male is infected: results from the European CREAThE network. *AIDS* 2007; 21: 1909–14.
60. Sauer MV, Wang JG, Douglas NC et al. Providing fertility care to men seropositive for human immunodeficiency virus: Reviewing 10 years of experience and 420 consecutive cycles of *in vitro* fertilization and intracytoplasmic sperm injection. *Fertil Steril* 2009; 91: 2455–60.
61. Nicopoullos JD, Almeida P, Vourliotis M, Goulding R, Gilling-Smith C. A decade of sperm washing: Clinical correlates of successful insemination outcome. *Hum Reprod* 2010; 25: 1869–76.
62. Zafer M, Horvath H, Mmeje O et al. Effectiveness of semen washing to prevent human immunodeficiency virus (HIV) transmission and assist pregnancy in HIV-discordant couples: A systematic review and meta-analysis. *Fertil Steril* 2016; 105: 645–55.e2.
63. Vitorino RL, Grinsztejn BG, de Andrade CA et al. Systematic review of the effectiveness and safety of assisted reproduction techniques in couples serodiscordant for human immunodeficiency virus where the man is positive. *Fertil Steril* 2011; 95: 1684–90.
64. Nicopoullos JD, Frodsham LC, Ramsay JW, Almeida PA, Rozis G, Gilling-Smith C. Synchronous sperm retrieval and sperm washing in an intracytoplasmic sperm injection cycle in an azoospermic man who was positive for human immunodeficiency virus. *Fertil Steril* 2004; 81: 670–4.
65. Bujan L, Daudin M, Moinard N, Plante P, Parinaud J, Pasquier C. Azoospermic HIV-1 infected patients wishing to have children: Proposed strategy to reduce HIV-1 transmission risk during sperm retrieval and intracytoplasmic sperm injection: Case report. *Hum Reprod* 2007; 22: 2377–81.
66. Centers for Disease Control (CDC). HIV-1 infection and artificial insemination with processed sperm. *MMWR Morb Mortal Wkly Rep* 1990; 39: 249–56.
67. Cohan D, Weber S, Aaron E. CDC should reverse its recommendation against semen washing-intrauterine insemination for HIV-serodifferent couples. *Am J Obstet Gynecol* 2013; 209: 284.
68. Fakoya A, Lamba H, Mackie N et al. British HIV Association, BASHH and FSRH guidelines for the management of the sexual and reproductive health of people living with HIV infection 2008. *HIV Med* 2008; 9: 681–720.
69. Redd AD, Quinn TC, Tobian AA. Frequency and implications of HIV superinfection. *Lancet Infect Dis* 2013; 13: 622–8.
70. Nicopoullos JD, Almeida PA, Ramsay JW, Gilling-Smith C. The effect of human immunodeficiency virus on sperm parameters and the outcome of intrauterine insemination following sperm washing. *Hum Reprod* 2004; 19: 2289–97.
71. Nicopoullos JD, Almeida P, Vourliotis M, Gilling-Smith C. A decade of the sperm-washing programme: Correlation between markers of HIV and seminal parameters. *HIV Med* 2011; 12: 195–201.
72. Kehl S, Weigel M, Muller D, Gentili M, Hornemann A, Sutterlin M. HIV-infection and modern antiretroviral therapy impair sperm quality. *Arch Gynecol Obstet* 2011; 284: 229–33.
73. Dulioust E, Du AL, Costagliola D et al. Semen alterations in HIV-1 infected men. *Hum Reprod* 2002; 17: 2112–8.
74. Bujan L, Sergerie M, Moinard N et al. Decreased semen volume and spermatozoa motility in HIV-1-infected patients under antiretroviral treatment. *J Androl* 2007; 28: 444–52.
75. Frapsauce C, Grabar S, Leruez-Ville M et al. Impaired sperm motility in HIV-infected men: An unexpected adverse effect of efavirenz? *Hum Reprod* 2015; 30: 1797–806.
76. Coll O, Lopez M, Vidal R et al. Fertility assessment in non-infertile HIV-infected women and their partners. *Reprod Biomed Online* 2007; 14: 488–94.
77. Lopez S, Coll O, Durban M et al. Mitochondrial DNA depletion in oocytes of HIV-infected antiretroviral-treated infertile women. *Antivir Ther* 2008; 13: 833–8.
78. Massad LS, Evans CT, Minkoff H et al. Effects of HIV infection and its treatment on self-reported menstrual abnormalities in women. *J Womens Health (Larchmt)* 2006; 15: 591–8.
79. Brunham RC, Cheang M, McMaster J, Garnett G, Anderson R. Chlamydia trachomatis, infertility, and population growth in sub-Saharan Africa. *Sex Transm Dis* 1993; 20: 168–73.
80. Brunham RC, Garnett GP, Swinton J, Anderson RM. Gonococcal infection and human fertility in sub-Saharan Africa. *Proc Biol Sci* 1991; 246: 173–7.
81. Coll O, Lopez M, Hernandez S. Fertility choices and management for HIV-positive women. *Curr Opin HIV AIDS* 2008; 3: 186–92.
82. David MC, Ha SH, Paynter S, Lau C. A systematic review and meta-analysis of management options for adults who respond poorly to hepatitis B vaccination. *Vaccine* 2015; 33: 6564–9.
83. Mast EE, Margolis HS, Fiore AE et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. *MMWR Recomm Rep* 2005; 54(RR-16): 1–31.

84. Schillie S, Murphy TV, Fenlon N, Ko S, Ward JW. Update: Shortened interval for postvaccination serologic testing of infants born to hepatitis B-infected mothers. *MMWR Morb Mortal Wkly Rep* 2015; 64: 1118–20.
85. Terrault NA, Dodge JL, Murphy EL et al. Sexual transmission of hepatitis C virus among monogamous heterosexual couples: The HCV partners study. *Hepatology* 2013; 57: 881–9.
86. Chan DP, Sun HY, Wong HT, Lee SS, Hung CC. Sexually acquired hepatitis C virus infection: A review. *Int J Infect Dis* 2016; 49: 47–58.
87. Pasquier C, Daudin M, Righi L et al. Sperm washing and virus nucleic acid detection to reduce HIV and hepatitis C virus transmission in serodiscordant couples wishing to have children. *AIDS* 2000; 14: 2093–9.
88. Halfon P, Giorgetti C, Bourliere M et al. Medically assisted procreation and transmission of hepatitis C virus: Absence of HCV RNA in purified sperm fraction in HIV co-infected patients. *AIDS* 2006; 20: 241–6.
89. Benova L, Mohamoud YA, Calvert C, Abu-Raddad LJ. Vertical transmission of hepatitis C virus: Systematic review and meta-analysis. *Clin Infect Dis* 2014; 59: 765–73.
90. Checa Cabot CA, Stoszek SK, Quarleri J et al. Mother-to-child transmission of hepatitis C virus (HCV) among HIV/HCV-coinfected women. *J Pediatr Infect Dis Soc* 2013; 2: 126–35.
91. Di Domenico C, Di Giacomo C, Marinucci G, Di Paolo A. Vertical transmission of HCV infection: Prospective study in infants born to HIV-1 seronegative women. *Ig Sanita Pubbl* 2006; 62: 129–42.
92. Steyaert SR, Leroux-Roels GG, Dhont M. Infections in IVF: Review and guidelines. *Hum Reprod Update* 2000; 6: 432–41.
93. Coll O, Fiore S, Floridia M et al. Pregnancy and HIV infection: A European consensus on management. *AIDS* 2002; 16(Suppl 2): S1–18.
94. Martinet V, Manigart Y, Rozenberg S, Becker B, Gerard M, Delvigne A. Ovarian response to stimulation of HIV-positive patients during IVF treatment: A matched, controlled study. *Hum Reprod* 2006; 21: 1212–7.
95. Stora C, Epelboin S, Devouche E et al. Women infected with human immunodeficiency virus type 1 have poorer assisted reproduction outcomes: A case–control study. *Fertil Steril* 2016; 105: 1193–201.
96. Coll O, Suy A, Figueras F et al. Decreased pregnancy rate after *in-vitro* fertilization in HIV-infected women receiving HAART. *AIDS* 2006; 20: 121–3.
97. Chen H, Ge HS, Lv JQ et al. Chronic hepatitis B virus infection in women is not associated with IVF/ICSI outcomes. *Arch Gynecol Obstet* 2014; 289: 213–7.
98. Lam PM, Suen SH, Lao TT, Cheung LP, Leung TY, Haines C. Hepatitis B infection and outcomes of *in vitro* fertilization and embryo transfer treatment. *Fertil Steril* 2010; 93: 480–5.
99. Lorusso F, Palmisano M, Chironna M et al. Impact of chronic viral diseases on semen parameters. *Andrologia* 2010; 42: 121–6.
100. Kajiyama W, Kashiwagi S, Ikematsu H, Hayashi J, Nomura H, Okochi K. Intrafamilial transmission of adult T cell leukemia virus. *J Infect Dis* 1986; 154: 851–7.
101. Malm K, Ekermo B, Hillgren K, Britton S, Fredlund H, Andersson S. Prevalence of human T-lymphotropic virus type 1 and 2 infection in Sweden. *Scand J Infect Dis* 2012; 44: 852–9.
102. Gessain A, Cassar O. Epidemiological aspects and world distribution of HTLV-1 infection. *Front Microbiol* 2012; 3: 388.
103. Centers for Disease Control and Prevention, U.S. Public Health Service Working Group. Recommendations for counseling persons infected with human T-lymphotrophic virus, types I and II. *MMWR Recomm Rep* 1993; 42(RR-9): 1–13.
104. Cao-Lormeau VM, Blake A, Mons S et al. Guillain–Barre syndrome outbreak associated with Zika virus infection in French Polynesia: A case–control study. *Lancet* 2016; 387: 1531–9.
105. D'Ortenzio E, Matheron S, Yazdanpanah Y et al. Evidence of sexual transmission of Zika virus. *N Engl J Med* 2016; 374: 2195–8.
106. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika virus. *N Engl J Med* 2016; 374: 1552–63.
107. Mansuy JM, Dutertre M, Mengelle C et al. Zika virus: High infectious viral load in semen, a new sexually transmitted pathogen? *Lancet Infect Dis* 2016; 16: 405.
108. Turmel JM, Abgueguen P, Hubert B et al. Late sexual transmission of Zika virus related to persistence in the semen. *Lancet* 2016; 387: 2501.
109. Gaskell KM, Houlihan C, Nastouli E, Checkley AM. Persistent Zika virus detection in semen in a traveler returning to the United Kingdom from Brazil, 2016. *Emerg Infect Dis* 2017; 23: 137–9.
110. Oster AM, Russell K, Stryker JE et al. Update: Interim guidance for prevention of sexual transmission of Zika virus—United States, 2016. *MMWR Morb Mortal Wkly Rep* 2016; 6: 323–5.
111. Gilling-Smith C, Emiliani S, Almeida P, Liesnard C, Englert Y. Laboratory safety during assisted reproduction in patients with blood-borne viruses. *Hum Reprod* 2005; 20: 1433–8.
112. Lesourd F, Izopet J, Mervan C et al. Transmissions of hepatitis C virus during the ancillary procedures for assisted conception. *Hum Reprod* 2000; 15: 1083–5.
113. Cobo A, Bellver J, de Los Santos MJ, Remohi J. Viral screening of spent culture media and liquid nitrogen samples of oocytes and embryos from hepatitis B, hepatitis C, and human immunodeficiency virus chronically infected women undergoing *in vitro* fertilization cycles. *Fertil Steril* 2012; 97: 74–8.

114. Benifla JL, Letur-Konirsch H, Collin G et al. Safety of cryopreservation straws for human gametes or embryos: a preliminary study with human immunodeficiency virus-1. *Hum Reprod* 2000; 15: 2186–9.
115. Tedder RS, Zuckerman MA, Goldstone AH et al. Hepatitis B transmission from contaminated cryopreservation tank. *Lancet* 1995; 346: 137–40.
116. Clarke GN. Sperm cryopreservation: Is there a significant risk of cross-contamination? *Hum Reprod* 1999; 14: 2941–3.
117. Tomlinson M, Sakkas D. Is a review of standard procedures for cryopreservation needed?: Safe and effective cryopreservation-should sperm banks and fertility centres move toward storage in nitrogen vapour? *Hum Reprod* 2000; 15: 2460–3.
118. Nicopoullos JD, Almeida P, Vourliotis M, Gilling-Smith C. A decade of the United Kingdom sperm-washing program: Untangling the transatlantic divide. *Fertil Steril* 2010; 94: 2458–61.

Severe ovarian hyperstimulation syndrome

65

ZALMAN LEVINE and INNA BERIN

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS) is the gravest complication of controlled ovarian stimulation (COS) (1). From a perspective of priorities in reproductive medicine in general and assisted reproduction technologies (ARTs) in particular, OHSS is second only to high-order multiple birth on the list of adverse outcomes that need to be minimized or completely eliminated.

OHSS consists of ovarian enlargement accompanied by an overproduction of ovarian hormones and a host of other ovarian vasoactive substances, which alone or in concert may produce the hyperpermeability state responsible for the signs, symptoms, and complications of OHSS (Figure 65.1).

CLASSIFICATION

Like many other diseases, OHSS exists in a clinical spectrum. Some patients, at one end of the spectrum, exhibit only mild signs and symptoms of the disease; others, at the other extreme, require intensive management and may even be at risk of death from the disease (2–6). Diseases that can manifest in a range of severities need classification systems for two reasons. First, if clinicians are to evaluate and treat patients with the disease, parameters must exist that can be applied to each patient to assess the extent of disease and to plan an appropriate management strategy. Just as congestive heart failure is classified based on level of functional ability to help clinicians determine whether the patient can be managed medically or should be placed on a heart transplant list, so must OHSS be classified to help clinicians determine whether the patient should be managed supportively or intensively, medically or surgically, at home or in the hospital.

Second, if clinical researchers are to study disease epidemiology and investigate various strategies for treatment and prevention, a uniform classification scheme will ensure consistency by allowing researchers to speak in a common language about the disease and by enabling clinicians to apply the results of these studies to individual patients. Just as the revised American Fertility Society classification system for endometriosis enables standardized research into the disease and ensures the relevance of clinical trials for clinical practice, so must OHSS be classified to ensure uniform definitions in clinical research and to maximize the application of the research in everyday clinical care.

Classification schemes by disease severity

Over the past 25 years, several classification systems have been suggested in order to better categorize OHSS and disseminate uniform guidelines for prevention and treatment. Most of these classification systems are based on the severity of the disease, based on a combination of the severity of the patient's symptoms as well as the severity of the physical, laboratory, and radiologic signs of the disease. Five major severity-based schemes have been suggested to classify OHSS.

Rabau et al. (1967)

Although pregnant mare serum gonadotropin was used clinically as early as the 1930s to induce ovulation, the results of these early trials were disappointing. Gonadotropin treatments began to enter the clinical armamentarium in 1958, when a combination of follicle-stimulating hormone (FSH) derived from cadaveric human pituitary glands and human chorionic gonadotropin (hCG) successfully induced ovulation and pregnancy (7). With the use of these agents, and with the later introduction of clomiphene citrate (8) and human menopausal gonadotropins (hMGs) (9), experience with the spectrum of OHSS unfortunately grew.

The original classification, suggested by Rabau et al. in 1967 (10), categorizes the syndrome into six grades by levels of increasing severity. Grade 1 disease is defined by the presence of supraphysiologic levels of estradiol (E2) and pregnanediol, as measured by 24-hour urinary excretion greater than 150 μg and 10 mg, respectively. Grade 2 adds to these laboratory measurements the presence of enlarged ovaries and questionably palpable cysts. Interestingly, Rabau et al. did not define grades 1 and 2 as OHSS at all, and felt that these grades were expected by-products of ovarian stimulation and required no attention or treatment.

In the Rabau et al. classification system, grade 3 disease adds the presence of abdominal distention and definitively palpable ovarian cysts, and grade 4 includes vomiting and possibly diarrhea. To Rabau et al., patients with grades 3 and 4 OHSS are at possible risk of future worsening and complications, and require observation but no intervention. Grades 5 and 6, in this system, require hospitalization with aggressive observation and intervention. Grade 5 is defined by fluid shifts and third spacing, leading to ascites and hydrothorax, and grade 6 is defined by hematologic changes in blood volume, blood viscosity, and coagulation time.

Schenker and Weinstein (1978)

Schenker and Weinstein (11) modified the Rabau et al. classification system to group the grades into a less

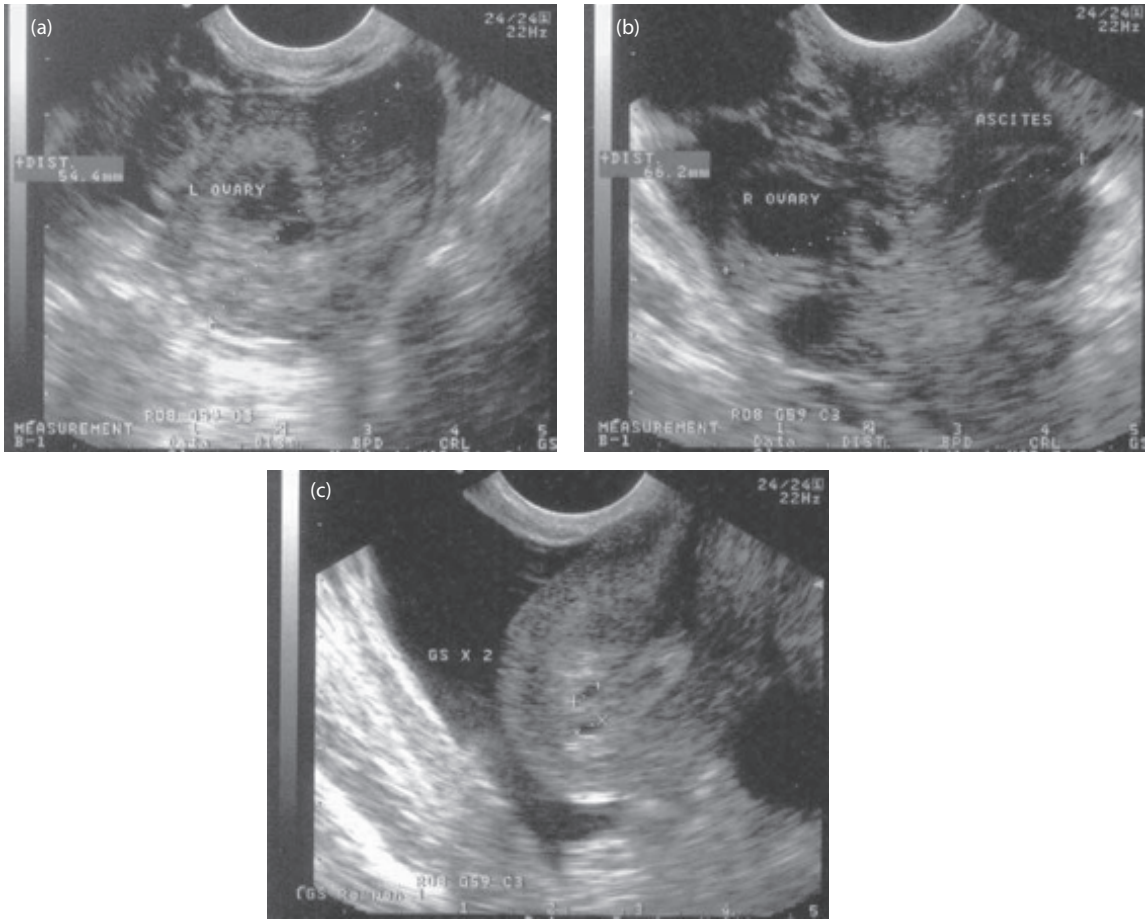

Figure 65.1 Transvaginal ultrasound depicting the ovaries and uterus of a 26-year-old woman who had undergone *in vitro* fertilization for unexplained infertility. Peak estradiol was 2336 pg/mL on the ninth day of stimulation using a gonadotropin-releasing hormone antagonist protocol. Human chorionic gonadotropin (hCG) 10,000 U was used for oocyte maturation. Twenty oocytes were retrieved, two blastocysts were transferred on the fifth day following oocyte retrieval, and six blastocysts were cryopreserved. Two days after embryo transfer, the patient presented with abdominal pain and nausea and was found to have ovarian enlargement, moderate ascites, hemoconcentration, and leukocytosis. She was diagnosed with moderate ovarian hyperstimulation syndrome (OHSS) and treated as an outpatient with isotonic fluids. Serum hCG was positive 10 days after embryo transfer. A clinical twin intrauterine pregnancy was observed ultrasonographically on the 17th day after embryo transfer, as seen in (c). An enlarged left ovary can be seen in (a) and an enlarged right ovary with ascites in (b). The patient continued to be monitored as an outpatient until resolution of her OHSS, and was discharged to obstetric care with an ongoing twin pregnancy at 11 weeks gestational age.

cumbersome mild/moderate/severe terminology, but maintained the grading system as well. In the Schenker and Weinstein scheme, grades 1 and 2 are termed mild OHSS, and include the same definitions—urinary excretion of E2 and pregnanediol for grade 1 and enlarged ovaries with small cysts for grade 2. Grades 3 and 4 are termed moderate OHSS, and included abdominal distention for grade 3 and nausea, vomiting, and/or diarrhea for grade 4. Grades 5 and 6 are defined as severe disease, including large ovarian cysts and ascites and/or hydrothorax for grade 5 and hemoconcentration, increased blood viscosity, and coagulation changes for grade 6.

Golan et al. (1989)

While the Rabau et al. and Schenker and Weinstein classification systems seemed at the time to be comprehensive, they suffer from several drawbacks. First, they focus more on ovarian response, particularly in the lower grades of the disease, than on the clinical syndrome. Second, they are difficult to incorporate into the clinical setting, as 24-hour urinary hormones are not routinely measured. Third, they incorporate unnecessarily cumbersome subdivisions; simple classification as mild, moderate, and severe OHSS would be adequately descriptive and more clinically useful. Finally, these schemes were developed through observation of women undergoing ovulation induction. More recently, with the evolution of ART and routine use of COS in ART patients, the laboratory and clinical findings in the lower grades of the Rabau et al. and Schenker and Weinstein classifications are routinely observed and may reflect not a syndrome, but an acknowledgement that COS has indeed been achieved.

Therefore, there was a need for a simpler, more clinically useful, and more relevant system. Golan et al. (12) in 1989

attempted such a revision of these older systems by classifying OHSS into mild, moderate, and severe, eliminating hormone measurements from the system, and including in the mild category clinical symptoms previously classified as moderate disease prior to the days of routine COS for ART. Golan et al.'s system does maintain a grading scheme, defining grades 1 and 2 as mild OHSS, grade 3 as moderate OHSS, and grades 4 and 5 as severe OHSS. In the mild category, grade 1 includes abdominal discomfort and distention and grade 2 adds enlarged ovaries to 5–12 cm and nausea, vomiting, and/or diarrhea. For moderate OHSS, Golan et al. introduced the use of ultrasound, defining ultrasound evidence of ascites as grade 3, even if the degree of ascites is not detectable clinically. Severe OHSS features respiratory symptoms such as dyspnea and tachypnea and clinical evidence of ascites and/or hydrothorax as grade 4, and hemoconcentration, oliguria, increased blood viscosity, coagulation abnormalities, hypotension, and renal hypoperfusion as grade 5. Perhaps one of the most important innovations of the Golan et al. system is the introduction of ultrasound as a tool in classifying OHSS, encouraging the use of this technology, already in widespread use in the monitoring of ovarian response, for the evaluation and monitoring of OHSS as well. The moderate form of OHSS includes significant ovarian enlargement (5–12 cm) and accompanying symptoms such as abdominal pain, significant bloating, nausea, and diarrhea.

Navot et al. (1992)

The major deficiency of the Golan et al. classification scheme is the absence of a distinction between forms of the disease that are severe but not life threatening and forms that are critical and potentially fatal. If one of the major purposes of the classification scheme is to aid the clinician in appropriately managing the patient with OHSS, such a distinction would be of great clinical importance. Additionally, clinicians routinely assess the severity and course of OHSS with laboratory measurements of hematologic parameters, electrolytes, and renal function, yet the classification system does not address these widely used measurements in a quantitative way.

To correct these deficiencies, Navot et al. (13) subdivided the "severe" category into "severe OHSS" and "critical OHSS." According to the Navot et al. scheme, patients with severe OHSS have variably enlarged ovaries, massive ascites with or without hydrothorax, a hematocrit greater than 45%, a leukocyte count greater than 15,000, clinically measured oliguria, serum creatinine 1.0–1.5 mg/dL, creatinine clearance at least 50 mL/minute, laboratory evidence of hepatic dysfunction, and anasarca. Patients with critical, life-threatening OHSS have a critically contracted blood volume and multiorgan failure. They exhibit more extreme forms of the same parameters, including a hematocrit greater than 55%, a leukocyte count greater than 25,000, serum creatinine at least 1.6 mg/dL, creatinine clearance 50 mL/minute or less, prerenal azotemia, thromboembolic phenomena, and acute respiratory distress syndrome (Table 65.1).

Notably, unlike the earlier schema, the Navot et al. system minimizes the significance of ovarian enlargement. In the past, ovulation induction was the primary cause of OHSS, and ovarian size may have been an important parameter in assessing the disease. However, now that much OHSS results from COS for ART, follicular aspiration and iatrogenic follicular trauma during oocyte retrieval may minimize ovarian size even in the face of severe OHSS. With COS, anasarca can frequently coexist with relatively minor ovarian enlargement. Therefore, the Navot et al. scheme downplays ovarian enlargement, relying more on the general clinical picture and on common laboratory parameters.

Rizk and Aboulghar (1999)

In an attempt to further subdivide Golan et al.'s severe category, Rizk and Aboulghar (14) defined three grades

Table 65.1 Classification of ovarian hyperstimulation syndrome

Mild	Moderate	Severe	Critical
• Bloating • Nausea • Abdominal distention • Ovaries $\leq$5 cm	• Vomiting • Abdominal pain • US evidence of ascites • Hct >41% • WBC count >10,000/mm^3 • Ovaries >5 cm	• Massive ascites • Hydrothorax • Hct >45% • WBC count >15,000/mm^3 • Oliguria • Creatinine 1–1.5 mg/dL • Creatinine clearance $\geq$50 mL/minute • Hepatic dysfunction • Anasarca • Ovaries variably enlarged	• Tense ascites • Hypoxemia • Pericardial effusion • Hct >55% • WBC count >25,000/mm^3 • Oliguria or anuria • Creatinine >1.5 mg/dL • Creatinine clearance <50 mL/minute • Renal failure • Thromboembolic phenomena • ARDS • Ovaries variably enlarged

Abbreviations: Hct, hematocrit; WBC, white blood cell; US, ultrasound; ARDS, acute respiratory distress syndrome.

of severe OHSS. Grade A severe OHSS features dyspnea, oliguria, nausea, vomiting, diarrhea, abdominal pain, clinical or ultrasound evidence of ascites, hydrothorax, and enlarged ovaries on ultrasound. Patients with grade A disease have a normal biochemical profile. Grade B severe OHSS adds massive ascites, markedly enlarged ovaries, severe dyspnea, severe oliguria, increased hematocrit, hepatic dysfunction, and elevated serum creatinine. Grade C OHSS, which features complications that Rizk and Aboulghar feel can also occur in the setting of moderate disease, resembles Navot et al.'s "critical" category, with end-organ complications such as renal failure, venous thrombosis, and acute respiratory distress syndrome.

CLASSIFICATION BY DISEASE ONSET: EARLY AND LATE OHSS

Since 1994, investigators (15–17) have recognized that what is commonly called OHSS actually includes two distinct disease entities: OHSS that occurs three to seven days after hCG triggering and OHSS that occurs more than 10 days after hCG triggering. As a disease, OHSS seems to include these two distinct forms based on the timing of its onset, and can consequently be classified into early OHSS and late OHSS. Both forms of OHSS share a common pathophysiology; in both, hCG triggers granulosa cells to produce vasoactive substances that induce the capillary permeability that yields the clinical sequelae of OHSS. What distinguishes the early and late forms of the disease is the source of the hCG. In early OHSS, the exogenously injected hCG drives the granulosa directly to secrete sufficient vasoactive substances to produce the syndrome within three to seven days, while in late OHSS, early pregnancy is responsible for the granulosa cell activity, as the implanting trophoblast produces increasing levels of endogenous hCG.

Clinically, these two entities ought to be distinguished, because they are distinct. Early OHSS, but not late OHSS, is dependent on ovarian stimulation; higher peak E2 levels and greater gonadotropin doses are correlated with an increased incidence of early OHSS, but not of late OHSS. Therefore, criteria related to ovarian response can be used to predict early OHSS, but not late OHSS. Early OHSS can occur in any stimulated cycle, while late OHSS only occurs in the setting of a pregnancy. Late OHSS is more likely to be severe; in fact, late OHSS may account for almost 70% of all cases of severe OHSS. Late OHSS can occur with either a singleton or multiple pregnancy. While some authors have suggested that multiple pregnancy has a stronger association with late OHSS than singleton pregnancy by virtue of higher trophoblastic hCG production, De Neubourg et al. found an equal association of singleton and multiple pregnancies with late OHSS (18).

ETIOLOGY

While the exact etiological factor responsible for the pathogenesis of OHSS is unknown, the syndrome is known to be dependent on hCG. OHSS does not occur if hCG is withheld, and ongoing hCG stimulation by early pregnancy is a significant risk factor for persistent and severe OHSS. This hCG dependence underlies some of the major preventive strategies for the syndrome. More recently, numerous vasoactive substances have been implicated in the pathophysiology of the disease, including prorenin, renin, prostaglandins, angiotensin II, vascular endothelial growth factor (VEGF), tumor necrosis factor-α, insulin-like growth factor-1, epidermal growth factor, basic fibroblast growth factor, platelet-derived growth factor, transforming growth factors-α and -β, and interleukins-1β, -2, and -6 (19–30). Many of these substances are proangiogenic, and are probably responsible for the physiologic neovascularization that occurs during folliculogenesis and leutinization within the ovary. VEGF seems to play a particularly critical role in the pathophysiology of OHSS. Evidence for this critical role includes the facts that VEGF is secreted by granulosa cells, VEGF levels correlate with OHSS severity, recombinant VEGF has been shown to induce OHSS, and specific VEGF antiserum has been shown to reverse the effects of VEGF-induced OHSS. Furthermore, hCG has been shown to increase VEGF secretion by granulosa cells, and to increase serum levels of VEGF (31–33). Indeed, many of the angiogenic factors implicated in the pathophysiology of OHSS probably act directly or indirectly through VEGF. Since angiogenic factors are so strongly associated with OHSS, ongoing research will likely identify antiangiogenic strategies for prevention and treatment.

OHSS has been reported in several women with spontaneous pregnancies (34–36), and the cause of this OHSS seems to be a familial mutation in the FSH receptor, increasing its sensitivity to trophoblastic hCG. The mutation allows for constitutive stimulation of the FSH receptor by hCG, triggering the ovarian cascade responsible for OHSS. This form of OHSS clearly illustrates the distinction noted above between early and late OHSS; these women did not have stimulated ovaries and did not have hCG triggering, yet developed OHSS due to endogenous production of hCG by the developing pregnancy. This spontaneous late OHSS in at least one report was severe, requiring hospitalization and intensive management. Whether FSH receptor mutations or polymorphisms will play a role in the onset or severity of iatrogenic OHSS will require further research.

Serum E2 also seems to play a role in the pathogenesis of OHSS, but the nature of this role is not clear because a high serum E2 level, while a known risk factor for OHSS, seems to be neither necessary nor sufficient for the development of the disease. On the one hand, the fact that most patients with OHSS have high E2 levels (37) may suggest that high E2 is necessary in the development of OHSS; but on the other hand, OHSS can occur in hypoestrogenic patients such as those with hypogonadotropic hypogonadism (38) Similarly, the fact that a high serum E2 level is a definitive risk factor for OHSS may suggest that it is sufficient for the development of OHSS; but on the other hand, OHSS does not occur when hCG is withheld, despite high E2 levels (39). E2 itself does not have direct vasoactivity

(40) and does not seem to cause the vascular dysfunction seen in OHSS (41). Recently, expression of the cystic fibrosis transmembrane conductance regulator (CFTR) has been studied in OHSS because of CFTR's known effects in increasing channel activity in the peritoneal cavity, which can cause fluid shifts and ascites. E2 is a known up-regulator of CFTR expression, which may provide a potential causative link between hyperestrogenemia and OHSS (42).

PREVENTION OF SEVERE OHSS

The role of the stimulatory agent and protocol

OHSS has intrigued clinicians for many years because of its devastating consequences in otherwise healthy young women. As an iatrogenic condition resulting from elective ovarian stimulation in the quest for pregnancy, the need to completely prevent the syndrome is evident. In order to promulgate safe COS, it is essential to first define the "at-risk population." Table 65.2 delineates the risk factors for severe OHSS that should alert the clinician contemplating COS. Because oocyte retrieval for *in vitro* fertilization (IVF) decreases the risk of OHSS, presumably due to the follicular trauma inherent in the procedure, the criteria defining high versus low risk may differ depending on whether the COS is for the purpose of IVF or for conventional ovulation induction or superovulation. The single most important risk factor for OHSS is a polycystic appearance of the ovaries on transvaginal ultrasound, having a high antral follicle count and a "necklace sign" or "string of pearls" appearance (Figure 65.2). In contrast, relatively quiescent ovaries with few antral follicles usually predict a low COS response with little risk of OHSS.

Recently, serum concentrations of anti-Mullerian hormone (AMH) have been investigated as a risk factor for OHSS. AMH is secreted by ovarian granulosa cells in preantral and small antral follicles and can be used to estimate ovarian reserve and predict ovarian response to gonadotropin stimulation (43). In one study, all cycle cancelations due to OHSS occurred in patients who were in the highest AMH quartile of greater than 7 ng/mL (44). Another cohort study found baseline serum AMH levels prior to stimulation to be highly correlated with OHSS, with an AMH concentration of greater than or equal to 3.36 ng/mL yielding a sensitivity of 90.5% and a specificity of 81.3% for the subsequent development of OHSS (45).

Early reports suggested a relationship between the type of gonadotropin preparation utilized and the risk of OHSS. More recent comparisons between recombinant FSH (rFSH) and human menopausal gonadotropins (hMGs) did not show significant differences among variable drug regimens. A large study by Bergh et al. (46) compared 119 cycles of rFSH (Gonal-F) to 114 cycles of urinary follicle stimulating hormone highly purified (uFSH-HP) (Metrodin HP). Both groups were down-regulated by a long gonadotropin releasing hormone agonist (GnRHa) protocol. All parameters studied, including E2 serum concentrations, ampules utilized, days of stimulation, number of oocytes retrieved, and number of embryos obtained, were significantly in favor of rFSH, and although clinical pregnancy rates and implantation rates were similar, significantly more embryos were frozen subsequent to rFSH stimulation. The respective rates of OHSS for rFSH and uFSH-HP were 5.2% and 1.7%. Another large study compared 585 patients receiving rFSH with 396 patients receiving uFSH (47). This report demonstrated similar advantages of rFSH regarding length of treatment and ampules utilized, but also showed a significantly higher ongoing pregnancy rate for rFSH when frozen–thawed embryos were added to the equation. The rate of OHSS in this study was 3.2% versus 2.0% for rFSH and uFSH, respectively, and the difference was not statistically significant.

The capacity of rFSH to enhance both follicular recruitment and serum E2 concentrations may indeed carry a slightly increased risk of OHSS. However, the seemingly increased risk in these studies may also be due to early inexperience with rFSH. With increased awareness and understanding of the unique features of rFSH, the actual rate of OHSS with rFSH use has since decreased, as has been borne out in subsequent published studies (48,49).

Table 65.2 Risk factors associated with ovarian hyperstimulation syndrome

High risk	Low risk
Young (<35 years of age)	Older (>35 years of age)
Polycystic-appearing ovaries	Hypogonadotropic
Asthenic habitus	Heavy build
High serum estradiol (ART >4000 pg/mL, OI >1700 pg/mL)	Low serum estradiol
Multiple stimulated follicles (ART >20, OI >6)	Poor response to gonadotropins
Necklace sign	Few antral follicles
Pregnancy	Elevated baseline FSH
hCG luteal supplementation	Progesterone or no luteal supplementation
GnRH agonist down-regulatory protocol	Clomiphene citrate and/or hMG protocol
High serum anti-Mullerian hormone	

Abbreviations: ART, assisted reproduction technology; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin; OI, ovulation induction.

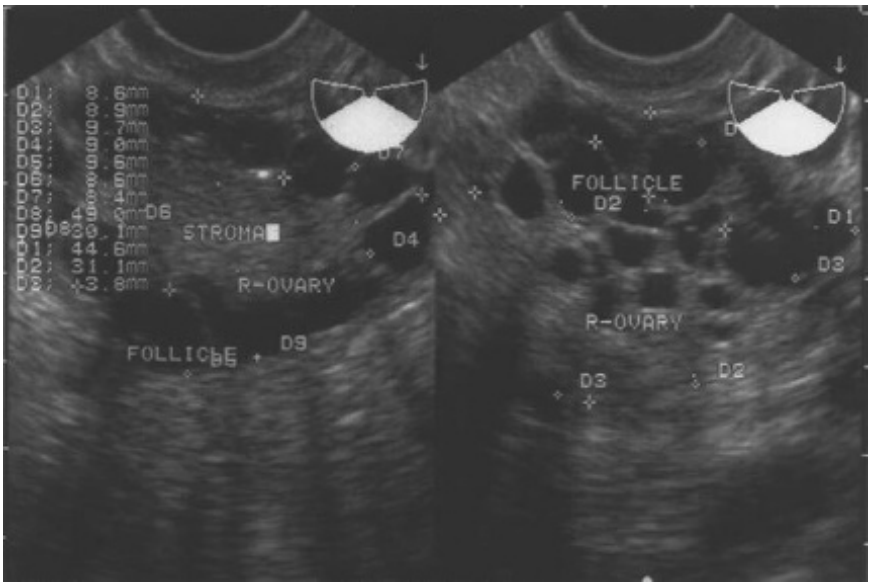

Figure 65.2 Transvaginal ultrasound depicting the right ovary of a 31-year-old woman with amenorrhea and polycystic ovary syndrome. The picture features major high-risk factors for the development of severe ovarian hyperstimulation syndrome: (1) "String of pearls" appearance of antral follicles on the left panel; (2) Dense stroma occupying the center of the ovary; (3) Enlarged ovary measuring 49 × 44.6 mm; (4) Total of 60 antral follicles in one ovary.

Indeed, numerous studies have shown that the method of stimulation (chronic low dose, step up, or step down) carries far more weight as a risk factor than the type of injectable gonadotropin used (50,51). Specifically, the so-called chronic low-dose regimen is more likely to result in a mono- or bi-follicular response and therefore a significantly lower rate of OHSS. Similarly, unlike a step-up protocol, which continuously rescues follicles from atresia, a step-down protocol will allow more follicles to undergo atresia, thus reducing the overall number of follicles capable of secretory activity by the time hCG is administered. A reduction in the rate of OHSS will naturally follow.

An extension of the step-down concept is "coasting" or "controlled drift," championed by Sher and colleagues (52,53), and later practiced widely by several other researchers with variable results. Coasting may work to prevent or reduce the severity of OHSS by altering the capacity of the granulosa cells to produce VEGF (54), and seems to confer this benefit without compromising cycle outcomes. Benadiva et al. and Tortoriello et al., for example, reported a significant reduction in OHSS with coasting (55–58), and a 2003 review of 10 studies showed that <2% of women developed OHSS while maintaining acceptable pregnancy rates (36.5%–63%) when coasting was continued until serum E2 levels fell below 3000 pg/mL (59). A retrospective analysis by Kovács et al., in which gonadotropins were withheld for an average of 2.2 days, showed that pregnancy rates in the coasting group were comparable to those in non-coasted control cycles (60). Two other recent retrospective studies similarly demonstrated the absence of any adverse effect of coasting on cycle outcome, including implantation rate, pregnancy rate, and live birth rate (61,62). In contrast, however, other studies have found either no benefit in coasting (63,64) or have shown diminished oocyte collection rates (65) and implantation and pregnancy rates (66) when coasting is prolonged, particularly for greater than three days (67). Most of these discrepancies in the benefit and outcome of coasting likely stem from interstudy differences in coasting protocols. Additional research is required to evaluate the efficacy of coasting and to determine the optimal protocol for delaying hCG administration in high-risk patients (68).

The use of GnRHa in conjunction with COS, either as a "long" or "short" protocol, profoundly affects the risk of OHSS. GnRHas play a paradoxical role in OHSS by virtue of the control they afford, despite their overall suppressive effect on ovarian stimulation. Both the long and the short GnRHa protocols uniformly abolish the mid-cycle luteinizing hormone (LH) surge. This suppression of the LH surge allows continued stimulation by gonadotropins, which in turn will drive more follicles to either full or quasi-maturation with a consequent rise in serum E2 values and a markedly increased risk of OHSS (13). In contrast, during cycles without GnRHa suppression, either a significant LH surge or at least marked luteinization will limit continued gonadotropin stimulation and thus lead to a concomitantly lesser risk of OHSS. On the other hand, because of the suppressive effect of GnRHa on ovarian function, some have advocated continuing GnRHa administration for one week following hCG administration in GnRHa down-regulation cycles where all embryos are electively cryopreserved because of a high risk of OHSS (69). This strategy can be extended easily to oocyte donation cycles.

The role of hCG and its substitutes

Once the prerequisite for severe hyperstimulation is established, namely a multifollicular, high-estrogenic milieu, the occurrence of OHSS is utterly hCG dependent; either exogenously administered or pregnancy-derived hCG is absolutely essential for the development of OHSS. In contrast, avoidance of hCG or substitution by a low-affinity, shorter-acting compound are the mainstays for the prevention of OHSS. Indeed, the acknowledgement of the role of hCG in OHSS has led to all but complete discontinuation of the foul habit of hCG administration for luteal supplementation. Cessation of this practice eliminated a major risk factor for OHSS in assisted reproduction.

However, hCG as a surrogate for the mid-cycle LH surge is still universally used in COS for both ovulation induction and IVF. The standard dosage of hCG used to trigger ovulation is 5000–10,000 IU, or 250 μg of recombinant hCG (rhCG). hCG in these dosages takes six to nine days to clear from the circulation, thus exerting continuous ovarian stimulation up to the stage in which endogenous pregnancy-derived hCG is perceived. Since hCG has a very long half-life and high affinity for the ovarian LH receptor, it sustains the function of multiple corpora lutea to the point of rescue by endogenous hCG. This ovarian action of hCG exerts a stimulatory effect on the putative ovarian substances that directly promote, or may even be the causal factors in, ovarian hyperstimulation. Indeed, angiotensin II, VEGF, tumor necrosis factor-α, and interleukins-1β, -2, or -6 are all either directly or indirectly enhanced by hCG (70,71). Because of these OHSS-promoting actions of hCG, one simple preventive strategy is to administer lower-dose hCG. As expected, triggering ovulation or oocyte maturation with lower-dose hCG on a sliding scale, with the administration of between 3300 and 5000 U depending on serum E2 concentration on the day of triggering, has been shown to decrease the risk of OHSS (72,73).

Recombinant LH and OHSS

The critical role of hCG in OHSS has prompted many researchers to look for an alternate substance to trigger ovulation while reducing the prolonged and often excessive stimulation of hCG. Although exogenous native LH would constitute a physiological replacement, it has several theoretical disadvantages, including its very short half-life of about 20 minutes. Because of this short half-life, either huge doses or repeated administration would be needed to create a sustained surge of at least 24 hours. Recombinant LH (rLH) is now commercially available. A dose-finding study, in which rLH was utilized to trigger ovulation, showed the engineered product to be highly sialylated, greatly extending its half-life *in vivo* (74). Because of this, the use of rLH as a mid-cycle substitute to the natural LH surge is now feasible in all kinds of COS, whether using gonadotropins alone, clomiphene citrate in conjunction with gonadotropins, down-regulation with GnRHa, or LH suppression with GnRH antagonists (GnRH-ant).

While this prolonged half-life, on the one hand, allows rLH to be clinically useful, it might also theoretically render the rLH similar to hCG, and therefore may not substantially reduce the incidence of OHSS. One preliminary comparison of rLH and rhCG in IVF demonstrated a lower incidence of moderate to severe OHSS in women receiving a single dose of rLH (75). Loumaye et al. used doses of rLH between 5000 IU and 30,000 IU to compare with 5000 IU of hCG (74). All doses of rLH successfully induced final follicular maturation and yielded similar numbers of oocytes and equal fertilization rates, but the incidence of ovarian enlargement and some ascites seemed to be directly dose dependent. Whereas no ovarian enlargement or ascites were seen up to 10,000 IU of rLH, 1 of 26 women (3.8%) who took 30,000 IU rLH, and 13 of 121 women (10.7%) who took hCG, had ovarian enlargement, ascites, or accompanying symptomatology. One patient in the hCG group had severe OHSS. The authors concluded that rLH may be safer than hCG as far as OHSS is concerned. This dose-finding study suggests that the rLH used probably has a relatively long half-life compared with native LH. Alternately, it is possible that a single peak of rLH is sufficient to induce final oocyte maturation as opposed to the 24-hour naturally occurring LH surge.

GnRHa trigger and OHSS

Alternatively, final follicular maturation and ovulation may be triggered using a GnRHa to stimulate an endogenous LH surge in patients at risk for OHSS. Early attempts to elicit an LH surge with synthetic GnRH in an hMG-stimulated cycle yielded variable results (76,77). Subsequently, however, attempts to trigger ovulation with GnRH analogs have been more consistent in their results. Lanzone et al. (78) and Imoedemhe et al. (79) reported the successful use of GnRHa for induction of an endogenous LH/FSH surge for final follicular maturation following exogenous gonadotropin stimulation of the ovaries, and numerous other studies have specifically reported the use of GnRHa to successfully induce follicular maturation in IVF cycles (80,81) and ovulation in non-IVF cycles (82,83).

Several authors have addressed the efficacy of GnRHa in preventing OHSS. Most reports support the hypothesis that GnRHa induces adequate ovulation while avoiding OHSS. Emperaire and Ruffie studied 37 of 126 cycles in 48 patients undergoing ovulation induction with a regimen of either hMG or hMG with clomiphene citrate (84). All cycles were considered to be at high risk for OHSS and/or multiple pregnancy (E2 level >1000–1200 pg/mL and more than three follicles of >17 mm mean diameter). In these at-risk cycles, an endogenous LH surge was provoked by intranasal buserelin 200 mg three times at eight-hourly intervals. Ovulation was documented in all cycles except one (97%). Eight pregnancies resulted (21.6%), and there were no cases of OHSS. Imoedemhe et al. (79) used two doses of GnRHa (Suprefact 100 mg) by nasal spray eight hours apart to induce follicular maturation 34–36 hours prior to oocyte recovery in 38 women considered at risk of OHSS (E2 >4000 pg/mL) in an IVF program. Of the 707 oocytes recovered at egg retrieval,

93% were scored as mature, and 46% were successfully fertilized. Twenty-six women had embryos replaced; 11 pregnancies occurred (28.9%) and there were no cases of OHSS. Itskovitz et al. used buserelin acetate in dosages of either 250 or 500 mg injected subcutaneously in either a single or two divided doses 12 hours apart (81). Approximately 78% of all eggs recovered were considered mature. Three of 13 patients conceived (21.4%) and none developed any signs or symptoms of OHSS. Shalev et al. (83) and Balasch et al. (85) used a single subcutaneous injection of triptorelin or leuprolide (0.5 mg), respectively, to trigger ovulation in gonadotropin-stimulated cycles that would otherwise have been cancelled due to a high risk of OHSS. Overall, 50% and 17.4% conception rates were achieved, respectively, while no patient developed OHSS. Kulikowski and colleagues used a single dose of 0.3 mg GnRHa subcutaneously in 32 patients undergoing ovulation induction for IVF and in 16 patients undergoing ovulation induction for ovulatory disturbances, all of whom they felt were at risk of OHSS (E2 >2500 pg/mL) (86). All patients had ovulation induced with a clomiphene/hMG protocol. There were no cases of OHSS in the GnRHa group and four pregnancies occurred (12.5%). In the control IVF group, there were four cases of OHSS and three pregnancies occurred (8.8%). In the 16 patients who had undergone ovulation induction with clomiphene/hMG/GnRHa, no OHSS was detected, while four patients became pregnant (25%). Engmann et al. randomized 66 IVF patients at risk of OHSS to a GnRH-ant protocol with GnRHa trigger, or a GnRHa down-regulatory protocol with hCG trigger (87). All patients received luteal support using intramuscular progesterone, and the GnRHa trigger group received luteal estrogen supplementation as well. A total of 31% of patients in the hCG trigger group developed some form of OHSS, compared with none in the GnRHa trigger group. Moreover, there were no significant differences in implantation, clinical pregnancy, and ongoing pregnancy rates. The study concluded that the use of a GnRHa trigger reduces, if not eliminates, the risk of OHSS in high-risk patients, without compromising pregnancy outcomes. O'Neill et al. compared endocrine profiles and reproductive outcomes with use of GnRHa trigger in women with polycystic ovary syndrome (PCOS) and use of GnRHa trigger in other hyper-responders, and found no differences in the number of oocytes retrieved, percentage mature oocytes, fertilization rates, or implantation rates, and no patients in either group developed OHSS (88). In summary, a number of investigators have used mid-cycle GnRHa, in varying dosages and time intervals, for cycles considered to be at high risk for development of OHSS. Pregnancy rates of 12.5%–50% were achieved, with a 0% incidence of OHSS. Because some studies have suggested a degradation in pregnancy outcomes with the use of GnRHa triggers, Griesinger et al. recently proposed the use of a GnRHa trigger to prevent OHSS, together with pronuclear cryopreservation and frozen–thawed embryo transfer at a later date. In their prospective observational study, cumulative ongoing pregnancy rates were high and no cases of moderate or severe OHSS were observed (89).

In contrast to the above reports showing an absence of development of OHSS in high-risk patients given GnRHa for follicular maturation and ovulation, Van der Meer et al. published a study in which three patients who used buserelin to induce a pre-ovulatory endogenous LH surge in lieu of hCG nevertheless developed moderate OHSS (90). Severe ascites, hypovolemia, or electrolyte imbalance did not occur, and no patients were hospitalized. These authors concluded that OHSS is due to a massive luteinization of the follicles after exaggerated follicular stimulation, and can occur independently of the ovulation-triggering agent. Gerris et al. also reported the occurrence of moderate OHSS in one patient following GnRHa administration (82), but in this case native GnRH was used, resulting in successful ovulation triggering but without the critical ovarian suppression that is thought to be at least equally important in the prevention of OHSS (91). Casper surveyed a total of 163 cycles in which GnRHa was used to trigger ovulation in the context of preventing OHSS (92). He stipulated that 900 cycles should have been randomized in order to detect a significant difference between GnRHa and hCG. However, his analysis relies on an assumed 2% risk for severe OHSS, and while a 2% risk may be applicable to the average woman undergoing COS, most women in his survey likely had far greater risk, possibly in the 10%–20% range. The preponderance of evidence to date supports a decreased incidence of OHSS with GnRHa compared with hCG as the triggering agent for ovulation, although a small possibility of moderate OHSS remains, particularly in conception cycles. Most importantly, there have been no reports of severe or critical OHSS after triggering ovulation with mid-cycle GnRHa.

Clinicians using GnRHa to trigger ovulation must realize that the ensuing luteal phase is dramatically deficient, and full luteal progesterone support must be employed. In oocyte donation cycles, in cycles using a gestational carrier, and in cycles in which all embryos or oocytes are expected to be cryopreserved, such as for purposes of preimplantation genetic testing of embryos, luteal sufficiency is of no concern. In such cases, GnRHa triggering is widely accepted, with all studies to date showing a nearly absolute elimination of any risk of OHSS (93).

Clinicians must also be aware that because of pituitary desensitization, GnRHa cannot be used as an ovulation trigger for cycles in which GnRHa was previously used for down-regulation. If a patient at high risk of OHSS is identified and GnRHa triggering is contemplated, a GnRH-ant protocol, rather than a long GnRHa protocol, should be used for suppression of the endogenous mid-cycle LH surge.

GnRH-ants and OHSS

GnRH-ants seem to be associated with a decreased risk of OHSS compared with the GnRHa long protocol in patients undergoing IVF. Although one meta-analysis found no differences in the incidence of OHSS with the use of GnRH-ant compared with long GnRHa down-regulatory protocols (94), another more recent meta-analysis of 45 randomized controlled trials revealed a significant reduction in the

incidence of OHSS with the use of GnRH-ant compared with GnRHa down-regulation protocols, with an odds ratio of 0.43 (95). Furthermore, fewer interventions to prevent OHSS were administered in GnRH-ant protocols versus GnRHa protocols (odds ratio 0.44). Another meta-analysis confirmed a decreased risk of OHSS with the use of GnRH-ant, but particularly for cetrorelix and less so for ganirelix (96). A prospective multicenter study demonstrated a reduction in the incidence of OHSS and a decreased cycle cancellation rate due to risk of OHSS in high-risk IVF patients treated with a GnRH-ant protocol as compared with historical controls down-regulated with GnRHa (97). Interestingly, since the degree of ovarian suppression with GnRH-ant may be more profound at high doses, the dose of GnRH-ant may therefore be adjusted to minimize the development of OHSS in high-risk patients. de Jong et al. employed this strategy when they used the GnRH-ant ganirelix to prevent OHSS by increasing the dose of the antagonist when target E2 values were inadvertently exceeded (98). Indeed, E2 values rapidly decreased with a concomitant decrease in ovarian size. Although this group's suggestion is novel, far more intriguing is the potential use of GnRH-ant in conjunction with either rLH or GnRHa to trigger ovulation. Because of the competitive nature of GnRH-ant suppression and lack of desensitization, it is possible to trigger ovulation with GnRHa during co-treatment with gonadotropins and GnRH-ant. The respective dosages of each agent still await further studies, although 0.25 mg of ganirelix or cetrorelix daily seems to be sufficient to eliminate the LH surge and seems to result in favorable clinical outcomes. Theoretically, a larger dose of GnRHa would be needed to induce an LH surge in cycles suppressed by a GnRH-ant than in cycles using gonadotropins alone without a GnRH-ant. It cannot be stressed enough that full progesterone support is mandatory throughout the luteal phase when GnRHa is used to trigger ovulation.

Embryology strategies

Liberal application of embryo cryopreservation for patients showing early signs of hyperstimulation can be an important safety net in guarding against severe OHSS (99), although the efficacy of cryopreservation as a preventive measure for OHSS has been questioned (100). With routine culture of embryos to the blastocyst stage, it is possible to accurately assess the degree of OHSS prior to embryo transfer; because blastocyst transfer takes place on the seventh day after hCG, absence of even a moderate degree of OHSS is reassuring, and one may safely proceed with embryo transfer (13). The higher implantation rates associated with blastocyst transfer have led some clinicians to employ single-blastocyst transfer in patients at risk of developing severe OHSS (101,102). Such a strategy results in a negligible multiple gestation rate, which purportedly is associated with more severe OHSS presumably secondary to higher hCG levels. Although theoretically plausible, the utility of such an approach remains to be confirmed.

Improvements in *in vitro* maturation of immature oocytes might also enable women, particularly those with PCOS who are at greatest risk of OHSS, to undergo assisted reproduction using minimal if any gonadotropin stimulation. This may dramatically reduce or eliminate the risk of OHSS (103,104).

Prophylactic use of colloid agents to prevent OHSS

Third spacing and intravascular volume depletion due to increased capillary leakage are hallmarks of OHSS. Several investigators have administered intravenous colloidal agents such as albumin and hydroxyethyl starch at the time of oocyte retrieval as prophylactic intravascular volume and oncotic pressure enhancers to minimize the risk of developing OHSS (105–107). In contrast to the significant value of albumin for treatment of the fully developed syndrome, colloids are of questionable benefit as preventive measures. A meta-analysis of five randomized clinical trials has validated the use of intravenous albumin administration at the time of oocyte retrieval in high-risk patients (108). In this meta-analysis, albumin infusion to 18 at-risk women was necessary to prevent one case of severe OHSS. Because albumin treatment leads to risks such as allergic reactions and transmission of viruses and prions, the relative merits of albumin infusion compared with other preventive strategies remain unclear.

Miscellaneous techniques to prevent OHSS

Other modalities that have been suggested for the prevention of OHSS include unilateral or bilateral follicular aspiration as a rescue for cycles not otherwise intended to undergo oocyte retrieval (109). Egbase advocated ovarian diathermy prior to initiation of COS (110). Ovarian diathermy should, however, be reserved to young patients with severe PCOS who tend to hyperstimulate even on a prolonged low-dose FSH regimen.

Metformin, the second-generation biguanide insulin sensitizer, has been advocated for the treatment of women with severe PCOS and insulin resistance. Although a more favorable response to ovulation enhancement would be expected, it is unclear yet whether a reduction in the incidence of OHSS will follow. One small study comparing combined treatment using clomiphene and metformin with clomiphene alone showed a lower incidence of OHSS with the addition of metformin, although this difference did not achieve statistical significance (111). A randomized prospective trial showed that adding metformin to gonadotropin regimens for ovulation induction yields fewer large follicles, lower E2 levels on the day of hCG administration, and a reduced rate of cycle cancellation for over-response (112). These effects would probably result in a reduced risk of OHSS as well. Indeed, a study of 287 women undergoing IVF demonstrated a significant reduction in the incidence of OHSS in those taking metformin (113). Clearly, though, more data are needed to fully elucidate the effects of metformin on OHSS in women with PCOS.

Suppression of ovarian steroidal secretion, either through continued administration of GnRHa following oocyte retrieval coupled with cryopreservation or through administration of intramuscular hydroxyprogesterone

caproate and E2 valerate following embryo transfer, has been suggested to minimize the risk of developing OHSS. Both approaches currently remain experimental (69,114). Aromatase inhibitors administered in the luteal phase can be used to reduce luteal E2 concentrations, and thus may be a promising approach in preventing OHSS or minimizing its attendant hyperestrogenemia-related risks in patients such as oocyte donors whose luteal sufficiency is of no concern (115).

The anti-inflammatory action of corticosteroids has also been hypothesized to be beneficial in preventing OHSS. However, because of conflicting reports in the literature, there are currently insufficient data to recommend such an approach (116,117). Nevertheless, there may be a preventive role for corticosteroids used in conjunction with aspirin: one randomized trial demonstrated a decreased incidence of OHSS among women undergoing IVF who received a combination of aspirin and prednisolone from the beginning of COS through to the day of the pregnancy test (118), and another trial demonstrated a benefit to aspirin alone in the prevention of OHSS (119). Recently published guidelines by the Practice Committee of the American Society for Reproductive Medicine considered the evidence for the use of aspirin in the prevention of OHSS to be fair (120).

Calcium infusion has also been hypothesized to prevent OHSS because of its inhibition of cyclic adenosine monophosphate (cAMP) synthesis and cAMP-dependent renin secretion from juxtaglomerular cells in the kidneys (121,122). Reduced renin secretion results in decreased angiotensin II production, with a consequent decrease in angiotensin II-mediated stimulation of VEGF synthesis. In two studies to date, IVF patients at risk for OHSS were infused intravenously with calcium gluconate 10%, 10 mL in 200 mL of normal saline, on the day of oocyte retrieval and for three days thereafter. Patients receiving the calcium infusion had a decreased incidence of both mild and severe OHSS (123,124). This prevention modality carries little risk and potential benefit, but because of insufficient data cannot yet be routinely recommended.

Recent novel research has focused on preventive strategies aimed particularly at VEGF as the critical ovarian mediator of the syndrome. Encouragingly, treatment with a VEGF receptor antagonist prevented the increase in capillary permeability seen in an OHSS rat model (125). Likewise, treatment with *fms*-like tyrosine kinase, a soluble agent that binds VEGF with high affinity and thus decreases its availability for its endothelial effects, demonstrated the same effect in a similar OHSS rat model (126). Dopamine agonists also decrease vascular permeability by preventing the phosphorylation of VEGF receptor 2 and thus reducing the release of vasoactive angiogenic agents (127,128). Cabergoline, a dopamine D2 receptor agonist, inactivates VEGF receptor 2 in animal models (129). A prospective, randomized, double-blind study investigated the effects of daily cabergoline treatment (0.5 mg/day) in oocyte donors at risk of OHSS (130). The study showed more than a 50% reduction in the incidence of moderate OHSS with the use of cabergoline from the day of hCG administration through to six days post-oocyte retrieval. Several other studies and meta-analyses demonstrated similar findings for cabergoline, as well as for the other dopamine agonists quinagolide and bromocriptine (131–137). Such novel options make pathophysiologic sense and have the potential to play a future role in OHSS prevention.

A promising approach may be the use of a kisspeptin as a novel trigger of oocyte maturation for women at high risk of OHSS undergoing IVF. Kisspeptins are a group of hypothalamic peptides coded by the *KISS1* gene; the gene was so named because of its discovery in 1996 in Hershey, PA, home of the famous chocolate delight known worldwide as the "Hershey Kiss." Originally identified as a tumor suppressor gene in its suppression of metastasis in malignant human melanoma (138), *KISS1* has more recently been shown to play a key role in reproductive pathways. Kisspeptins, the family of peptides encoded by *KISS1*, are expressed in the hypothalamus and are potent stimulators of the hypothalamic–pituitary–gonadal axis, directly modulating GnRH secretion and pulsatility by signaling hypothalamic neurons through a G-protein-coupled kisspeptin receptor to stimulate hypothalamic GnRH release and in turn pituitary FSH and LH secretion (139). A 54-amino acid kisspeptin peptide known as kisspeptin-54 has recently been studied in women at high risk of OHSS undergoing IVF; it was found to be an effective and safe trigger with high yields of oocyte maturation and a high implantation rate, and none of the 60 women in the study developed OHSS (140). While further study is required, including trials comparing kisspeptin-54 with other oocyte maturation triggers, before recommendations for clinical use can be developed, this approach holds significant promise as an OHSS prevention strategy.

Obviously, there are many strategic options for the prevention of OHSS in high-risk patients. The current increasing use of GnRH-ant in clinical practice holds great promise for preventing severe OHSS. As we master the complexity of GnRH-ant for LH surge suppression together with GnRHa for triggering ovulation, ovarian stimulation will likely become better controlled, and severe OHSS will become a rare if not forgotten entity.

TREATMENT OF SEVERE OHSS

Medical approach

There are two possible approaches to the treatment of OHSS: one pathogenesis oriented and one empiric. The former approach utilizes agents that specifically negate the putative causative factor(s) of OHSS. Indomethacin was hypothesized to be such an agent when prostaglandins were believed to play a role in OHSS. Angiotensin-converting enzyme (ACE) inhibitors are another group of specific pharmacological agents that were thought to have potential use in the treatment of OHSS because they inhibit the production of angiotensin II, a probable pathogenic factor for the syndrome. Unfortunately, indomethacin did not benefit the syndrome, and ACE inhibitors are teratogenic

and thus contraindicated whenever a pregnancy is contemplated. Just as VEGF antagonists may become useful for the prevention of OHSS, similar cytokine inhibitors are being studied for treatment of the syndrome. To date, such therapies remain investigational, largely preclinical, and not yet compelling. One study found pentoxifylline, an inhibitor of the synthesis of tumor necrosis factor-α, to be ineffective at limiting ascites formation in an OHSS rabbit model, although it did decrease ovarian weight compared with controls (141). Several case studies or small case reports suggest that cabergoline may have value not only in the prevention of OHSS, but also in its treatment as well. In these case reports, adjuvant treatment with high doses of cabergoline (1 mg daily), perhaps in conjunction with a GnRH-ant, seemed to facilitate a rapid resolution of even severe OHSS, with no side effects reported (142–144). However, until such interventions are validated in human trials, the treatment of OHSS remains largely empiric in nature.

The clinical manifestations of OHSS are a cascade of pathophysiologic events resulting from a global increase in vascular permeability. This increased vascular permeability causes a decrease in the colloid oncotic pressure (145), which results in a change in extracellular fluid equilibrium. Fluid consequently shifts into the extravascular or "third" space, depleting intravascular volume and causing hypotension. "Third spacing" causes abdominal ascites, pleural and pericardial effusions, and dependent edema. Cardiac preload falls due to a combination of hypovolemia caused by fluid shifts and compression of the inferior vena cava from the increasing intraperitoneal pressure due to ascites accumulation. Falling cardiac preload reduces cardiac output, which, together with a decrease in peripheral vascular resistance due to an arterial vasodilatation (146), in turn leads to a decrease in renal perfusion. Decreasing renal perfusion increases proximal tubule reabsorption of salt and water, leading to decreased urinary sodium excretion and oliguria. The proximal sodium reabsorption, and consequently diminished exposure of the distal tubule to sodium, impairs the sodium–hydrogen/potassium exchange in the distal tubule, causing hyperkalemic acidosis. Decreasing renal perfusion also decreases glomerular filtration rate, which can result in oliguria or anuria and a full-blown prerenal azotemia. OHSS also produces a hypercoagulable state, possibly due to a combination of hemoconcentration, high levels of ovarian steroids, and changes in liver perfusion, resulting in decreased hepatic protein synthesis with consequent depletion of circulating antithrombotic factors (147).

Individual treatment will depend on the severity of the syndrome. Mild forms of OHSS require little more than reassurance, since it is well established that mild symptoms usually resolve, in the absence of pregnancy, within two weeks of receiving hCG. If a pregnancy ensues, mild symptoms may progress, but rarely by more than one degree in severity. In patients with moderate ascites and mild hemoconcentration (hematocrit <45%), bed rest and abundant liquid intake should be prescribed. The tendency towards intravascular volume depletion and hyponatremia may be treated with oral isotonic electrolyte solutions; sports drinks, popular among athletes, are particularly suitable because they are engineered for optimal rehydration. The patient should be vigilant in noting any decreases in urine output, significant weight gain, or abdominal bloating as self-assessed by daily abdominal girth measurement. These findings, if present, may be the first warning signals of accumulation of ascitic fluid and worsening hemoconcentration. A hematocrit >45%, or 30% increased over baseline, indicates that the condition has entered the category of severe OHSS and that hospitalization is required.

Dramatic clinical deterioration is most likely to manifest eight to nine days after hCG administration, when endogenous, pregnancy-derived hCG becomes perceptible. The single most important variable that indicates the severity of the OHSS is hemoconcentration, as reflected in the hematocrit. Because the hematocrit is actually the ratio between red cell volume and total blood volume, where total blood volume = red cell volume + plasma volume, the change in plasma volume must always be larger than the change reflected by the hematocrit (70). Thus, a change of 2% in the hematocrit from 42% to 44% is four-times smaller than the actual 8% drop in plasma volume. This is extremely important to remember when one is treating patients with OHSS. Any increase in the hematocrit as it approaches 45% underestimates the magnitude of plasma volume depletion and thus the seriousness of the patient's condition. One should therefore not be lulled into a false sense of security when only a small incremental rise in hematocrit between 40% and 45% is observed. Similarly, in the face of hemoconcentration, small reductions in hematocrit may represent a significant improvement in plasma volume (13).

An additional measure of hemoconcentration is the magnitude of leukocytosis; white blood cell counts higher than 25,000/mm^3, largely reflecting a granulocytosis, may be seen. This massive neutrophilia may be attributed to hemoconcentration and generalized stress reaction. When oral isotonic fluid intake is insufficient to maintain plasma volume, intravenous fluid therapy becomes mandatory. Table 65.3 details the advantages and disadvantages of various therapies in the treatment of severe OHSS, and Figure 65.3 provides a clinical algorithm for the management of OHSS. Crystalloids alone, although seldom sufficient for restoring homeostasis because of massive protein loss through hyperpermeable capillaries, still remains the mainstay of treatment. Because of the tendency for hyponatremia, sodium chloride with or without glucose is the crystalloid of choice. The daily volume infused may vary from 1.5 L to greater than 3.0 L. Although some authors advocate fluid restriction to minimize the accumulation of ascites (148), one should rather deal with the discomfort of ascites than face the consequences of hemoconcentration with the attendant risks of thromboembolism and renal shutdown. In order to maintain fluid balance, the patient's urine output, oral and intravenous fluid intake, bodyweight, abdominal girth, hematocrit, and serum

Table 65.3 Pros and cons of various therapies of ovarian hyperstimulation syndrome

Therapy	Pros	Cons
Intravenous crystalloids	Alleviates hemoconcentration Improves renal perfusion	Lost immediately from vascular tree Aggravates ascites
Fluid restriction	Controls ascites	Reduces renal perfusion Promotes hemoconcentration
Albumin	Improves colloid-oncotic pressure Improves renal perfusion	Risks of human blood product
Furosemide	Reduces total body water	Further reduces intravascular volume
Indomethacin	May block prostanglandin-induced hyperpermeability	Implicated in renal failure
ACE inhibitors	May block angiotensin II-induced hyperpermeability	Teratogenic
Paracentesis	Alleviates tense ascites Improves renal perfusion	Risks of hemorrhage, infection, and leakage
Heparin	Decreases risk of thromboembolic phenomena	Increases risk of hemorrhage
Peritoneo-venous shunt	Replaces lost electrolytes and proteins	Risk of self-toxicity Elaborate setup and risk of infection
Dopamine drip	Improves renal perfusion	Need for Intensive Care Unit management

Abbreviation: ACE, angiotensin-converting enzyme.

electrolytes must be monitored. In addition, coagulation parameters and liver enzymes should be periodically assessed. Intravenous volume replacement should aim to improve renal perfusion before fluid escapes into the peritoneal and/or pleural cavities; this transient hemodilution is achieved at the expense of increased third spacing and increased total body water. Whenever adequate fluid balance cannot be restored by crystalloid alone, plasma expanders should be utilized. Since albumin is the main protein lost in OHSS, human albumin is physiologic and thus the colloid of choice (Table 65.3). Albumin at doses of 50–100 g at 25% concentration should be administered intravenously and repeated every 2–12 hours until the hematocrit falls below 45% and urine output increases.

At a relatively advanced stage of OHSS, during treatment with crystalloids and colloids, gradual hemodilution is obtained at the expense of a tightening abdominal wall with the rapid accumulation of ascitic fluid. At this stage of restored intravascular volume and improved renal perfusion, there may occur a sudden, paradoxical onset of oliguria, increasing serum creatinine, and rapidly falling creatinine clearance (149). This sudden deterioration in fluid balance is probably the result of a significant rise in intra-abdominal pressure produced by tense ascites. Increased intra-abdominal pressure may in turn impede renal venous outflow, causing congestion, renal edema, and decrease in renal function. Such tense ascites is best treated surgically via therapeutic paracentesis, although diuretics may also be effective. When oliguria persists despite evidence of adequate hemodilution, intravenous furosemide at a 10–20-mg dose is often beneficial. In practice, an albumin–furosemide chase protocol seems to yield the best results. Two units of albumin, 50 g each, followed immediately by intravenous furosemide will often result in diuresis. In states of volume contraction, hemoconcentration, and hypotension, furosemide should be strictly avoided. In this precarious stage of OHSS, with impending renal failure, renal dose dopamine drip should be used for renal rescue.

Paracentesis

The single most important treatment modality in life-threatening OHSS that cannot be controlled by medical therapy is paracentesis. Because much of the clinical pathophysiology of OHSS stems from the increase in intra-abdominal pressure caused by ascetic fluid shifts (150,151), OHSS can be considered to be an abdominal compartment syndrome (152). As such, reduction in intra-abdominal pressure alone can alleviate much of the clinical constellation of OHSS, and may even be considered the most important treatment modality for moderate or severe OHSS, and certainly for any OHSS with tense ascites. Rabau et al. first proposed the use of paracentesis in the treatment of severe OHSS (10). Paracentesis was temporarily discredited, but later regained popularity (Table 65.3 and Figure 65.3) (11). Thaler et al. (153), Borenstein et al. (149), and Forman et al. (154) have all promoted paracentesis as safe and exceptionally beneficial. Dramatic improvements in the clinical symptoms of severe OHSS, with almost instantaneous diuresis, were reported (149). In a series of seven patients in whom paracentesis was performed, urine output rose from 780 $\pm$ 407 mL to 1670 $\pm$ 208 mL ($p < 0.05$), creatinine clearance rose from 75.4 $\pm$ 16.0 mL/minute to 101 $\pm$ 15 mL/minute ($p < 0.05$), hematocrit decreased from 46.3% $\pm$ 2.2% to 37.1% $\pm$ 2.5% ($p < 0.05$), and a mean weight loss of 5.3 kg was observed (95). In the study by Forman et al. (154), 37 L of ascitic fluid with a protein content of 46–53 g/L (reflecting a total protein loss of 1.85 kg) was removed from a single patient, underscoring both the high protein content of ascitic fluid and the safety of the procedure.

The indications for paracentesis include the need for symptomatic relief, tense ascites, oliguria, rising serum

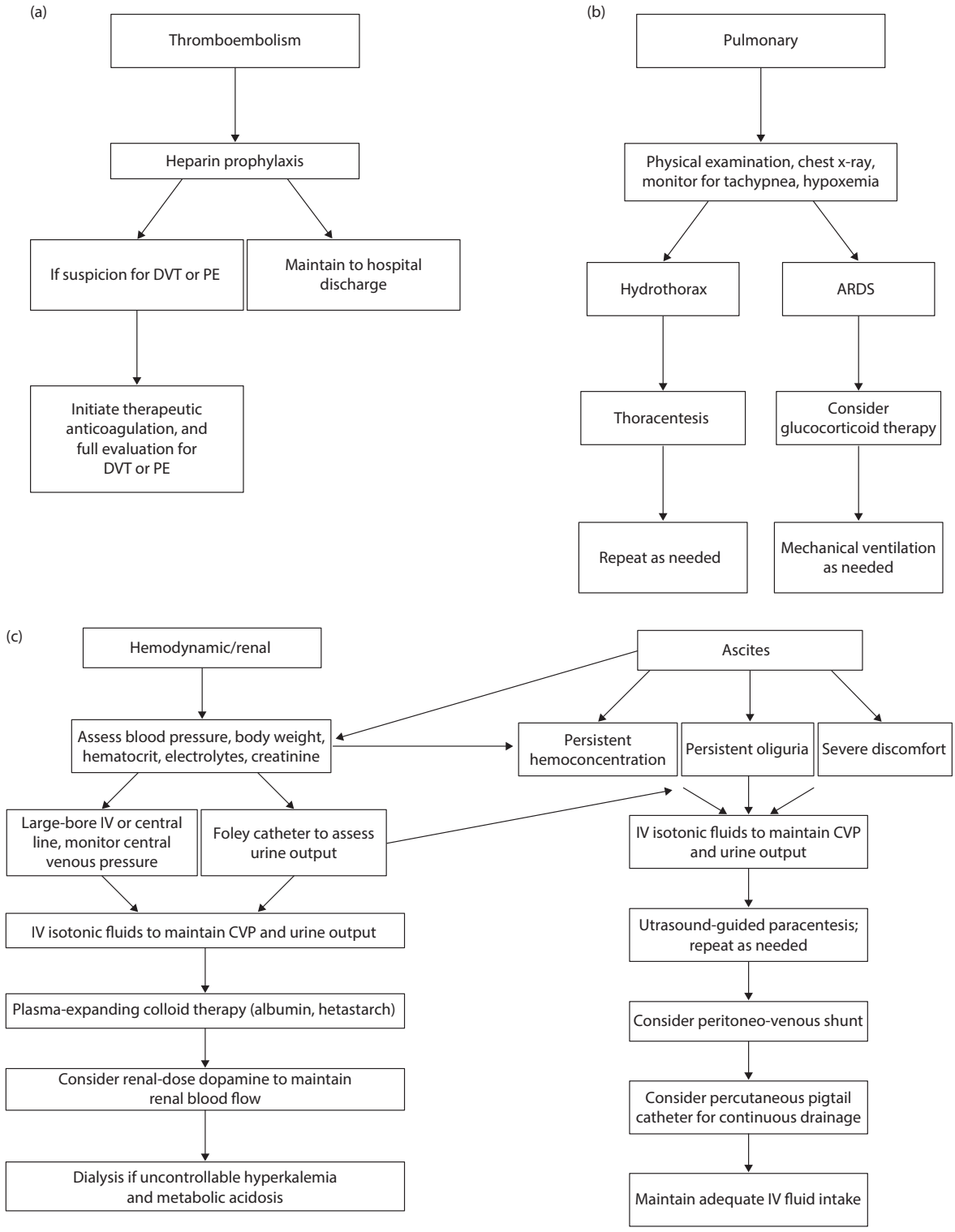

Figure 65.3 Algorithms for the intensive care of the patient with critical ovarian hyperstimulation syndrome: (a) thromboembolism; (b) pulmonary; (c) hemodynamic/renal and ascites. *Abbreviations*: IV, intravenous; DVT, deep vein thrombosis; CVP, central venous pressure; PE, pulmonary embolus; ARDS, acute respiratory distress syndrome.

creatinine concentration or falling creatinine clearance, and hemoconcentration unresponsive to medical treatment. Paracentesis should be performed aseptically under ultrasound guidance. Careful monitoring of hemodynamic stability is also mandatory. Rizk and Aboulghar advocated transvaginal ultrasonically guided aspiration of ascitic fluid as an effective and equally safe method (155), but a transabdominal approach can be used as well. Up to 4 L may be removed either by slow drainage to gravity (153) or with negative pressure using large evacuated containers. Paracentesis

is contraindicated in patients who are hemodynamically unstable or in the presence of suspected hemoperitoneum.

With aggressive use of paracentesis, including repeated paracenteses when ascitic re-accumulation is noted, patients with even severe OHSS can be treated effectively and safely as outpatients, without the need for hospitalization (156). In fact, outpatient management of moderate or severe OHSS with early and frequent paracentesis has been shown to be more effective than traditional inpatient therapy (157) and should therefore be considered for all such patients.

A new and innovative treatment for severe OHSS was suggested by Koike et al. (148). These authors describe continuous peritoneo-venous shunting in 18 patients with severe OHSS. This study group was compared with 36 control patients who had received intravenous albumin at a dose of 37.5 g/day. Recirculation of ascites fluid rich in proteins is not a novel idea (158); however, the reliance on a continuous shunt from the peritoneal cavity into the antecubital vein is a novel and logical way to replenish the vascular tree with the fluid, proteins, and electrolytes that were lost from the vasculature. The study reports faster hemodilution, shorter hospital stays, and prompt improvement in symptoms in the shunted patients due to diuresis and a reduction in the amount of ascites. There are, however, some problems with the study besides the complexity of the setup. First, the reinfused ascites may contain the very substances that might be responsible for the profound hyperpermeability of OHSS, and thus may exacerbate the syndrome. Second, the group advocates fluid restriction, which may aggravate hemoconcentration and thus contribute to renal failure and thromboembolic phenomena (148).

In addressing the hypercoagulable state of OHSS, most authors reserve anticoagulation for special circumstances in which thromboembolic events have already occurred, or in the setting of a hereditary coagulopathy. One study suggests that prophylactic screening for hereditary thrombophilias should perhaps be undertaken in all patients undergoing assisted reproduction, because of a higher prevalence of thrombophilia in women with severe OHSS (159). However, another conflicting study found no such increased prevalence and recommends against screening the general IVF population for thrombophilias (160). In any case, although prophylactic treatment of women with OHSS with unfractionated or low-molecular-weight heparin is of some theoretical value, rapid alleviation of the patient's hemoconcentration is far more important.

Rarely, as a last resort, when the critical stage of OHSS is complicated by renal failure, thromboembolism, acute respiratory distress syndrome, and multiorgan failure, there is no choice but to perform a potentially life-saving termination of pregnancy.

REFERENCES

1. Abramov Y, Elchalal U, Schenker JG. An epidemic of severe OHSS; a price we have to pay? *Hum Reprod* 1999; 14: 2181–3.
2. Moses M, Bogowsky H, Anteby E, Lunenfeld B, Rabau E, Serr DM, David A, Salomy M. Thromboembolic phenomena after ovarian stimulation with menopausal gonadotrophins. *Lancet* 1965; 2: 1213.
3. Esteban-Altirriba J. Le syndrome d'hyperstimulation massive des ovaries. *Rev Fr Gynecol Obstet* 1961; 56: 555.
4. Figueroa-Cases P. Reaccion ovarica monstruosa a las gonadotrofinas a proposito de un caso fatal. *Ann Cirug* 1958; 23: 116.
5. Cluroe AD, Synek BJ. A fatal case of ovarian hyperstimulation syndrome with cerebral infarction. *Pathology* 1995; 27: 344–6.
6. Semba S, Moriya T, Youssef EM, Sasano H. An autopsy case of ovarian hyperstimulation syndrome with massive pulmonary edema and pleaural effusion. *Pathol Int* 2000; 50: 549–52.
7. Gemzel CA, Diczfalusy E, Tillinger G. Clinical effect of human pituitary follicle-stimulating hormone. *J Clin Endocrinol Metab* 1958; 18: 1333.
8. Greenblatt RB, Barfield WE. Induction of ovulation with MRL/41. *JAMA* 1961; 178: 101.
9. Lunenfeld B. Treatment of anovulation by human gonadotropins. *J Int Fed Gynecol Obstet* 1963; 1: 153.
10. Rabau E, David A, Serr DM, Mashiach S, Lunenfeld B. Human menopausal gonadotrophin for anovulation and sterility: Results of 7 years of treatment. *Am J Obstet Gynecol* 1967; 98: 92–8.
11. Schenker JG, Weinstein D. Ovarian hyperstimulation syndrome: A current survey. *Fertil Steril* 1978; 30: 255–68.
12. Golan A, Ron-El R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: An update review. *Obstet Gynecol Surv* 1989; 44: 430–40.
13. Navot D, Bergh PA, Laufer N. Ovarian hyperstimulation syndrome in novel reproductive technologies: Prevention and treatment. *Fertil Steril* 1992; 58: 249–61.
14. Rizk B, Aboulghar MA. Classification, pathophysiology, and management of ovarian hyperstimulation syndrome. In: *In-vitro Fertilization and Assisted Reproduction*. Brinsden P (ed.). New York, NY and London, U.K.: The Parthenon Publishing Group, pp. 131–55, 1999.
15. Lyons CA, Wheeler CA, Frishman GN, Hackett RJ, Seifer DB, Haning RV, Jr. Early and late presentation of the ovarian hyperstimulation syndrome: Two distinct entities with different risk factors. *Hum Reprod* 1994; 9: 792–9.
16. Mathur RS, Akande VA, Keay SD, Hunt LP, Jenkins JM. Distinction between early and late ovarian hyperstimulation syndrome. *Fertil Steril* 2000; 73: 901–7.
17. Papanikolaou EG, Tournaye H, Verpoest W, Camus M, Vernaeve V, Van Steirteghem A, Devroey P. Early and late ovarian hyperstimulation syndrome: Early pregnancy outcome and profile. *Hum Reprod* 2005; 20: 636–41.
18. De Neubourg D, Mangelschots K, Van Royen E, Vercruyssen M, Gerris J. Singleton pregnancies are as affected by ovarian hyperstimulation syndrome as twin pregnancies. *Fertil Steril* 2004; 82: 1691–3.

19. Navot D, Margalioth EJ, Laufer N, Birkenfeld A, Relou A, Rosler A, Schenker JG. Direct correlation between plasma renin activity and severity of the ovarian hyperstimulation syndrome. *Fertil Steril* 1987; 48: 57–61.
20. McClure N, Healy DI, Rogers PA, Sullivan J, Beaton L, Haning RV Jr, Connolly DT, Robertson DM. Vascular endothelial growth factor as a capillary permeability agent in ovarian hyperstimulation syndrome. *Lancet* 1994; 344: 235–6.
21. Friedlander MA, Loret de Mola JR, Goldfarb JM. Elevated levels of interleukin-6 in ascites and serum from women with ovarian hyperstimulation syndrome. *Fertil Steril* 1993; 60: 826–33.
22. Abramov Y, Schenker JG, Lewin A, Friedler S, Nisman B, Barak V. Plasma inflammatory cytokines correlate to the ovarian hyperstimulation syndrome. *Hum Reprod* 1996; 11: 1381–6.
23. Revel A, Barak V, Lavy Y, Anteby E, Amit A, Finci-Yeheskel Z, Mayer M, Simon A, Laufer N, Hurwitz A. Characterization of intraperitoneal cytokines and nitrates in women with severe ovarian hyperstimulation syndrome. *Fertil Steril* 1996; 66: 66–71.
24. Orvieto R, Ben-Rafael Z. The immune system in severe ovarian hyperstimulation syndrome. *Isr J Med Sci* 1996; 32: 1180–2.
25. Sealey JE, Atlas SA, Glorioso N, Manapat H, Laragh JH. Cyclical secretion of prorenin during the menstrual cycle: Synchronization with luteinizing hormone and progesterone. *Proc Natl Acad Sci USA* 1985; 82: 8705–9.
26. Derkx FH, Alberda AT, Zeilmaker GH, Schalekamp MA. High concentrations of immunoreactive renin, prorenin and enzymatically-active renin in human ovarian follicular fluid. *Br J Obstet Gynaecol* 1987; 94: 4–9.
27. Geva E, Jaffe RB. Role of vascular endothelial growth factor in ovarian physiology and pathology. *Fertil Steril* 2000; 74: 429–38.
28. Whelan LG III, Vlahos NF. The ovarian hyperstimulation syndrome. *Fertil Steril* 2000; 73: 883–96.
29. Warren RS, Yuan H, Matli MR, Ferrara N, Donner DB. Induction of vascular endothelial growth factor by insulin-like growth factor I in colorectal carcinoma. *J Biol Chem* 1996; 271: 483–8.
30. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. *Endocrinol Rev* 1997; 18: 4–25.
31. Levin ER, Rosen GF, Cassidenti DL, Yee B, Meldrum D, Wisot A, Pedram A. Role of vascular endothelial growth factor in ovarian hyperstimulation syndrome. *J Clin Invest* 1998; 102: 1978–85.
32. Neulen J, Yan Z, Raczek S, Weindel K, Keek C, Weich HA, Marme D, Breckwoldt M. Human chorionic gonadotropin-dependent expression of vascular endothelial growth factor/vascular permeability factor in human granulosa cells: Importance in ovarian hyperstimulation syndrome. *J Clin Endocrinol Metab* 1995; 80: 1967–71.
33. Pellicer A, Albert C, Mercader A, Bonilla-Musoles F, Remohi J, Simon C. The pathogenesis of ovarian hyperstimulation syndrome: *In vivo* studies investigating the role of interleukin-1β, inerleukin-6, and vascular endothelial growth factor. *Fertil Steril* 1999; 71: 482–9.
34. Smits G, Olatunbosun O, Delbaere A, Pierson R, Vassart G, Costagliola S. Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. *N Engl J Med* 2003; 349: 760–6.
35. Vasseur C, Rodien P, Beau I et al. A chorionic gonadotropin-sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. *N Engl J Med* 2003; 349: 753–9.
36. Montanelli L, Delbaere A, Di Carlo C, Nappi C, Smits G, Vassart G, Costagliola S. A mutation in the follicle-stimulating hormone receptor as a cause of familial spontaneous ovarian hyperstimulation syndrome. *J Clin Endocrinol Metab* 2004; 89: 1255–8.
37. Haning RV, Jr., Austin CW, Carlson IH, Kuzma DL, Shapiro SS, Zweibel WJ. Plasma estradiol is superior to ultrasound and urinary estriol glucuronide as a predictor of ovarian hyperstimulation syndrome during induction of ovulation with menotropins. *Fertil Steril* 1983; 40: 31–6.
38. Levy T, Orvieto R, Homburg R, Peleg D, Dekel A, Ben Rafael Z. Severe ovarian hyperstimulation syndrome despite low plasma estrogen concentrations in a hypogonadotrophic, hypogonadal patient. *Hum Reprod* 1996; 11: 1177–9.
39. Schenker JG. Prevention and treatment of ovarian hyperstimulation syndrome. *Hum Reprod* 1993; 8: 653–9.
40. Delvigne A, Rozenberg S. Systematic review of data concerning etiopathology of ovarian hyperstimulation syndrome. *Int J Fertil Womens Med* 2002; 47: 211–26.
41. Manau D, Balasch J, Arroyo V, Jiménez W, Fábregues F, Casamitjana R, Creus M, Vanrell JA. Circulatory dysfunction in asymptomatic *in vitro* fertilization patients. Relationship with hyperestrogenemia and activity of endogenous vasodilators. *J Clin Endocrinol Metab* 1998; 83: 1489–93.
42. Ajonuma LC, Tsang LL, Zhang GH et al. Estrogen-induced abnormally high cystic fibrosis transmembrane conductance regulator expression results in ovarian hyperstimulation syndrome. *Mol Endocrinol* 2005; 19: 3038–44.
43. Gnoth C, Schuring AN, Friol K, Tigges J, Mallmann P, Godehardt E. Relevance of anti-Mullerian hormone measurement in a routine IVF program. *Hum Reprod* 2008; 23: 1359–65.
44. La Marca A, Giulini S, Tirelli A, Bertucci E, Marsella T, Xella S, Volpe A. Anti-Mullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. *Hum Reprod* 2007; 22: 766–71.

45. Lee TH, Liu CH, Huang CC, Shih YT, Ho HN, Yang YS, Lee MS. Serum anti-Mullerian hormone and estradiol levels as predictors of ovarian hyperstimulation syndrome in assisted reproductive technology cycles. *Hum Reprod* 2008; 23: 160–7.
46. Bergh C, Howles CM, Borg K, Hamberger L, Josefsson B, Nilsson L, Wikland M. Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F) versus highly purified urinary FSH (Metrodin HP): Results of a randomized comparative study in women undergoing assisted reproductive techniques. *Hum Reprod* 1997; 12: 2133–9.
47. Out HJ, Mannaerts BM, Driessen SG, Bennink HJ. A prospective, randomized, assessor-blind, multicentre study comparing recombinant and urinary follicle stimulating hormone (Puregon versus Metrodin) in *in-vitro* fertilization. *Hum Reprod* 1995; 10: 2534–40.
48. Frydman R, Howles C, Truong F. A double-blind, randomized study to compare recombinant follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Medtrodin HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection: On behalf of The French Multicentre Trialists. *Hum Reprod* 2000; 15: 520–5.
49. Schats R, Sutter P, Bassil S, Kremer JA, Tournaye H, Donnez J. Ovarian stimulation during assisted reproduction treatment: Comparison of recombinant and highly purified urinary human FSH: On behalf of The Feronia and Apis study group. *Hum Reprod* 2000; 15: 1691–7.
50. Hedon B, Hugues J. *A comparative prospective study of a chronic low dose versus a conventional ovulation stimulation regimen using r-FSH in anovulatory infertile women*. Report no. GF 8220. Geneva, Switzerland: Ares Serono International SA.
51. Homburg R, Levy T, Ben-Rafael Z. A comparative prospective study of conventional regimen with chronic low-dose administration of follicle-stimulating hormone for anovulation associated with polycystic ovary syndrome. *Fertil Steril* 1995; 63: 729–33.
52. Sher G, Salem R, Feinman M, Dodge S, Zouves C, Knutzen V. Eliminating the risk of life-endangering complications following overstimulation with menotropin fertility agents: A report on women undergoing *in vitro* fertilization and embryo transfer. *Obstet Gynecol* 1993; 81: 1009–11.
53. Sher G, Zouves C, Feinman M, Maassarani G. "Prolonged coasting": An effective method for preventing severe ovarian hyperstimulation syndrome in patients undergoing *in-vitro* fertilization. *Hum Reprod* 1995; 10: 3107–9.
54. Tozer AJ, Iles RK, Iammarrone E, Gillott CM, Al-Shawaf T, Grudzinskas JG. The effects of "coasting" on follicular fluid concentrations of vascular endothelial growth factor in women at risk for developing ovarian hyperstimulation syndrome. *Hum Reprod* 2004; 19: 522–8.
55. Benadiva CA, Davis O, Kligman I, Moomjy M, Liu HC, Rosenwaks Z. Withholding gonadotropin administration is an effective alternative for the prevention of ovarian hyperstimulation syndrome. *Fertil Steril* 1997; 67: 724–7.
56. Tortoriello DV, McGovern PG, Colon JM, Skurnick JH, Lipetz K, Santoro N. "Coasting" does not adversely affect cycle outcome in a subset of highly responsive *in vitro* fertilization patients. *Fertil Steril* 1998; 69: 454–60.
57. Grochowski D, Wolczynski S, Kuczynski W, Domitrz D, Szamatowicz J, Szamatowicz M. Correctly timed coasting reduces the risk of ovarian hyperstimulation syndrome and gives good cycle outcome in an *in vitro* fertilization program. *Gynecol Endocrinol* 2001; 15: 234–8.
58. Al-Shawaf T, Zosmer A, Hussain S, Tozer A, Panay N, Wilson C, Lower AM, Grudzinskas JG. Prevention of severe ovarian hyperstimulation syndrome in IVF with or without ICSI and embryo transfer: A modified "coasting" strategy based on ultrasound for identification of high-risk patients. *Hum Reprod* 2001; 16: 24–30.
59. Levinson-Tavor O, Friedler S, Schachter M, Raziel A, Strassburger D, Ron-El R. Coasting—What is the best formula? *Hum Reprod* 2003; 18: 937–40.
60. Kovács P, Mátyás S, Kaali SG. Effect of coasting on cycle outcome during *in vitro* fertilization/intracytoplasmic sperm injection cycles in hyper-responders. *Fertil Steril* 2006; 85: 913–7.
61. Yilmaz N, Uygur D, Ozgu E, Batioglu S. Does coasting, a procedure to avoid ovarian hyperstimulation syndrome, affect assisted reproduction cycle outcome? *Fertil Steril* 2010; 94: 189–93.
62. Abdalla H, Nicopoullos JDM. The effect of duration of coasting and estradiol drop on the outcome of assisted reproduction: 13 years of experience in 1,068 coasted cycles to prevent ovarian hyperstimulation. *Fertil Steril* 2010; 94: 1757–63.
63. Shapiro D, Craven G, Mitchell-Leef J, Massey JB, Wright G, Tucker MJ. Allowing estradiol levels to fall in a controlled drift does not affect pregnancy rates and offers no protection against ovarian hyperstimulation syndrome. *Fertil Steril* 1997; 68(Suppl 1): S169.
64. Lee C, Tummon I, Martin J, Nisker J, Power S, Tekpetey F. Does withholding gonadotrophin administration prevent severe ovarian hyperstimulation syndrome? *Hum Reprod* 1998; 13: 1157–8.
65. Delvigne A, Kostyla K, Murillo D, Van Hoeck J, Rozenberg S. Oocyte quality and IVF outcome after coasting to prevent ovarian hyperstimulation syndrome. *Int J Fertil Womens Med* 2003; 48: 25–31.
66. Isaza V, Garcia-Velasco JA, Aragones M, Remohi J, Simon C, Pellicer A. Oocyte and embryo quality after coasting: The experience from oocyte donation. *Hum Reprod* 2002; 17: 1777–82.

67. Ulug U, Bahceci M, Erden HF, Shalev E, Ben-Shlomo I. The significance of coasting duration during ovarian stimulation for conception in assisted fertilization cycles. *Hum Reprod* 2002; 17: 310–3.
68. D'Angelo A, Amso N. "Coasting" (withholding gonadotropins) for preventing ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev* 2002; 3: CD002811.
69. Endo T, Honnma H, Hayashi T, Chida M, Yamazaki K, Kitajima Y, Azumaguchi A, Kamiya H, Kudo R. Continuation of GnRH agonist administration for 1 week, after HCG injection, prevents ovarian hyperstimulation syndrome following elective cryopreservation of all pronucleate embryos. *Hum Reprod* 2002; 17: 2548–51.
70. Bergh PA, Navot D. Ovarian hyperstimulation syndrome—A review of pathophysiology. *J Assist Reprod Genet* 1992; 9: 429–38.
71. Orvieto R, Ben-Rafael Z. Ovarian hyperstimulation syndrome: A new insight into an old enigma. *J Soc Gynecol Investig* 1998; 5: 110–3.
72. Chen D, Burmeister L, Goldschlag D, Rosenwaks Z. Ovarian hyperstimulation syndrome: Strategies for prevention. *Reprod Biomed Online* 2003; 7: 43–9.
73. Kashyap S, Leveille M, Wells G. Low dose hCG reduces the incidence of early and severe ovarian hyperstimulation syndrome. *Fertil Steril* 2006; 86(Suppl 2): S182–3.
74. Loumaye E, Piazzi A, Engrand P. Results of a phase II, dose finding, clinical study comparing r-LH with hCG to induce final follicular maturation prior to IVF. *Sixteenth World Congress on Fertility and Sterility*, San Francisco, CA, October 4–9, 1998 (Abst.O-236).
75. European Recombinant LH Study Group. Human recombinant luteinizing hormone is as effective as, but safer than, urinary human chorionic gonadotropin in inducing final follicular maturation and ovulation in *in vitro* fertilization procedures: Results of a multicenter double-blind study. *J Clin Endocrinol Metab* 2001; 86: 2607–18.
76. Breckwoldt M, Czygan PJ, Lehmann F. Synthetic LH-RH as a therapeutic agent. *Acta Endocrinol (Copenh)* 1974; 75: 209–20.
77. Crosignani PG, Trojsi L, Attanasio A, Tonani E. Hormonal profiles in anovulatory patients treated with gonadotropins and synthetic luteinizing hormone releasing hormone. *Obstet Gynecol* 1975; 46: 15–22.
78. Lanzone A, Fulghesu AM, Apa R, Caruso A. LH surge induction by GnRH agonist at the time of ovulation. *Gynecol Endocrinol* 1989; 3: 213–20.
79. Imoedemhe D, Chan R, Sigue A, Pacpaco E. A new approach to the management of patients at risk of ovarian hyperstimulation in an *in-vitro* fertilization programme. *Hum Reprod* 1991; 6: 1088–91.
80. Gonen Y, Balakier H, Powell W, Casper RF. Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for *in vitro* fertilization. *J Clin Endocrinol Metab* 1990; 71: 918–22.
81. Itskovitz J, Boldes R, Levron J, Erlik Y, Kahana L. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. *Fertil Steril* 1991; 56: 213–20.
82. Gerris J, De Vits A, Joostens M. Triggering of ovulation in human menopausal gonadotrophin-stimulated cycles: Comparison between intravenously administered gonadotrophin-releasing hormone (100 and 500 ug), GnRH agonist (buserelin, 500 μg) and human chorionic gonadotrophin (10,000 IU). *Hum Reprod* 1995; 10: 56–62.
83. Shalev E, Geslevich Y, Ben-Ami M. Induction of preovulatory luteinizing hormone surge by gonadotrophin-releasing hormone agonist for women at risk for developing the ovarian hyperstimulation syndrome. *Hum Reprod* 1994; 9: 417–9.
84. Emperaire JC, Ruffie A. Triggering ovulation with endogenous luteinizing hormone may prevent the ovarian hyperstimulation syndrome. *Hum Reprod* 1991; 6: 506–10.
85. Balasch J, Tur R, Creus M, Buxaderas R, Fabregues F, Ballesca JL, Barri PN, Vanrell JA. Triggering of ovulation by a gonadotropin releasing hormone agonist in gonadotropin-stimulated cycles for prevention of ovarian hyperstimulation syndrome and multiple pregnancy. *Gynecolog Endocrinol* 1994; 8: 7–12.
86. Kulikowski M, Wolczynski S, Kuczynski W, Grochowski D. Use of GnRH analog for induction of the ovulatory surge of gonadotropins in patients at risk of the ovarian hyperstimulation syndrome. *Gynecol Endocrinol* 1995; 9: 97–102.
87. Engmann L, Diluigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing *in vitro* fertilization prevents the risk of ovarian hyperstimulation syndrome: A prospective randomized controlled study. *Fertil Steril* 2008; 89: 84–91.
88. O'Neill KE, Senapati S, Dokras A. Use of gonadotropin-releasing hormone agonist trigger during *in vitro* fertilization is associated with similar endocrine profiles and oocyte measures in women with and without polycystic ovarian syndrome. *Fertil Steril* 2015; 103: 264–9.
89. Griesinger G, von Otte S, Schroer A, Ludwig AK, Diedrich K, Al-Hasani S, Schultze-Mosgau A. Elective cryopreservation of all pronuclear oocytes after GnRH agonist triggering of final oocyte maturation in patients at risk of developing OHSS: A prospective, observational proof-of-concept study. *Hum Reprod* 2007; 22: 1348–52.
90. Van der Meer S, Gerris J, Joostens M. Triggering of ovulation using a gonadotrophin-releasing hormone agonist does not prevent ovarian hyperstimulation syndrome. *Hum Reprod* 1993; 8: 1628–31.

91. Kol S, Lewit N, Itskovitz-Eldor J. Ovarian hyperstimulation: Effects of GnRH analogues. Ovarian hyperstimulation syndrome after using gonadotrophin-releasing hormone analogue as a trigger of ovulation: Causes and implications. *Hum Reprod* 1996; 11: 1143–4.
92. Casper RF. Ovarian hyperstimulation: Effects of GnRH analogues. Does triggering ovulation with gonadotrophin-releasing hormone analogue prevent severe ovarian hyperstimulation syndrome? *Hum Reprod* 1998; 11: 1144–6.
93. Bodri D, Guillén JJ, Galindo A, Mataró D, Pujol A, Coll O. Triggering with human chorionic gonadotropin or a gonadotropin-releasing hormone agonist in gonadotropin-releasing hormone antagonist-treated oocyte donor cycles: Findings of a large retrospective cohort study. *Fertil Steril* 2009; 91: 365–71.
94. Griesinger G, Diedrich K, Tarlatzis BC, Kolibianakis EM. GnRH-antagonists in ovarian stimulation for IVF in patients with poor response to gonadotrophins, polycystic ovary syndrome, and risk of ovarian hyperstimulation: A meta-analysis. *Reprod Biomed Online* 2006; 13: 628–38.
95. Al-Inany H, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, Abou-Setta AM. Gonadotropin-releasing hormone antagonists for assisted reproductive technology. *Cochrane Database Syst Rev* 2011; 5: CD001750.
96. Ludwig M, Katalinic A, Diedrich K. Use of GnRH antagonists in ovarian stimulation for assisted reproductive technologies compared to the long protocol. *Arch Gynecol Obstet* 2001; 265: 175–82.
97. Ragni G, Vegetti W, Riccaboni A, Engl B, Brigante C, Crosignani PG. Comparison of GnRH agonists and antagonists in assisted reproduction cycles of patients at high risk of ovarian hyperstimulation syndrome. *Hum Reprod* 2005; 20: 2421–5.
98. de Jong D, Macklon NS, Mannaerts BM, Coelingh Bennink HJ, Fauser BC. High dose gonadotrophin-releasing hormone antagonist (ganirelix) may prevent ovarian hyperstimulation syndrome caused by ovarian stimulation for *in-vitro* fertilization. *Hum Reprod* 1998; 13: 573–5.
99. Garrisi G, Navot D. Cryopreservation of semen, oocytes, and embryos. *Curr Opin Obstet Gynecol* 1992; 4: 726–31.
100. D'Angelo A, Amso NN. Embryo freezing for preventing ovarian hyperstimulation syndrome: A Cochrane review. *Hum Reprod* 2002; 17: 2787–94.
101. Kinget K, Nijs M, Cox AM, Janssen M, Jacobs P, Bosmans E, Ombelet W. A novel approach for patients at risk for ovarian hyperstimulation syndrome: Elective transfer of a single zona-free blastocyst on day 5. *Reprod Biomed Online* 2002; 4: 51–5.
102. Trout SW, Bohrer MK, Deifer DB. Single blastocyst transfer in women at risk of ovarian hyperstimulation syndrome. *Fertil Steril* 2001; 76: 1066–7.
103. Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of *in vitro* maturation and *in vitro* fertilization for women with polycystic ovaries. *Obstet Gynecol* 2002; 100: 665–70.
104. Tan SL, Child TJ. *In vitro* maturation of oocytes from unstimulated polycystic ovaries. *Reprod Biomed Online* 2002; 4(Suppl 1): 18–23.
105. Shalev E, Giladi Y, Matilsky M, Ben-Ami M. Decreased incidence of severe ovarian hyperstimulation syndrome in high risk *in-vitro* fertilization patients receiving intravenous albumin: A prospective study. *Hum Reprod* 1995; 10: 1373–6.
106. Isik AZ, Gokmen O, Zeyneloglu HB, Kara S, Gulekli B. Intravenous albumin prevents moderate-severe ovarian hyperstimulation in *in-vitro* fertilization patients: A prospective, randomized and controlled study. *Eur J Obstet Gynecol Reprod Biol* 1996; 70: 179–83.
107. Gokmen O, Ugur M, Ekin M, Keles G, Turan C, Oral H. Intravenous albumin versus hydroxyethyl starch for the prevention of ovarian hyperstimulation in an *in-vitro* fertilization programme: A prospective randomized placebo controlled study. *Eur J Obstet Gynecol Reprod Biol* 2001; 96: 187–92.
108. Aboulghar M, Evers JH, Al-Inany H. Intravenous albumin for preventing severe ovarian hyperstimulation syndrome: A Cochrane review. *Hum Reprod* 2002; 17: 3027–32.
109. Egbase PE, Al Sharhan M, Grudzinskas JG. Early unilateral follicular aspiration compared to coasting for the prevention of severe OHSS; a prospective randomized study. *Hum Reprod* 1999; 14: 1421–5.
110. Egbase PE. Severe OHSS; how many cases are preventable? *Hum Reprod* 2000; 15: 8–10.
111. Malkawi HY, Qublan HS. The effect of metformin plus clomiphene citrate on ovulation and pregnancy rates in clomiphene-resistant women with polycycstic ovarian syndrome. *Saudi Med J* 2002; 23: 663–6.
112. DeLeo D. Effects of metformin on gonadotropin-induced ovulation in women with polycystic ovary syndrome. *Fertil Steril* 1999; 72: 282–5.
113. Khattab S, Fotouh IA, Mohesn IA, Metwally M, Moaz M. Use of metformin for prevention of ovarian hyperstimulation syndrome: A novel approach. *Reprod Biomed Online* 2006; 13: 194–7.
114. Rjosk HK, Abendstein BJ, Kreuzer E, Schwartzler P. Preliminary experience with steroidal ovarian suppression for prevention of severe ovarian hyperstimulation syndrome in IVF patients. *Hum Fertil* 2001; 4: 246–8.
115. Garcia-Velasco JA, Quea G, Piró M, Mayoral M, Ruiz M, Toribio M, Requena A. Letrozole administration during the luteal phase after ovarian stimulation impacts corpus luteum function: A randomized, placebo-controlled trial. *Fertil Steril* 2009; 92: 222–5.

116. Lainas T, Petsas G, Stavropoulou G, Alexopoulou E, Iliadis G, Minaretzis D. Administration of methylprednisolone to prevent severe ovarian hyperstimulation syndrome in patients undergoing *in vitro* fertilization. *Fertil Steril* 2002; 78: 529–33.
117. Tan SL, Balen A, el Hussein E, Campbell S, Jacobs HS. The administration of glucocorticoids for the prevention of ovarian hyperstimulation syndrome in *in vitro* fertilization: A prospective randomized study. *Fertil Steril* 1992; 58: 378–83.
118. Revelli A, Dolfin E, Gennarelli G, Lantieri T, Massobrio M, Holte JG, Tur-Kaspa I. Low-dose acetylsalicylic acid plus prednisolone as an adjuvant treatment in IVF: A prospective, randomized study. *Fertil Steril* 2008; 90: 1685–91.
119. Varnagy A, Bodis J, Manfai Z, Wilhelm F, Busznyak C, Koppan M. Low-dose aspirin therapy to prevent ovarian hyperstimulation syndrome. *Fertil Steril* 2010; 93: 2281–4.
120. Practice Committee of the American Society for Reproductive Medicine. Prevention and treatment of moderate and severe ovarian hyperstimulation syndrome: A guideline. *Fertil Steril* 2016; 106: 1634–47.
121. Ortiz-Capisano MC, Ortiz PA, Harding P, Garvin JL, Beierwaltes WH. Decreased intracellular calcium stimulates renin release via calcium-inhibitable adenylyl cyclase. *Hypertension* 2007; 49: 162–9.
122. Beierwaltes WH. The role of calcium in the regulation of renin secretion. *Am J Physiol Renal Physiol* 2010; 298: F1–11.
123. Yakovenko SA, Sivozhelezov VS, Zorina IV, Dmitrieva NV, Apryshko VP, Voznesenskaya JV. Prevention of OHSS by intravenous calcium. *Hum Reprod* 2009; 24(Suppl 1): i61.
124. Gurgan T, Demirol A, Guven S, Benkhalifa M, Girgin B, Li TC. Intravenous calcium infusion as a novel preventive therapy of ovarian hyperstimulation syndrome for patients with polycystic ovarian syndrome. *Fertil Steril* 2011; 96: 53–7.
125. Gomez R, Simon C, Remohi J, Pellicer A. Vascular endothelial growth factor receptor-2 activation induces vascular permeability in hyperstimulated rats, and this effect is prevented by receptor blockade. *Endocrinology* 2002; 143: 4339–48.
126. NcElhinney B, Ardill J, Caldwell C, McClure N. Preventing ovarian hyperstimulation syndrome by inhibiting the effects of vascular endothelial growth factor. *J Reprod Med* 2003; 48: 243–6.
127. Busso C. Symposium: Update on prediction and management of OHSS. Prevention of OHSS—Dopamine agonists. *Reprod Biomed Online* 2009; 19: 43–51.
128. Garcia-Velasco J. How to avoid ovarian hyperstimulation syndrome: A new indication for dopamine agonists. *Reprod Biomed Online* 2009; 19: 71–5.
129. Gomez R, Gonzalez-Izquierdo M, Zimmermann RC, Novella-Maestre E, Alonso-Muriel I, Sanchez-Criado J, Remohi J, Simon C, Pellicer A. Low-dose dopamine agonist administration blocks vascular endothelial growth factor (VEGF)-mediated vascular hyperpermeability without altering VEGF receptor 2-dependent luteal angiogenesis in a rat ovarian hyperstimulation model. *Endocrinology* 2006; 147: 5400–11.
130. Alvarez C, Marti-Bonmati L, Novella-Maestre E, Sanz R, Gomez R, Fernandez-Sanchez M, Simon C, Pellicer A. Dopamine agonist cabergoline reduces hemoconcentration and ascites in hyperstimulated women undergoing assisted reproduction. *J Clin Endocrinol Metab* 2007; 92: 2931–7.
131. Spitzer D, Wogatzky J, Murtinger M, Zech MH, Haidbauer R, Zech NH. Dopamine agonist bromocriptine for the prevention of ovarian hyperstimulation syndrome. *Fertil Steril* 2011; 95: 2742–4.
132. Soares S, Gomez R, Simon C, Garcia-Velasco J, Pellicer A. Targeting the endothelial growth factor system to prevent ovarian hyperstimulation syndrome. *Hum Reprod Update* 2008; 14: 321–33.
133. Humaidan P, Quartarolo J, Papanikolaou G. Preventing ovarian hyperstimulation syndrome: Guidance for the clinician. *Fertil Steril* 2010; 94: 389–400.
134. Carizza C, Abdelmassih V, Abdelmassih S, Ravizzini P, Salgueiro L, Salgueiro TP, Jine LT, Nagy P, Abdelmassih R. Cabergoline reduces the early onset of ovarian hyperstimulation syndrome: A prospective randomized study. *Reprod Biomed Online* 2008; 17: 751–5.
135. Youssef M, van Wely M, Hassan MA, Al-Inany HG, Mochtar M, Khattab S, van der Veen F. Can dopamine agonists reduce the incidence and severity of OHSS in IVF/ICSI treatment cycles? A systematic review and meta-analysis. *Hum Reprod Update* 2010; 16: 459–66.
136. Busso C, Fernandez-Sanchez M, Garcia-Velasco J, Landeras J, Ballesteros A, Muñoz E, Gonzalez S, Simon C, Arce JC, Pellicer A. The non-ergot derived dopamine agonist quinagolide in prevention of early ovarian hyperstimulation syndrome in IVF patients: A randomized, double-blind, placebo-controlled trial. *Hum Reprod* 2010; 25: 995–1004.
137. Busso C, Fernandez-Sanchez M, Garcia-Velasco J, Landeras J, Ballesteros A, Muñoz E, Gonzalez S, Simon C, Arce JC, Pellicer A. Quinagolide is effective in preventing moderate/severe OHSS in IVF patients: A randomised, double-blind, placebo-controlled trial. *Hum Reprod* 2008; 23(Suppl): i60.
138. Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR. KISS-1, a novel human malignant melanoma metastasis-suppressor gene. *J Natl Cancer Inst* 1996; 23: 1731–7.
139. Skorupskaite K, George JT, Anderson RA. The kisspeptin–GnRH pathway in human reproductive health and disease. *Hum Reprod Update* 2014; 20: 485–500.

140. Abbara A, Jayasena CN, Christopoulos C et al. Efficacy of kisspeptin-54 to trigger oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS) during *in vitro* fertilization (IVF) therapy. *J Clin Endocrinol Metab* 2015; 100: 3322–31.
141. Serin IS, Ozcelik B, Bekyurek T, Ozturk F, Koc N, Sahin Y. Effects of pentoxifylline in the prevention of ovarian hyperstimulation syndrome in a rabbit model. *Gynecol Endocrinol* 2002; 16: 355–9.
142. Ata B, Seyhan A, Orhaner S, Urman B. High dose cabergoline in management of ovarian hyperstimulation syndrome. *Fertil Steril* 2009; 92: 1168.e1–4.
143. Rollene NL, Amols MH, Hudson SB, Coddington CC. Treatment of ovarian hyperstimulation syndrome using a dopamine agonist and gonadotropin releasing hormone antagonist: A case series. *Fertil Steril* 2009; 92: 1169.e15–7.
144. Saharkhiz N, Akbari Sene A, Salehpur S, Tamimi M, Vasheghani Farahani M, Sheibani K. Treatment results of high dose cabergoline as an adjuvant therapy in six patients with established severe hyper stimulation syndrome. *Iran J Reprod Med* 2014; 12: 713–6.
145. Tollan A, Holst N, Forsdahl F, Fadnes H, Oian P, Maltau JM. Transcapillary fluid dynamics during ovarian stimulation for *in vitro* fertilization. *Am J Obstet Gynecol* 1990; 162: 554–8.
146. Balasch J, Arroyo V, Fábregues F, Sálo J, Jiménez W, Paré JC, Vanrell JA. Neurohormonal and hemodynamic changes in severe cases of ovarian hyperstimulation syndrome. *Ann Intern Med* 1994; 121: 27–33.
147. Fábregues F, Balasch J, Gìnès P, Manau D, Jiménez W, Arroyo V, Creus M, Vanrell JA. Ascites and liver test abnormalities during severe ovarian hyperstimulation syndrome. *Am J Gastroenterol* 1999; 94: 994–9.
148. Koike T, Araki S, Minakami H, Ogawa S, Sayama M, Shibahara H, Sato I. Clinical efficacy of peritoneovenous shunting for the treatment of severe OHSS. *Hum Reprod* 2000; 15: 113–7.
149. Borenstein R, Elhalal U, Lunenfeld B, Shoham Z. Severe OHSS; a reevaluated therapeutic approach. *Fertil Steril* 1989; 51: 791–5.
150. Cil T, Tummon IS, House AA, Taylor B, Hooker G, Franklin J, Rankin R, Carey M. A tale of two syndromes: Ovarian hyperstimulation and abdominal compartment. *Hum Reprod* 2000; 15: 1058–60.
151. Maslovitz S, Jaffa A, Eytan O, Wolman I, Many A, Lessing JB, Gamzu R. Renal blood flow alteration after paracentesis in women with ovarian hyperstimulation. *Obstet Gynecol* 2004; 104: 321–6.
152. Grossman LC, Michalakis KG, Browne H, Payson MD, Segars JH. The pathophysiology of ovarian hyperstimulation syndrome: An unrecognized compartment syndrome. *Fertil Steril* 2010; 94: 1392–8.
153. Thaler I, Yoffe M, Kaftory JK, Brandes JM. Treatment of OHSS; the physiologic basis for a modified approach. *Fertil Steril* 1981; 36: 110–3.
154. Forman RG, Frydman R, Egan D, Ross C, Barlow DH. Severe OHSS using agonists of gonadotropin-releasing hormone for *in vitro* ferilization; a European series and a proposal for prevention. *Fertil Steril* 1990; 53: 502–9.
155. Rizk B, Aboulghar MA. Modern management of OHSS. *Hum Reprod* 1991; 6: 1082–7.
156. Smith LP, Hacker MR, Alper MM. Patients with severe ovarian hyperstimulation syndrome can be managed safely with aggressive outpatient transvaginal paracentesis. *Fertil Steril* 2009; 92: 1953–9.
157. Csokmay JM, Yauger BJ, Henne MB, Armstrong AY, Queenan JT, Segars JH. Cost analysis model of outpatient management of ovarian hyperstimulation syndrome with paracentesis: "Tap early and often" versus hospitalization. *Fertil Steril* 2010; 93: 167–73.
158. Fukaya T, Chida S, Terada Y, Funamaya Y, Yajima A. Treatment of severe OHSS by ultrafiltration and reinfusion of ascitic fluid. *Fertil Steril* 1994; 61: 561–4.
159. Dulitzky M, Cohen SB, Inbal A, Seidman DS, Soriano D, Lidor A, Mashiach S, Rabinovici J. Increased prevalence of thrombophilia among women with severe ovarian hyperstimulation syndrome. *Fertil Steril* 2002; 77: 463–7.
160. Fabregues F, Tassies D, Reverter JC, Carmona F, Ordinas A, Balasch J. Prevalens of thrombophilia in women with severe ovarian hyperstimulation syndrome and cost-effectiveness of screening. *Fertil Steril* 2004; 81: 989–95.

Bleeding, severe pelvic infection, and ectopic pregnancy

66

RAOUL ORVIETO

Transvaginal ultrasound-guided aspiration of oocytes is a well-accepted and universally used method in assisted reproduction (1,2). Its major advantages include easy access to ovarian follicles with excellent oocyte yield and good visualization of the major pelvic vessels. It is done as a day care procedure, either under intravenous analgesia and sedation or under general anesthesia, and is usually atraumatic. Nevertheless, there are some inherent risks, namely puncture of blood vessels and hemoperitoneum, bleeding from the vaginal vault puncture site, rupture of adnexal cystic masses, bowel perforation, trauma to pelvic organs, and pelvic infection. In addition, embryo transfer (ET) itself may be associated with complications such as pelvic infection, multiple pregnancy (which is directly related to the number of transferred embryos), spontaneous abortion, and extrauterine pregnancy (EUP). Maxwell et al. (3) have reported on the incidence of both serious and minor complications in young women undergoing 886 oocyte retrievals for oocyte donation. While the rate of serious complications, which included ovarian hyperstimulation syndrome, ovarian torsion, infection, and ruptured ovarian cyst, was 0.7%, the rate of minor complications severe enough to prompt the donor to seek medical attention after retrieval was 8.5%.

The aim of the present review is to discuss comprehensively three of these complications: bleeding, pelvic inflammatory disease (PID), and EUP.

BLEEDING

Vaginal bleeding

During ultrasound-guided transvaginal oocyte aspiration, multiple punctures of the vaginal vault or inappropriate handling and rotation of the ultrasound vaginal probe while inserting an aspiration needle through the vaginal vault can injure or tear the vaginal mucosa, ovaries, intra-abdominal organs, or blood vessels (1,4–8). Bleeding from the vaginal vault is a common consequence of oocyte pickup (OPU), with a reported incidence of 1.4%–18.4% (5). In most cases, vaginal bleeding as a result of OPU stops spontaneously at the end of the procedure (6). In cases in which it does not, the bleeding site needs to be identified by vaginal exploration with a large speculum, followed by application of pressure with sponge forceps or vaginal packing with a large gauze roll. If this is unsuccessful or the tear is wide and deep, suturing is necessary. The use of a thinner-tipped needle (0.9 mm in diameter) during OPU resulted in significantly less vaginal bleeding when compared to a standard needle (1.4 mm in diameter) (7).

Intraperitoneal or retroperitoneal bleeding

Transvaginal oocyte aspiration can also cause bleeding if intraperitoneal or retroperitoneal pelvic blood vessels are injured or if there is damage to the fine vascular network surrounding the punctured ovarian follicle. The reported incidence of severe intra- or retro-peritoneal bleeding varies from 0% to 1.3% (1,6,8–10); a recent report described one case of intra-abdominal bleeding complicating the aspiration of 1000 oocyte donors (11). Young, lean patients and those with polycystic ovary syndrome or a history of previous surgery were specifically demonstrated to be at much higher risk of this complication (12,13). Intraperitoneal bleeding tends to be severe with acute hemodynamic deterioration, whereas retroperitoneal bleeding usually has a later and more indolent presentation. Yih et al. (14) studied serial complete blood counts before and after OPU in 93 *in vitro* fertilization (IVF) cycles and demonstrated a non-significant change in hematocrit levels, indicating that clinically significant blood loss after OPU is actually uncommon.

Azem et al. (15) described a patient who presented to the emergency room 10 hours after OPU with severe lower abdominal pain, vomiting, and tenesmus. Examination revealed a distended abdomen with severe tenderness in the pouch of Douglas; on transvaginal sonography, a minimal, 3–4-cm collection of fluid was noted. Laparoscopy followed by laparotomy, which was performed on the basis of the clinical profile, revealed a retroperitoneal hematoma 7 cm in diameter. After evacuation and hemostasis, active bleeding from the mid-sacral vein occurred and was controlled by a metal clip. This case demonstrates the indolent course of retroperitoneal bleeding and physicians should be alerted to the possibility of retroperitoneal hematoma despite an absence of free fluid in the pouch of Douglas. Notably, a similar case with no significant intraperitoneal fluid collection was also described as a result of ureteral injury with the consequent uroretroperitoneum (16).

Intra-abdominal bleeding should be suspected immediately after OPU upon the development of signs and symptoms of anemia—specifically weakness, dizziness, dyspnea, or persistent tachycardia. Early management consists of intense hemodynamic monitoring, together with serial measurement of blood hemoglobin concentrations and ultrasonographic evaluation for the presence of intra-abdominal fluid. It should be emphasized, however, that intra-abdominal blood clots or retroperitoneal bleeding might be invisible even to an experienced ultrasound operator. A drop in hemoglobin concentration is an indication for prompt blood transfusion. If hemodynamic

deterioration continues or acute abdominal pain develops, diagnostic laparoscopy or exploratory laparotomy with subsequent hemostasis of the bleeding site(s) is required. The clinician must make sure to handle the fragile hyperstimulated ovaries very cautiously. Notably, a longer time interval between OPU and surgical intervention was noted to put the patient at risk of ovariectomy (17).

Dicker et al. described three cases of severe intra-abdominal bleeding from ovarian puncture sites during OPU, leading to acute abdominal complications (1). In two of the patients, symptoms developed three hours after OPU (hemoglobin 9.0 g/100 mL and 8.1 g/100 mL, respectively), and laparoscopic drainage and hemostasis were sufficient. The third patient became symptomatic after four hours (hemoglobin 7.3 g/100 mL) and required exploratory laparotomy and hemostasis in addition to the transfusion of four units of blood as a life-saving procedure. Later, Battaglia et al. (18) reported severe intra-abdominal bleeding from the surfaces of both ovaries in a patient with coagulation factor XI deficiency. As expected, the patient became symptomatic three hours after OPU and required laparotomy, partial resection of stuffed ovaries, and hemostasis. Physicians should be aware of the presence of concomitant coagulopathy and might therefore consider intense coagulation factor replacement before or during abdominal exploration.

Massive delayed intra-abdominal hemorrhage was also reported following OPU (two and four days later) in patients at risk of thromboembolic events who concomitantly used a therapeutic dose of low-molecular-weight heparin (19). These cases should direct physicians' attention and keep them alert while conducting an IVF treatment in this subgroup of patients. Moreover, the authors recommended that the patient be kept in the ward for observation for at least two to four days following OPU.

Can we prevent severe intra-abdominal bleeding from ovarian puncture sites during OPU? In a cross-sectional retrospective study, Revel et al. (20) questioned the utility of coagulation screening before OPU. Among the 1032 patients evaluated, they found that 534 coagulation tests were needed to prevent one case of bleeding associated with an abnormal coagulation test result. Moreover, while the use of color Doppler sonography during OPU was suggested to reduce the risk of blood vessel injury by the guiding needle (21), its routine use could not predict all cases with moderate peritoneal bleeding (22).

Description of the intraoperative measures needed to control intra-abdominal hemorrhage is beyond the scope of this text, and the reader is referred elsewhere for a detailed review (23).

PELVIC INFLAMMATORY DISEASE

PID is an infrequent complication of ultrasound-guided transvaginal aspiration of oocytes or ET, with a reported incidence of 0.2%–0.5% per cycle (9,24–26). Signs or symptoms of pelvic infection, such as pyrexia, continuous low abdominal pain, dysuria, or offensive vaginal discharge, are infrequent (24). However, this does not exclude occult, subclinical bacterial colonization, which may influence the success of the IVF–ET treatment, or slowly progress throughout pregnancy (27). Ashkenazi et al. evaluated the outcomes of all IVF–ET procedures performed in their unit between 1986 and 1992 (26). Of the 4771 patients who underwent transvaginal OPU, 28 (0.58%) had symptoms of PID within one to seven days. The diagnosis was established by a rise in body temperature to 38°C for more than 48 hours, signs of pelvic peritonitis on physical examination, leukocyte counts of >12,000 cells/m^3, and elevated erythrocyte sedimentation rates. All patients were admitted to hospital for treatment with intravenous antibiotics. Notably, ovarian abscess following oocyte retrieval may manifest late during pregnancy with low-grade fever or vague abdominal pain (28).

OPU can also lead to severe abdominal complications. Our group reported on nine patients (0.24%) with tubo-ovarian or pelvic abscess after transvaginal-guided OPU (26). Three patients required laparotomy and adnexectomy, whereas in six patients, culdocentesis was performed for adequate pelvic abscess drainage. Kelada and Ghani (29) have described a case of bilateral ovarian abscesses following transvaginal oocyte retrieval, complicated by early signs of consumption coagulopathy. The latter is a serious and life-threatening complication of pelvic infection and sepsis, which should be diagnosed and corrected immediately.

Mechanisms underlying pelvic infection

During transvaginal aspiration, accidental needle transport of cervicovaginal flora into ovarian tissue can cause unilateral or bilateral oophoritis, and accidental puncture of a contaminated or sterile hydrosalpinx can cause salpingitis. Some authors have attributed pelvic infection to infected endometriotic cysts or tubo-ovarian abscesses after aspiration of endometriomas (30,31) or, rarely, to inadvertent puncture of the bowel. Pelvic infection in women with endometriosis was shown to be more serious and resistant to antibiotic treatment, and frequently required surgical intervention (32). Pelvic infection can occur as a direct consequence of transcervical ET. This is evidenced by reported cases of PID following ET in an agonadal donor egg recipient (33), or during cryopreserved ET (34); it may also occur as a result of the reaction of a silent or persistent subclinical infection, as seen occasionally after hysterosalpingography. Another possible cause during ET is catheterization of the uterus, which may force bacteria-laden air or fluid into one or both tubes by a piston-like effect.

Effect of acute pelvic infection on IVF–ET outcome

The first study of the impact of pelvic infection on IVF–ET outcome was reported by Ashkenazi et al. in 1994 (26). We found that the number of oocytes recovered, fertilized, and cleaved in 28 patients undergoing IVF in whom PID developed was similar to that of a comparison group with mechanical infertility. However, there were no pregnancies in the PID group, as compared with the 23%–31%

pregnancy rate per transfer in the whole group of patients treated by IVF, indicating that the appearance of PID at the critical time of implantation may cause a failure to conceive. This finding has several possible explanations, as outlined in detail below.

Endotoxemia

Endotoxin-releasing bacteria can be introduced into the peritoneal cavity during transvaginal oocyte recovery and into the uterine cavity or tubes during ET. Ng et al. (35) described a case in which human oocytes were degenerated and fragmented, with no evidence of fertilization, in the presence of *Klebsiella*-derived endotoxin. In a study of the effects of endotoxin infusion on the circulating levels of eicosanoids, progesterone, and cortisol and on abortions, Giri et al. (36) found that first-trimester cows were more sensitive to the abortifacient effect of endotoxin than second- and third-trimester cows. The mechanism of the endotoxin-induced abortion apparently involved the prolonged release of prostaglandin F2α, which has a stimulant effect on uterine smooth muscle contractions and a luteolytic effect resulting in a gradual decline in the plasma level of progesterone (36). In addition, high endotoxin doses can induce the release of various autacoids, catecholamines, and cortisol, which directly or indirectly lead to metabolic and circulatory failures and, thereby, termination of pregnancy.

Local inflammatory reaction

Bacteria trigger a chain of events that lead to the activation, proliferation, and differentiation of lymphocytes, and the production of specific antibodies and various cytokines. This excessive production of cytokines may disrupt the delicate balance between the immune and reproductive systems and result in reproductive failure (37–39).

Temperature elevation

Apart from their direct role on implantation and early embryonic development, cytokines may mediate temperature elevation and indirectly affect the outcome of IVF–ET. The febrile reaction is an integrated endocrine, autonomic, and behavioral response coordinated by the hypothalamus. The actions of circulating cytokines, such as interleukin-1 and tumor necrosis factor, on the central nervous system result in the secretion of prostaglandin E2, which initiates an elevation in body temperature together with corticosteroid secretion (40), which is also a component of the stress response. Some authors have suggested that fever is essential for amplifying the emergence of T-cell immunity in peripheral tissues (41). *In vitro* experiments have shown that temperature elevation leads to disintegration of the cytoskeleton (42) and may affect the transport of organelles. In pregnancy, maternal heat exposure can cause intracellular embryonic damage (43) and inhibit cell mitosis, proliferation, and migration, resulting in cell death. In a study of guinea pig embryos, Edwards et al. (44) reported cell damage within minutes and cell death within hours after heating. Other mechanisms of heat-induced cell injury are microvascular lesions, placental necrosis, and placental infarction (45).

Treatment

The role of prophylactic antibiotics in IVF–ET

The potential for intraperitoneal bacterial contamination during transvaginal oocyte recovery is well known and has led to the routine use of prophylactic antibiotics and vaginal disinfection (46). Meldrum (47) found no cases of pelvic infection among 88 transvaginal retrievals with the use of intravenous cefazolin and vaginal preparation with povidone–iodine and saline irrigation; nor did Tsai et al. (48) in patients with ovarian endometrioma using only vaginal douching with aqueous povidone–iodine followed by normal saline irrigation. Borlum and Maiggard (49) reported two cases of serious pelvic infection in almost 400 transvaginal aspirations. They used only two vaginal douchings with sterile saline and noted that minimizing the number of repeated vaginal penetrations may have helped with lowering the risk of infection. However, the appropriate type of antibiotic administration, the timing or duration of therapy, and the efficacy of therapy have not yet been established (47,50). Indeed, some authors claim that these measures may not only further reduce the incidence of PID after oocyte retrieval, but can even increase the risks of both an adverse reaction and of colonization with resistant organisms. Our experience with vaginal douchings with sterile saline in approximately 1100–1200 OPUs per year revealed a very low rate of PID after OPU. Peters et al. (51) suggested that only women with a tubal abnormality and a history of pelvic infection should receive prophylactic antibiotics before oocyte aspiration, and also possibly after ET. Others have suggested that such patients may benefit from transabdominal or transvesical rather than transvaginal procedures (52,53).

It is also noteworthy that Egbase et al. (54), in a study of the effects of prophylactic antibiotics in OPU on the endocervical microbial inoculation of the endometrium during ET, found that prophylactic antibiotics not only reduced the number of positive microbiology cultures of embryo catheter tips, but also significantly increased implantation and clinical pregnancy rates. On the other hand, in their prospective randomized study, Peikrishvili et al. (55) could not demonstrate any beneficial effects of antibiotic prescription (amoxicillin + clavulanic acid 1 g/125 mg) for six days following oocyte retrieval on implantation, pregnancy, or miscarriage rates.

Curative

PID or tubo-ovarian abscesses after OPU require accurate diagnosis and prompt treatment with broad-spectrum antibiotics. In the presence of a pelvic abscess that is larger than 8 cm or unresponsive to medication, transvaginal or percutaneous drainage is the treatment of choice (46), with or without ultrasound-guided intracavitary instillation of a combination of antibiotics (56). Patients who received antibiotics alone are more likely to require further surgical

intervention when compared with patients who additionally received image-guided drainage (57). Sometimes, surgical laparoscopy or laparotomy is needed to evacuate the abscess or remove the infected tubes or adnexae.

Summary

The appearance of PID at the critical time of implantation results in failure to conceive. This effect may be mediated by bacterial endotoxins, a local inflammatory reaction against bacteria with the involvement of cytokines that affects implantation and early embryonic development, or temperature elevation that directly affects the conceptus. Although the role of prophylactic antibiotics is still controversial, they can be considered in the presence of risk factors for PID; aspiration of hydrosalpinx or endometriomas during OPU might be a risk factor for infection and should be avoided. Furthermore, to prevent total failure, if PID develops before ET, cryopreservation and ET in subsequent cycles should be considered. However, if PID develops after ET, the bacterial infection and fever should be treated rigorously to prevent reproductive failure.

EXTRAUTERINE PREGNANCY

EUP is the implantation of a blastocyst anywhere except in the endometrial lining of the uterine cavity. In recent years, EUPs have shown a marked increase in both absolute number and rate of occurrence (58). By 1992, almost 2% of all pregnancies in the U.S.A. were EUPs, and ectopic pregnancies accounted for 10% of all pregnancy-related deaths (58,59). The rates of abortions, multiple pregnancies, and EUPs are higher in pregnancies resulting from assisted reproduction technology (ART) than in spontaneous pregnancies.

Other factors associated with the development of EUP include previous EUP, salpingitis, previous surgery to the fallopian tube, peritubal adhesions, pelvic lesions that distort the tube, developmental abnormalities of the tube, and altered tubal motility.

EUP after ART

The first IVF–ET pregnancy reported was an ectopic pregnancy (60). Today, the incidence of EUPs after IVF ranges from 2.1% to 9.4% of all clinical pregnancies (61,62). In 1996, the Society for Assisted Reproductive Technology (SART) (63) reported a decrease in the incidence of EUPs to 0.8% of transfers and 1.6% of pregnancies, compared with 0.9% and 2.8%, respectively, in 1995. This finding was attributable to the decrease in the proportion of couples with tubal factor infertility undergoing IVF treatment and a concomitant increase in couples with male factor infertility. Later, the SART reported the outcomes of ART initiated in the U.S.A. in 2001 (64). The incidence of EUP for all ART procedures was 0.8% per transfer and 1.6% per clinical pregnancy, which compares favorably with the estimated overall incidence of EUP in the U.S.A. of 2% per reported pregnancy (58). Perkins et al. assessed the risk of EUP associated with ART in the U.S.A. between 2001 and 2011. While a decline in the incidence of EUP was observed over the study period, with the most pronounced decline seen with frozen ETs, multiple ETs increase the risk of ectopic pregnancy (65).

Risk factors

Data on risk factors for EUP after IVF are still unclear. Martinez and Trounson (66) failed to identify any risk factors, whereas Karande et al. (67) pointed to a prior ectopic pregnancy. Verhulst et al. (68) found a significantly higher rate of EUP after IVF in patients with tubal disease (3.6%) compared with those with normal tubes (1.2%); this finding was confirmed by several other studies (62,69–71). Cohen et al. (72) showed that the number of patent tubes at the time of transfer was a risk factor, with a higher EUP rate in patients with zero or two patent tubes than in patients with one. In an analysis of the Bourn Hall Clinic data, Marcus and Brinsden (73) noted that the main risk factor was a history of PID. Though they found EUP to be more prevalent in patients with tubal factor infertility, those who received a higher culture medium volume and those with a higher progesterone/estradiol ratio on the day of ET had no associated history of EUP. Acharya et al. found that an increased oocyte yield correlated with a significantly increased EUP rate (74). Since this association was not found in oocyte recipients, they suggested that this increased EUP rate may be related to the supraphysiologic hormone levels achieved during ovarian stimulation (74). Finally, in a meta-analysis of risk factors for EUP, Ankum et al. (75) concluded that the four most significant were previous EUP, documented tubal pathology, previous tubal surgery, and *in utero* exposure to diethylstilbestrol. These results were confirmed by Lesny et al., who also added one more: a difficult ET on day 2 rather than day 3 (76). Clayton et al. (77) have recently analyzed the EUP risk among 94,118 patients who conceived with ART procedures. A total of 2009 (2.1%) were ectopic. In comparison with the ectopic rate (2.2%) among pregnancies conceived with IVF (fresh, non-donor cycles), the ectopic rate was significantly increased when zygote intrafallopian transfer was used (3.6%) and significantly decreased when donor oocytes were used (1.4%) or when a gestational surrogate carried the pregnancy (0.9%). Among fresh non-donor IVF–ET procedures, the risk of ectopic pregnancy was significantly increased among women with tubal factor infertility, endometriosis, and other non-tubal female factors of infertility, and significantly decreased among women with a previous live birth. Moreover, transfer of high-quality embryos was associated with a decreased ectopic risk when two or fewer embryos were transferred, but not when three or more embryos were transferred. By analyzing the SART database from 2008 to 2011, Londra et al. (78) found that the odds of EUP were 65% lower in women who had a frozen compared with a fresh transfer in autologous cycles. Moreover, frozen–thawed day-5 blastocyst transfer was associated with a lower EUP rate than frozen–thawed day-3 transfer and fresh transfer (79).

There are many theories on the manner by which embryos implant in the fallopian tube following ET: by

the hydrostatic force of the transfer medium containing the embryos in the fallopian tube ostia; by the gravitational pull of the embryos to the hanging tubes, which are located lower than the uterine fundus; or by reflux expulsion of the embryo due to embryonic migration to the fallopian tubes, either spontaneously or secondary to uterine contractions (80). The technique of ET itself may also be a culprit in EUP, although this is controversial (81). For example, while Yovich et al. (82) noted a significantly higher rate of EUP when the embryos were placed high near the uterine fundus or into the tube itself, rather than in the lower uterus, Friedman et al. (83) have demonstrated that blastocyst transfer closer to the fundus (<10 mm) is associated with a higher pregnancy rate. However, although in the latter study no EUP occurred in the <10-mm group, this outcome should be monitored closely in larger studies.

The transfer volume of culture media containing embryos may play a role in embryonic migration into the fallopian tubes. While most clinicians contend that more than 80 μL of media is needed for the embryo to reach the fallopian tube (62), Knutzen et al. (84), using a mock intrauterine ET with 50 μL of radiopaque dye, demonstrated easy passage of all or part of the material in 44% of patients. Lesny et al. (85) explained these findings as due to the propulsion of the embryo from the uterine fundus into the tubes by the junctional zone contractions. Therefore, as the likelihood of tubal placement is very high, the development of tubal pregnancy is not due solely to embryos reaching the tubes, but rather to an additional pathological process that prevents their movement back into the uterine cavity. Potential mechanisms may involve tubal disease affecting the luminal surface and thereby delaying or blocking embryonic passage into the uterine cavity, external factors that interfere with tubal motility, and abnormal embryos (69), such as those derived from chromosomally abnormal gametes (86).

To ameliorate the role of abnormal fallopian tubes in the pathogenesis of EUP after IVF, several authors have recommended that the tubes be occluded at the level of the uterotubal junction (87,88). However, this measure does not prevent the development of an interstitial pregnancy (71), although it certainly prevents the well-known phenomenon of spontaneous pregnancies after IVF treatment, which occur in 30% of patients with patent tubes (89).

Another potential interfering factor in tubal function and ET is the different hormonal milieus resulting from ovulation induction protocols, particularly those including clomiphene citrate (68,90). This may result from the effect of the high estradiol levels on tubal peristalsis through the control of tubal smooth muscle contractility and ciliary activity (82,90). Pygriotis et al. (71), however, did not demonstrate a difference in estradiol levels on the day of human chorionic gonadotropin (hCG) administration between IVF patients with and without EUP. Furthermore, they found an increased proportion of EUPs in frozen ETs following natural cycles in which the estradiol levels were comparatively low.

In summary, the reproductive health characteristics of infertile women, the different hormonal milieus, the technical issues of IVF procedures, and the estimated embryo implantation potential were all suggested as possible risk factors (91); however, the mechanisms are still uncertain and need further investigation.

Heterotopic pregnancy following ART

The general incidence of combined intrauterine and extrauterine (heterotopic) pregnancy is 1:15,000–30,000, and it increases dramatically to 1:100 in pregnancies following ART or ovulation induction (92–94). Although a distorted pelvic anatomy is responsible for the predisposition to both extrauterine and heterotopic pregnancy (95–97), heterotopic pregnancies are associated with a greater number of embryos transferred, whereas EUP is not. Tummon et al. (98) reported that when four or more embryos were transferred, the odds ratio for the development of a heterotopic pregnancy versus EUP was 10. The difficult diagnosis of this potentially life-threatening complication is often made during emergency surgery following tubal rupture and hemoperitoneum. In about 70% of cases, the outcome of the intrauterine pregnancy is favorable (live birth) once the EUP is terminated (99,100). A high index of suspicion and early intervention are mandatory to salvage the viable intrauterine pregnancy and prevent maternal mortality.

Diagnosis and treatment

Noninvasive diagnostic measures using transvaginal ultrasonography combined with serum hCG monitoring have proved to be a reliable tool in the diagnosis of EUP. Since most pregnancies following ART are monitored at an early stage before the onset of symptoms, early diagnosis of the condition and improved management and care have resulted in a decline in the morbidity and mortality of EUP. Of note is the fact that treating EUP with methotrexate has no influence on patients' serum anti-Mullerian hormone levels (101), nor patients' performance in the following IVF cycle (102,103). The diagnosis and treatment of EUP are beyond the scope of this chapter, and readers are referred elsewhere for detailed reviews (104,105).

BRIEF SUMMARY

Transvaginal ultrasound-guided aspiration of oocytes is a well-accepted and universally used method in assisted reproduction. Its major advantages include easy access to ovarian follicles with excellent oocyte yield and good visualization of the major pelvic vessels, and it is usually atraumatic. Nevertheless, there are some inherent risks, namely puncture of blood vessels and intra-abdominal or retroperitoneal bleeding, bleeding from the vaginal vault puncture site, rupture or perforation of pelvic organs, and pelvic infection. In addition, ET itself may be associated with complications such as pelvic infection, multiple pregnancy, or EUP. This chapter has comprehensively presented and discussed three of these complications: bleeding, PID, and EUP.

REFERENCES

1. Dicker D, Ashkenazi J, Feldberg D et al. Severe abdominal complications after transvaginal ultrasonographically-guided retrieval of oocytes for *in vitro* fertilization and embryo transfer. *Fertil Steril* 1993; 59: 1313–5.
2. Feldberg D, Goldman JA, Ashkenazi J et al. Transvaginal oocyte retrieval controlled by vaginal probe for *in vitro* fertilization: A comparative study. *J Ultrasound Med* 1988; 7: 726–8.
3. Maxwell KN, Cholst IN, Rosenwaks Z. The incidence of both serious and minor complications in young women undergoing oocyte donation. *Fertil Steril* 2008; 90: 2165–71.
4. Modder J, Kettel LM, Sakamoto K. Hematuria and clot retention after transvaginal oocyte aspiration: A case report. *Fertil Steril* 2006; 86: e1–2.
5. Tureck RW, Garcia C, Blasco L, Mastroianni L. Perioperative complications arising after transvaginal oocyte retrieval. *Obstet Gynecol* 1993; 81: 590–3.
6. Lenz S, Leeton J, Renou P. Transvaginal recovery of oocytes for *in vitro* fertilization using vaginal ultrasound. *J In Vitro Fert Embryo Transf* 1987; 4: 51–5.
7. Wikland M, Blad S, Bungum L, Hillensjo T, Karlstrom PO, Nilsson S. A randomized controlled study comparing pain experience between a newly designed needle with a thin tip and a standard needle for oocyte aspiration. *Hum Reprod* 2011;.26:1377–83.
8. Serour GI, Aboulghar M, Mansour R et al. Complica tions of medically assisted conception in 3500 cycles. *Fertil Steril* 1998; 70: 638–42.
9. Govaert I, Devreker F, Delbaere A et al. Short-term medical complications of 1500 oocyte retrievals for *in vitro* fertilization and embryo transfer. *Eur J Obstet Gynecol Reprod Biol* 1998; 77: 239–43.
10. Aragona C, Mohamed MA, Espinola MS et al. Clinical complications after transvaginal oocyte retrieval in 7,098 IVF cycles. *Fertil Steril* 2011; 95: 293–4.
11. Sauer MV. Defining the incidence of serious complications experienced by oocyte donors: A review of 1000 cases. *Am J Obstet Gynecol* 2001; 184: 277–8.
12. Liberty G, Hyman JH, Eldar-Geva T et al. Ovarian hemorrhage after transvaginal ultrasonographically guided oocyte aspiration: A potentially catastrophic and not so rare complication among lean patients with polycystic ovary syndrome. *Fertil Steril* 2010; 93: 874–9.
13. Zhen X, Qiao J, Ma C, Fan Y, Liu P. Intraperitoneal bleeding following transvaginal oocyte retrieval. *Int J Gynaecol Obstet* 2010;108:31–4.
14. Yih MC, Goldschlag D, Davis OK et al. Complete blood counts (CBC) after oocyte retrieval: What is normal? *Fertil Steril* 2001; 76: S115–6.
15. Azem F, Wolf Y, Botchan A et al. Massive retroperitoneal bleeding: A complication of transvaginal ultrasonography-guided oocyte retrieval for *in vitro* fertilization-embryo transfer. *Fertil Steril* 2000; 74: 405–6.
16. Fiori O, Cornet D, Darai E, Antoine JM, Bazot M. Uro-retroperitoneum after ultrasound-guided transvaginal follicle puncture in an oocyte donor: A case report. *Hum Reprod* 2006; 21: 2969–71.
17. Nouri K, Walch K, Promberger R, Kurz C, Tempfer CB, Ott J. Severe haematoperitoneum caused by ovarian bleeding after transvaginal oocyte retrieval: A retrospective analysis and systematic literature review. *Reprod Biomed Online* 2014;29:699–707.
18. Battaglia C, Regnani G, Giulini S et al. Severe intraabdominal bleeding after transvaginal oocyte retrieval for IVF-ET and coagulation factor XI deficiency: A case report. *J Assist Reprod Genet* 2001; 3: 178–81.
19. Mashiach R, Stockheim D, Zolti M, Orvieto R. Delayed intra-abdominal bleeding following transvaginal ultrasonography guided oocyte retrieval for *in vitro* fertilization in patients at risk for thrombo-embolic events under anticoagulant therapy. *F1000Res* 2013; 2: 189.
20. Revel A, Schejter-Dinur Y, Yahalomi SZ et al. Is routine screening needed for coagulation abnormalities before oocyte retrieval? *Fertil Steril* 2011; 95: 1182–4.
21. Shalev J, Orvieto R, Meizner I. Use of color Doppler sonography during follicular aspiration in patients undergoing *in vitro* fertilization may reduce the risk of blood vessel injury. *Fertil Steril* 2004; 81: 1408–10.
22. Rísquez F, Confino E. Can doppler ultrasound-guided oocyte retrieval improve IVF safety? *Reprod Biomed Online* 2010; 21: 444–5.
23. Thompson JD, Rock WA. Control of pelvic hemorrhage. In: *Te Linde's Operative Gynecology*. 8th edn. Rock JA, Thompson JD (eds). Philadelphia, PA: Lippincott-Raven, 1997, pp. 197–232.
24. Howe RS, Wheeler C, Mastroianni L, Jr. et al. Pelvic infection after transvaginal ultrasound-guided ovum retrieval. *Fertil Steril* 1988; 49: 726–8.
25. Curtis P, Amso N, Keith E et al. Evaluation of the risk of pelvic infection following transvaginal oocyte recovery. *Hum Reprod* 1992; 7: 625–6.
26. Ashkenazi J, Farhi J, Dicker D et al. Acute pelvic inflammatory disease after oocyte retrieval: Adverse effects on the results of implantation. *Fertil Steril* 1994; 61: 526–8.
27. Al-Kuran O, Beitawi S, Al-Mehaisen L. Pelvic abscess complicating an *in vitro* fertilization pregnancy and review of the literature. *J Assist Reprod Genet* 2008; 25: 341–3.
28. Sharpe K, Karovitch AJ, Claman P, Suh KN. Transvaginal oocyte retrieval for *in vitro* fertilization complicated by ovarian abscess during pregnancy. *Fertil Steril* 2006; 86: 219.e11–3.
29. Kelada E, Ghani R. Bilateral ovarian abscesses following transvaginal oocyte retrieval for IVF: A case report and review of literature. *J Assist Reprod Genet* 2007; 24: 143–5.

30. Yaron Y, Peyser MR, Samuel D et al. Infected endometriotic cysts secondary to oocyte aspiration for *in vitro* fertilization. *Hum Reprod* 1994; 9: 1759–60.
31. Nargund G, Parsons J. Infected endometriotic cysts secondary to oocyte aspiration for *in vitro* fertilization. *Hum Reprod* 1995; 10: 1555.
32. Elizur SE, Lebovitz O, Weintraub AY, Eisenberg VH, Seidman DS, Goldenberg M, Soriano D. Pelvic inflammatory disease in women with endometriosis is more severe than in those without. *Aust N Z J Obstet Gynaecol* 2014;54:162–5.
33. Sauer MV, Paulson RJ. Pelvic abscess complicating transcervical embryo transfer. *Am J Obstet Gynecol* 1992; 166: 148–9.
34. Friedler S, Ben-Shachar I, Abramov Y et al. Ruptured tubo-ovarian abscess complicating transcervical cryopreserved embryo transfer. *Fertil Steril* 1996; 65: 1065–6.
35. Ng SC, Edirisinghe WR, Sathanathan AH, Ratnam SS. Bacterial infection of human oocytes during *in vitro* fertilization. *Int J Fertil* 1987; 32: 298–301.
36. Giri SN, Emau P, Cullor JS et al. Effect of endotoxin on circulating levels of eicosanoids, progesterone, cortisol, glucose and lactic acid, and abortion in pregnant cows. *Vet Microbiol* 1990; 21: 211–31.
37. Ben-Rafael Z, Orvieto R. Cytokine involvement in reproduction. *Fertil Steril* 1992; 58: 1093–9.
38. Tartakovski B, Ben-Yair E. Cytokines modulate preimplantation development and pregnancy. *Dev Biol* 1991; 146: 345–52.
39. Romero R, Espinoza J, Mazor M. Can endometrial infection/inflammation explain implantation failure, spontaneous abortion, and preterm birth after *in vitro* fertilization? *Fertil Steril* 2004; 82: 799–804.
40. Saper CB, Breder CD. Endogenous pyrogens in the CNS: Role in the febrile response. *Prog Brain Res* 1992; 93: 419–28.
41. Hanson DF. Fever and the immune response. the effects of physiological temperatures on primary murine splenic T-cell responses *in vitro*. *J Immunol* 1993; 151: 436–48.
42. Kitano Y, Okada N. Organization and disorganization of actin filament in human epidermal keratinocyte: Heat, shock treatment and recovery process. *Cell Tissue Res* 1990; 261: 269–74.
43. Milunski A, Ulcickas M, Rothman KJ et al. Maternal heat exposure and neural tube defects. *JAMA* 1992; 268: 882–5.
44. Edwards MJ, Mulley R, Ring S, Wanner RA. Mitotic cell death and delay of mitotic activity in guinea-pig embryos following brief maternal hyperthermia. *J Embryol Exp Morphol* 1974; 32: 593–602.
45. Hendricks AG, Stone GW, Hendrickson RV, Matayoshi K. Teratogenic effects of hyperthermia in the bonnet monkey (Macaca radiata). *Teratology* 1979; 19: 177–82.
46. Russell JB, DeCherney AH, Hobbins JC. A new transvaginal probe and biopsy guide for oocyte retrieval. *Fertil Steril* 1987; 47: 350–2.
47. Meldrum DR. Antibiotics for vaginal oocyte aspiration. *J In Vitro Fert Embryo Transf* 1989; 6: 1–2.
48. Tsai YC, Lin MY, Chen SH et al. Vaginal disinfection with povidone iodine immediately before oocyte retrieval is effective in preventing pelvic abscess formation without compromising the outcome of IVF-ET. *J Assist Reprod Genet* 2005; 22: 173–5.
49. Borlum KG, Maiggard S. Transvaginal oocyte aspiration and pelvic infection. *Lancet* 1989; 2: 53.
50. Van Os HC, Roozenburg BJ, Janssen-Caspers HAB et al. Vaginal disinfection with povidone iodine and the outcome of *in vitro* fertilization. *Hum Reprod* 1992; 7: 349–50.
51. Peters AJ, Hecht B, Durinzi K et al. Salpingitis or oophoritis: What causes fever following oocyte aspiration and embryo transfer? *Obstet Gynecol* 1993; 81: 876–7.
52. Wren M, Parson J. Ultrasound directed follicle aspiration in IVF. In: *Recent Advances in the Management of Infertility*. Chen C, Tan SL, Cheng WC (eds). New York, NY: McGraw-Hill, 1989, pp. 105–81.
53. Ashkenazi J, Ben-David M, Feldberg D et al. Abdominal complications following ultrasonically-guided percutaneous transvesical collection of oocytes for *in vitro* fertilization. *J In Vitro Fert Embryo Transf* 1987; 4: 316–8.
54. Egbase PE, Udo EE, Al-Shharhan M, Grudzinskas JG. Prophylactic antibiotics and endocervical microbial inoculation of the endometrium at embryo transfer. *Lancet* 1999; 354: 651–2.
55. Peikrishvili R, Evrard B, Pouly JL, Janny L. Prophylactic antibiotic therapy (amoxicillin + clavulanic acid) before embryo transfer for IVF is useless. results of a randomized study. *J Gynecol Obstet Biol Reprod* 2004; 33: 713–9.
56. Caspi B, Zalel Y, Or Y et al. Sonographically-guided aspiration: An alternative therapy for tubo-ovarian abscess. *Ultrasound Obstet Gynecol* 1996; 7: 439–42.
57. To J, Aldape D, Frost A, Goldberg GL, Levie M, Chudnoff S. Image-guided drainage versus antibiotic-only treatment of pelvic abscesses: Short-term and long-term outcomes. *Fertil Steril* 2014;102:1155–9.
58. Centers for Disease Control and Prevention. Ectopic pregnancy—United States, 1990–1992. *MMWR Recomm Rep* 1995; 1: 46.
59. Berg CJ, Atrash HR, Koonin LM, Tucker M. Pregnancy-related mortality in the United States, 1987–1990. *Obstet Gynecol* 1996; 88: 161.
60. Steptoe P, Edwards R. Reimplantation of a human embryo with subsequent tubal pregnancy. *Lancet* 1976; 1: 830–2.
61. Azem F, Yaron Y, Botchan A et al. Ectopic pregnancy after *in vitro* fertilization-embryo transfer (IVF/ET): The possible role of the ET technique. *J Assist Reprod Genet* 1993; 10: 302–4.

62. Zouves C, Erenus M, Gomel V. Tubal ectopic pregnancy after *in vitro* fertilization and embryo transfer: A role for proximal occlusion or salpingectomy after failed distal tubal surgery. *Fertil Steril* 1991; 56: 691–5.
63. SART, ASRM. Assisted reproductive technology in the United States: 1996 results generated from the American society for reproductive medicine/society for assisted reproductive technology registry. *Fertil Steril* 1999; 71: 798–807.
64. SART, ASRM. Assisted reproductive technology in the United States: 201 results generated from the American society for reproductive medicine/society for assisted reproductive technology registry. *Fertil Steril* 2007; 87: 1253–66.
65. Perkins KM, Boulet SL, Kissin DM, Jamieson DJ, National ART Surveillance (NASS) Group. Risk of ectopic pregnancy associated with assisted reproductive technology in the United States, 2001–2011. *Obstet Gynecol* 2015; 125: 70–8.
66. Martinez F, Trounson A. An analysis of factors associated with ectopic pregnancy in a human *in vitro* fertilization program. *Fertil Steril* 1986; 45: 79–87.
67. Karande VC, Flood JT, Heard N et al. Analysis of ectopic pregnancies resulting from *in vitro* fertilization and embryo transfer. *Hum Reprod* 1991; 6: 446–9.
68. Verhulst G, Camus M, Bollen N et al. Analysis of the risk factors with regard to the occurrence of ectopic pregnancy after medically assisted procreation. *Hum Reprod* 1993; 8: 1284–7.
69. Herman A, Ron-El R, Golan A et al. The role of tubal pathology and other parameters in ectopic pregnancies occurring in *in vitro* fertilization and embryo transfer. *Fertil Steril* 1990; 54: 864–8.
70. Correy JF, Watkins RA, Bradfield GF et al. Spontaneous pregnancies and pregnancies as a result of treatment on an *in vitro* fertilization program terminating in ectopic pregnancies or spontaneous abortions. *Fertil Steril* 1988; 50: 85–8.
71. Pygriotis E, Sultan KM, Neal GS et al. Ectopic pregnancies after *in vitro* fertilization and embryo transfer. *J Assist Reprod Genet* 1994; 11: 80–3.
72. Cohen J, Mayaux MJ, Guihard-Moscato ML. Pregnancy outcomes after *in vitro* fertilization. A collaborative study on 2342 pregnancies. *Ann NY Acad Sci* 1988; 54: 1–6.
73. Marcus SF, Brinsden PR. Analysis of the incidence and risk factors associated with ectopic pregnancy following *in-vitro* fertilization and embryo transfer. *Hum Reprod* 1995; 10: 199–203.
74. Acharya KS, Acharya CR, Provost MP, Yeh JS, Steward RG, Eaton JL, Muasher SJ. Ectopic pregnancy rate increases with the number of retrieved oocytes in autologous *in vitro* fertilization with non-tubal infertility but not donor/recipient cycles: An analysis of 109,140 clinical pregnancies from the Society for Assisted Reproductive Technology registry. *Fertil Steril* 2015; 104: 873–8.
75. Ankum WM, Mol BW, Van der Veen et al. Risk factors for ectopic pregnancy: A meta-analysis. *Fertil Steril* 1996; 66: 513–6.
76. Lesny P, Killick SR, Robinson J, Maguiness SD. Transcervical embryo transfer as a risk factor for ectopic pregnancy. *Fertil Steril* 1999; 72: 305–9.
77. Clayton HB, Schieve LA, Peterson HB et al. Ectopic pregnancy risk with assisted reproductive technology procedures. *Obstet Gynecol* 2006; 107: 595–604.
78. Londra L, Moreau C, Strobino D, Garcia J, Zacur H, Zhao Y. Ectopic pregnancy after *in vitro* fertilization: Differences between fresh and frozen-thawed cycles. *Fertil Steril* 2015; 104: 110–8.
79. Fang C, Huang R, Wei LN, Jia L. Frozen–thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. *Fertil Steril* 2015; 103: 655–61.
80. Russell JB. The etiology of ectopic pregnancy. *Clin Obstet Gynecol* 1987; 30: 181–90.
81. Schoolcraft WB, Surrey ES, Gardner DK. Embryo transfer: Techniques and variables affecting success. *Fertil Steril* 2001; 76: 863–70.
82. Yovich JL, Turner SR, Murphy AJ. Embryo transfer technique as a cause of ectopic pregnancies in *in vitro* fertilization. *Fertil Steril* 1985; 44: 318–21.
83. Friedman BE, Lathi RB, Henne MB, Fisher SL, Milki AA. The effect of air bubble position after blastocyst transfer on pregnancy rates in IVF cycles. *Fertil Steril* 2011; 95: 944–7.
84. Knutzen UK, Sotto-Albors CE, Fuller D et al. Mock embryo transfer (MET) in early luteal phase, the cycle prior to *in vitro* fertilization and embryo transfer (IVF/ ET). *Proceedings of the 45th Annual Meeting of the American Fertility Society*. San Francisco, CA, November 13–16, 1989. American Fertility Society, Program Supplement, pS152: 299.
85. Lesny P, Killick SR, Tetlow RL et al. Embryo transfer—Can we learn anything new from the observation of junctional zone contraction? *Hum Reprod* 1998; 13: 1540–6.
86. Job-Spira N, Coste J, Boue J et al. Chromosomal abnormalities and ectopic pregnancy? New directions for aetiological research. *Hum Reprod* 1996; 11: 239–43.
87. Svare J, Norup P, Grove Thomsen S et al. Heterotopic pregnancies after *in vitro* fertilization and embryo transfer—A Danish survey. *Hum Reprod* 1993; 8: 116–8.
88. Tucker M, Smith D, Pike I et al. Ectopic pregnancy following *in vitro* fertilization and embryo transfer. *Lancet* 1981; 2: 1278.
89. Ben-Rafael Z, Mashiach S, Dor J et al. Treatment-independent pregnancy after *in vitro* fertilization and embryo transfer trial. *Fertil Steril* 1986; 45: 564–7.
90. Fernandez H, Coste J, Job-Spira N. Controlled ovarian hyperstimulation as a risk factor for ectopic pregnancy. *Obstet Gynecol* 1991; 78: 656–9.

91. Chang HJ, Suh CS. Ectopic pregnancy after assisted reproductive technology: What are the risk factors? *Curr Opin Obstet Gynecol* 2010; 22: 202–7.
92. Ben-Rafael Z, Carp HJ, Mashiach S et al. The clinical features and incidence of concurrent intra and extra uterine pregnancies. *Acta Eur Fertil* 1985; 16: 199–202.
93. Dimitry ES, Subak-Sharpe R, Mills M et al. Nine cases of heterotopic pregnancies in 4 years of *in vitro* fertilization. *Fertil Steril* 1990; 53: 107–10.
94. Tal J, Hadad S, Gordon M et al. Heterotopic pregnancy after ovulation induction and assisted reproduction technologies: A literature review from 1971 to 1993. *Fertil Steril* 1996; 66: 1–12.
95. Goldman GA, Fisch B, Ovadia J, Tadpir Y. Heterotopic pregnancy after assisted reproductive technologies. *Obstet Gynecol Surv* 1992; 47: 217–21.
96. Molloy D, Deambrosis W, Keeping D et al. Multiple-sited (heterotopic) pregnancy after *in vitro* fertilization and gamete intrafallopian transfer. *Fertil Steril* 1990; 53: 1068–71.
97. Li HP, Balmaceda JP, Zouves C et al. Heterotopic pregnancy associated with gamete intra-fallopian transfer. *Hum Reprod* 1992; 7: 131–5.
98. Tummon IS, Whitmore NA, Daniel SAJ et al. Transferring more embryos increases risk of heterotopic pregnancy. *Fertil Steril* 1964; 61: 1065–7.
99. Rizk B, Tan SL, Morcos S et al. Heterotopic pregnancies after *in vitro* fertilization and embryo transfer. *Am J Obstet Gynecol* 1991; 164: 161–4.
100. Rojanski N, Schenker JG. Heterotopic pregnancy and assisted reproduction—An update. *J Assist Reprod Genet* 1996; 13: 594–601.
101. Oriol B, Barrio A, Pacheco A et al. Systemic methotrexate to treat ectopic pregnancy does not affect ovarian reserve. *Fertil Steril* 2008; 90: 1579–82.
102. Orvieto R, Kruchkovich J, Zohav E et al. Does methotrexate treatment for ectopic pregnancy influence the patient's performance during a subsequent *in vitro* fertilization/embryo transfer cycle? *Fertil Steril* 2007; 88: 1685–6.
103. Wiser A, Gilbert A, Nahum R et al. Effects of treatment of ectopic pregnancy with methotrexate or salpingectomy in the subsequent IVF cycle. *Reprod Biomed Online* 2013;26:449–53.
104. Rock JA, Damario MA. Ectopic pregnancy. In: *Te Linde's Operative Gynecology*. 8th edn. Rock JA, Thompson JD (eds). Philadelphia, PA: Lippincott-Raven, 1997, pp. 501–27.
105. Yao M, Tulandi T. Current status of surgical and non-surgical management of ectopic pregnancy. *Fertil Steril* 1997; 67: 421–33.

Iatrogenic multiple pregnancies

The risk of assisted reproduction technology

67

ISAAC BLICKSTEIN and NATASA TUL

INTRODUCTION

The common denominator of most assisted reproduction technologies (ARTs) is ovarian (hyper)stimulation. The scheme to expose excess female gametes to abundant sperm intended to increase fertilization may inadvertently produce multiple zygotes. In ovulation induction, the number of fertilized eggs is uncontrolled and unpredicted. By contrast, the number of zygotes transferred in ART cycles has been always under control. Consequently, multiple pregnancies following ART cycles are almost exclusively physician-made (i.e., iatrogenic multiple pregnancies [IMPs]).

There are two exceptions to this statement. First, single-embryo transfer (SET) may still be associated with an increased risk of monozygotic (MZ) twins since ART augments the rate of zygotic splitting (1,2). Second, observations from the East Flanders Prospective Twin Survey suggest that a genetic familial trait for spontaneous twins may also be involved in induced conceptions. Hence, women with "twins in the family" undergoing infertility treatment (3) may be at increased risk of having multiples as compared with women without that characteristic.

Regardless of the mechanism involved in IMP, ART undoubtedly increases the risk of multiple births. In 1998, the incidence of multiples in the U.K. was 25% twins and 5% triplets (4). Whilst globally there have been reductions over recent years in these rates following ART cycles, it is still an issue. Roughly, these reference figures represent 20- and 50-fold increased frequencies of iatrogenic twins and triplets, respectively, as compared with naturally occurring multiples. Table 67.1 shows a simple model of an obstetrical service with 4000 live births/year, including 5% following iatrogenic pregnancies (5). In this model, the number of twins is doubled and that of triplets is 3.5-fold increased. Importantly, 5% of iatrogenic pregnancies will produce an excess of 31.5/1000 multiple-pregnancy neonates over the expected rate in spontaneous pregnancies.

ART and ovulation induction, the major contributors to the epidemic of multiple pregnancies, did not arise *ex vacuo*. In a modern society, women rely on efficient modern fertility treatment when deciding on postponing childbirth. It follows that advanced maternal age, by itself an accepted risk factor for natural multiples, is also a significant risk factor for reduced fecundity and increased need for fertility treatment. Thus, social trends act in concert with available ARTs to increase the risk of multiple pregnancies. Figure 67.1 shows the ratio of spontaneous to induced twins in East Flanders over the last two decades. Except for the unexplained "hump" in 1980, there is a clear change in the rate of induced twins from 1:46 to one in every two to three twins (6). This population-based trend might be even more accentuated in hospital-based data.

The wide spectrum of issues encompassed in IMP deserves a separate volume (7,8). In this chapter, several risks of multiple pregnancies following ART cycles will be specifically addressed.

Important information related to IMPs comes from the Israeli national registry of very low birthweight (VLBW; <1500 g) twins. Figure 67.2 shows that over the years, the frequency of VLBW twins as a result of ovulation induction did not increase. At the same time, however, the frequency of VLBW twins as a result of *in vitro* fertilization (IVF) is steadily increasing, suggesting that the "disease burden" of small twins is clearly man-made and potentially avoidable.

THE PREGNANCY

It is beyond the scope of this chapter to describe in detail the risks associated with multiple pregnancies (9,10). It is generally accepted that the human female is programmed for mono-ovulation, monofetal development, and nursing only one neonate. Consequently, pregnancies with more than one fetus overwhelm the uterine capacity to adequately nurture the fetuses. Animal and human models have repeatedly demonstrated the reciprocal relationship between birthweight and gestational age at delivery and litter size. Using singleton standards, a significant proportion of twins and all high-order multiple pregnancies (HOMPs) will be delivered preterm and will be small for gestational age. In addition to absolute growth restriction, relative (discordant) growth is common (11).

As a result of the limited uterine capacity, a natural reduction in fetal number is frequently seen. At the early stages, the embryo may disappear ("vanishing twin syndrome") in one of every six to seven twin pregnancies following ART cycles (12,13). Vanishing twin syndrome, which is considered by many to be natural multifetal pregnancy reduction (MFPR), gained special attention when Pharoah and Cooke hypothesized that single embryonic death may be implicated in cerebral palsy (CP) in the survivor (14,15). However, Matias et al. (16) summarized several case–control studies on plurality-dependent spontaneous embryonic loss rates after ART and found that twin pregnancies have a two- to five-fold lower miscarriage rate of the entire pregnancy compared with singletons. At present, it is unclear if this advantage is a chance event or related to the presence of a higher placental (and hormonal) support of the early pregnancy.

Table 67.1 Estimating the contribution of 5% iatrogenic conceptions in an obstetrical service with 4000 deliveries/year (spontaneous, 1.2% twins and 0.1% triplets; iatrogenic, 25% twins and 5% triplets)

	Singles	Twins	Triplets	Births	Neonates
100% spontaneous	3948	48	4	4000	4056
5% iatrogenic	140	50	10	200	270
95% spontaneous	3750	46	4	3800	3854
Total	3890	96	14	4000	4124

Source: Adapted from Blickstein I. Perinatal implications of iatrogenic multiple pregnancies. In: *4th World Congress of Perinatal Medicine*. Voto LS, Margulies M, Cosmi EV (eds). Bologna, Italy: Monduzzi Editore, 1999, pp. 167–72.

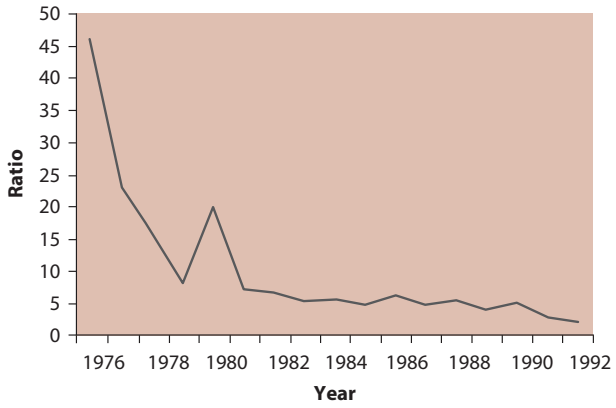

Figure 67.1 Ratio of spontaneous to induced twins. Since the implementation of effective infertility treatment, the ratio changed from one induced for every 40–50 spontaneous twin pregnancies to one induced for every two to three spontaneous twin pregnancies. (Adapted from the East Flanders Prospective Twin Survey: Blickstein I. Perinatal implications of iatrogenic multiple pregnancies. In: *4th World Congress of Perinatal Medicine*. Voto LS, Margulies M, Cosmi EV (eds). Bologna, Italy: Monduzzi Editore, 1999, pp. 167–72.)

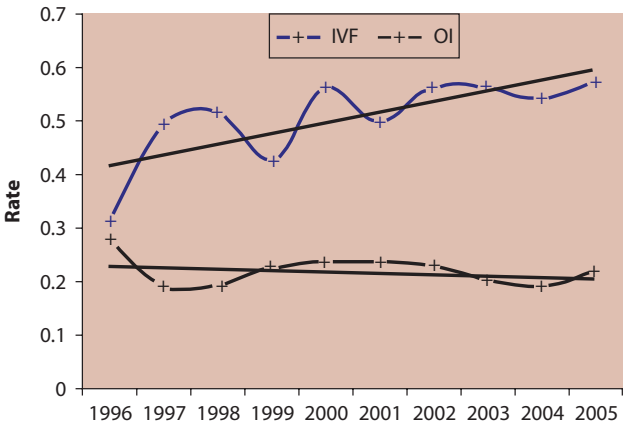

Figure 67.2 The rates of very low birthweight twins as a result of IVF and of OI (Isaac Blickstein, unpublished observation). *Abbreviations*: IVF, in vitro fertilization; OI, ovulation induction.

Multiples are associated with higher frequencies of malformations of varied etiology. The as-yet unknown factor(s) that causes zygotic splitting have been implicated in causing structural malformations in MZ twins. In the subset with monochorionic (MC) placentas, also encountered in HOMPs, twin–twin transfusion syndrome (TTTS) may affect as many as 10%–15% of the pairs and may result in major morbidity of one or both twins. Later in pregnancy, single fetal demise associated with MC placentas may result in severe end-organ damage in the survivor.

Finally, it has been shown that the risk of CP is five- to six-fold and 23-fold increased in twins and triplets, respectively, compared with singletons (17). A model based on British data related to the transfer of two and three embryos (4) and on British data related to CP in multiples (18) suggested a significantly lower estimated CP rate (2.7/1000 neonates) after spontaneous pregnancies compared with transfer of three embryos (odds ratio [OR] = 6.3), two embryos (OR = 3.3), and three embryos in which all triplets had been reduced to twins (OR = 3.8) (Figure 67.3) (19).

Three additional aspects deserve further consideration. First, as mentioned above, there is an increased risk of zygotic splitting following ART cycles. It is not known

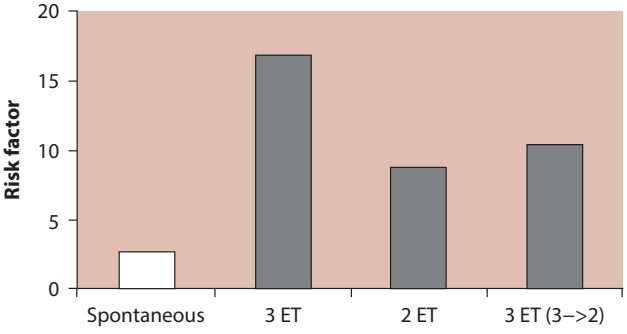

Figure 67.3 Estimated risk of cerebral palsy following transfer of three and two embryos, and following natural multifetal pregnancy reduction of all triplets to twins. A three- to six-fold increased risk of cerebral palsy is expected. *Abbreviation*: ET, embryo transfer. (Adapted from Blickstein I, Weissman A. *N Engl J Med* 1999; 341: 1313–4.)

why MZ twins are more frequent in conceptions after ART cycles. The most common cause-and-effect speculation suggests that the exposure of the zona pellucida to biochemical or mechanical trauma leads to herniation of the blastocyst and splitting of the zygote. Zygotic splitting is not only a biological enigma, but is also a major area of clinical importance, primarily because of the confirmed increased morbidity and mortality associated with MZ twinning.

Currently, zygotic splitting is inferred when the number of fetuses exceeds that of transferred embryos, or when monoamniotic twins are diagnosed. Evidently, the reported figures underestimate the true incidence, since bichorionic (BC) MZ twins cannot be clinically differentiated from same-sex BC dizygotic (DZ) twins. In addition, previous reports did not mention the number of transferred embryos and/or the method of ART. In an initial study (1), the data indicated that splitting is expected in 4.9% after IVF without intracytoplasmic sperm injection (ICSI) at a 12-times higher rate than the 0.4% rate of MZ twins in spontaneous conceptions. In a subsequent study (2) of a much larger data set from British IVF centers, Blickstein et al. found "only" a six-fold increased splitting rate. Blickstein and Keith (20) postulated that, given the remarkably constant frequency of MZ twins in different populations, there might be splitting-prone oocytes after fertilization. Thus, the higher the number of ovulation events (i.e., following ovarian stimulation), the greater the chance of recruiting a splitting-prone oocyte for ovulation, as is indeed the case with all methods of assisted conceptions. In addition, the more fecund patients with a better chance to conceive are significantly more likely to have MZ twins, as seen in those receiving a less "aggressive" regimen such as clomiphene citrate as the sole treatment, compared with other ovulation-enhancing agents (21). It follows that the chance of a follicle that contains an oocyte with a propensity to undergo splitting is quasi-"dose dependent," where the term "dose" refers to the combined effect of the patient's fecundity and the specific treatment administered. This finding is supported by the possibility that ovarian stimulation—the common denominator of all assisted procreation—may affect oocyte development, which could predispose to splitting (Figure 67.4). Irrespective of the mode of generation of MZ–MC twins after ART, these pregnancies remain rare. In a recent study, Simões et al. (22) found that MC twin pregnancies comprised 7.2% of all ART twins and 4.9% of all MC twins in their data set. These twins have a significantly worse outcome compared with bichorionic sets in terms of lower gestational age and birthweight. ART appears to increase the already high risk of monochorionicity compared with

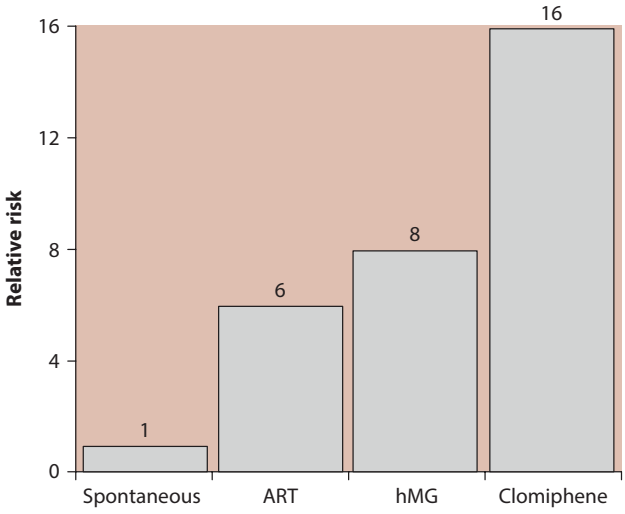

Figure 67.4 Zygotic splitting. Frequency of monozygotic twins following various methods of assisted reproduction. The accepted 0.4% rate of spontaneous zygotic splitting was used as a reference. Data from the East Flanders Prospective Twin Survey. *Abbreviations*: ART, assisted reproduction technology; hMG, human menopausal gonadotropin. (Adapted from Blickstein I et al. *N Engl J Med* 1999; 340: 738–9.)

spontaneous conception in terms of preterm birth at <32 weeks and birthweight <1500 g. These data should nonetheless be interpreted with caution because of the small sample size.

Second, one must also reconsider mortality figures in HOMPs undergoing MFPR. There is little doubt that MFPR is among the ultimate paradoxes of medicine, whereby infertile patients undergo intricate treatments and, when at last successful, may have to consider reduction (= termination) of their "surplus" fetuses (= success). At the same time, there is little doubt that MFPR may be the only solution for a potentially successful outcome of a high-order multiple gestation. MFPR, discussed elsewhere in this volume, is indeed associated with improved perinatal outcome, as expected from comparing HOMPs with twins or singletons. However, given that all fetuses have a similar survival potential, it is argued that the reduced fetus(es) should be included in the mortality figures of MFPR (23). Table 67.2 shows the minimal mortality rates associated with various MFPR procedures, which suggest, quite bluntly, that MFPR is in fact a lethal iatrogenic sequence of iatrogenic multiples.

The third point to consider is the frequently overlooked risk of chromosomal disorders in IMP. Although each

Table 67.2 Minimal mortality rates in various natural multifetal pregnancy reduction (MFPR) combinations

MFPR	4 → 2	5 → 2	3 → 2	4 → 1	3 → 1	2 → 1
Minimal mortality (%)	50	60	33	75	66	50

of the fetuses in a polyzygotic multiple gestation has the same chance for an aberration, as does a singleton with similar risk variables, there is an increased risk for the mother that one of her multiples will be affected. Data have clearly substantiated older calculations that showed that a 31-year-old mother of DZ twins carries a similar risk of having one twin with Down's syndrome as a 35-year-old mother of a singleton (24). Given that IMPs are more common in older mothers and that biochemical markers are less useful for twins and unavailable for HOMPs, one must rely on nuchal translucency measurements (25,26) or on invasive cytogenetic procedures (amniocentesis or chorionic villus sampling [(CVS]). Regrettably, little information exists about the former in HOMPs, and the latter carries an increased risk of miscarriage in these premium pregnancies.

Considering all of the risks associated with IMP, one undoubtedly should prefer a singleton to a multiple pregnancy. To minimize risks, no more than a single embryo should be transferred. This policy has been implemented in recent years in many countries, and a full discussion of the results is beyond the scope of this chapter. In general, the balance between the risk of multiples, the success rate of single ET, and the potential need for reimbursement of additional cycles have been considered, and the net result seems to favor single ET. However, there are two additional partners to the triangle. IMP following ART cycles is usually achieved after long-standing infertility and is usually the "end-stage" procedure. At this phase of reproductive life, most couples would consider a multiple pregnancy as compensation for their efforts (27). No wonder that most couples will support or even persuade the physician to increase the chances of pregnancy by increasing the number of transferred embryos.

THE PATIENT

The optimism at the beginning of an ART cycle changes quite often to severe psychological morbidity. From the outset, couples are faced with dilemmas that they have never faced before. For instance, couples initiating therapy were given questionnaires to determine attitudes regarding multiple pregnancy and MFPR (27,28). The results suggested declining ratings as the number of fetuses increased. Intrauterine insemination patients felt more favorable than the IVF group toward all gestational outcomes and less favorable toward MFPR. Baor and Blickstein (27) suggested that young adults who postpone childbearing may presume that fertility is granted, but when all other measures fail, the use of ART is considered the ultimate salvation for these couples. However, ART is highly stressful and may lead to significant negative psychological consequences (loss of self-esteem, confidence, health, close relationships, security, and hope). The risks of multiple pregnancies are frequently overlooked or underappreciated by infertile couples. Despite the real risks associated with a multiple pregnancy and birth, infertile patients often express a desperate wish to have twins or triplets, thereby accomplishing an instant family. The authors highlighted the need to provide infertile couples with detailed information on the risks of multiple pregnancy and birth.

In the next step, couples may confront the dilemma of donating or destroying supernumerary embryos. This seeming impasse was investigated in 200 couples embarking on IVF–ET treatment (29). Couples' opinions on genetic lineage and education were more determinant in their decision to destroy or to donate their supernumerary embryos than their opinions on the *in vitro* embryo status. The couples expressed various attitudes toward risks of twins and triplets, whereby twins were much more desired than triplets, which are often refused.

The psychological morbidity following MFPR and/or raising high-order multiples has been documented. When confronting the dilemma of potential loss of the entire pregnancy following MFPR, couples may experience considerable emotional distress. Nevertheless, many viewed this option as their "least bad" alternative (30). A French group that followed couples during pregnancy and for four years postpartum provided some important clues to understanding this complex situation (31,32). They first studied the effects of MFPR on the mothers' emotional well-being and the relationship with the children during the two years following intervention. Then, at two years, they compared mothers who had a reduction with mothers who had not and had delivered triplets. At one year, a third of the women in the reduction group reported persistent depressive symptoms related to the reduction, mainly sadness and guilt. The others made medical and rational comments expressing no emotion. At two years, all but two women seemed to have overcome the emotional pain associated with the reduction. The comparison with mothers of triplets indicated that the mothers' anxiety and depression, as well as difficult relationships with the children, were less acute in the reduction group. At four years after delivery, all mothers of triplets reported emotional distress, mainly fatigue and stress. A third of the mothers had a high score of depression and used psychotropic medication. The relationships with the children and difficulties in coping with their behavior and conflicts were the main reasons for psychological distress. Difficulties had not decreased over the years, to the extent that a third of the mothers spontaneously expressed regrets about having triplets.

A Swedish study found similar results (33). Couples ($n = 21$) with complete sets of triplets aged four to six years were interviewed about their experiences of being "triplet parents." The diagnosis of triplets had been a shock for most. All triplets were born prematurely. The first time at home was chaotic for most of the parents. Eventually, "triplet parents" spent more time organizing their lives and less time on emotional care than did parents of singletons.

The psychological effects are often superimposed on maternal complications, which are common in multiple pregnancies. The list of serious morbidity associated with twins and HOMPs has not been specified for IMP. However, risks of hypertensive disorders, eclampsia,

complications of treatment for premature contractions, prolonged bed rest, prolonged hospitalization, and operative deliveries are significantly higher in multiples than in singletons. Thus, the possibility of serious maternal morbidity associated with IMP should be considered to the same extent that ovarian hyperstimulation syndrome is considered before ART cycles.

Since maternal morbidity is undoubtedly increased in multiple gestations, it has been proposed that maternal mortality is also increased (34). However, since a multiple pregnancy is not registered as the direct cause of death, the risk is unknown. For example, eclampsia, tocolysis, and delivery-related deaths were more common in twins (34). Data from the Perinatal Information System, including over 700 Latin America and Caribbean hospitals, have clearly shown that multiple pregnancy increases the risk of significant maternal morbidity in nulliparas and maternal mortality in multiparas (35). It is believed that IMPs are not spared these risks.

The epidemic of iatrogenic HOMPs provided some insight into the increased maternal morbidity in these cases. The most significant morbidities found in triplets were pregnancy-induced hypertension (27%–33%), hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome (9.0%–10.5%), anemia (27.0%–58.1%), and postpartum hemorrhage (9.0%–12.3%) (36,37).

Since maternal morbidity clearly increases with plurality, it is expected that maternal morbidity will decrease following MFPR. Skupski et al. (38) found that severe pre-eclampsia was more common among IVF triplet pregnancies (26.3%) than among IVF triplets reduced to twins (7.9%). The prevalence of all pre-eclampsia cases was also higher among the triplet group (44.7%) than among the twin group (15.8%). Since all pregnancies were successfully implanted triplets, this finding suggests that plurality and placental mass are probably more important to the development of pre-eclampsia than successful implantation alone.

Similar findings were reported for gestational diabetes mellitus (GDM) (39). It was hypothesized that GDM, by virtue of it increasing fetal size, might have a beneficial effect in twins. A matched-control study found the pre-gravid obesity was associated with diabetes during a twin pregnancy. The twin neonates from the GDM group had more respiratory distress syndrome (OR 2.2; 95% confidence interval 1.3, 3.7) and had a three-fold, but not significantly increased perinatal mortality rate. Importantly, birthweight characteristics were similar in both groups (40).

Maternal morbidity should also be considered in the context of maternal age. ART has enabled pregnancies beyond the range of reproductive years, when underlying diseases are more common and pregnancy complications are expected to be intensified.

Data from the U.S. National Center for Health Statistics and the Center for Disease Control and Prevention (NCHS/CDC press release, September 14, 1999) suggest that: (i) between 1980–1982 and 1995–1997, the twin birth rate rose 63% for women aged 40–44 years and nearly 1000% for women aged 45–49 years; and (ii) the HOMP birth rate rose nearly 400% for women in their 30s and more than 1000% for women in their 40s. In 1997, there were more twins born to women aged 45–49 years than during the whole decade of the 1980s. Obviously, motherhood at or beyond the edge of reproductive age is a new aspect of what clinicians previously referred to as pregnancy in the "older gravida" (41). With ART, the boundary between "old" and "young" no longer exists. Generally, the majority of the published studies have been unanimous about the special and perhaps super-cautious attitude required for the older mother, an approach that translates to higher rates of peripartum interventions. Despite the fact that some complications may occur more frequently in older mothers as a result of accumulated prior diseases, there is no direct evidence that older age, per se, complicates either gestation or parturition. Quite unexpectedly, Keith et al. found that older age has an advantage of better perinatal outcomes (mainly in terms of birthweight) of twins and triplets (42). It is unclear if this is a result of the better socioeconomic status of older mothers or if it is related to some uterine "programming" effect.

In contrast to these skyrocketing rates, there are few series describing such "geriatric gravidas," and therefore the true prevalence of various complications may be underestimated. In one study, 4.5 ± 1.1 cleaving embryos were transferred per cycle to 45–59-year-old patients, resulting in 74 delivered pregnancies (34.9%). There were 29 (39.2%) multiple gestations, including 20 twins, seven triplets, and two quadruplets. Two of the triplet and both of the quadruplet pregnancies underwent MFPR to twins. Antenatal complications occurred in 28 women (37.8%), including preterm labor, hypertension, diabetes, pre-eclampsia, HELLP syndrome, and fetal growth retardation. Cesarean section was done in 64.8% (43).

The age-related risk for trisomy, depending on the source of the female gametes, is of primary importance when ART cycles are performed in the elderly. For those who conceive without donor eggs, this risk might be exceptionally high. However, in the case of a polyzygotic multiple gestation, the risk of pregnancy loss following cytogenetic studies might be unacceptably high. Thus, the timing of these studies becomes pertinent. In countries where feticide is permitted only before the 24th week of gestation, the only options are first-trimester CVS or second-trimester amniocentesis. In some countries, feticide is not restricted to gestational age, and late feticide is a clear option. In such instances, amniocentesis is scheduled during the 30th–32nd week, with the possibility of feticide at 33–35 weeks. This logical scheme eliminates the risk of losing the entire pregnancy at an unsalvageable age. However, this scheme provokes three major problems: first, the patient might deliver during the time interval before the cytogenetic results; second, legitimization of third-trimester feticide is a formidable ethical dilemma and does not imply that physicians will agree to terminate a viable fetus; and third, late termination is psychologically more difficult for couples. These intricacies may be

settled if preimplantation diagnosis should become a useful option.

Surrogate motherhood is a good example how ART may change all we know about IMP. Consider the "Angela" case, in which two embryos of unrelated couples were transferred to a surrogate uterus. The newborn twins, whose parentage was confirmed postpartum, were non-siblings who shared no common genes and, of course, shared nothing with the surrogate mother (44).

It goes without saying that the most common and the most risky complication of multiple pregnancies is preterm birth, for which no remedy is available. However, irrespective of plurality, an association between preterm birth and ART has long been suspected and found to be related to causes such as iatrogenic preterm birth (in the so-called "premium" pregnancies), fertility history, and past obstetric performance, as well as underlying medical conditions of the female partner (45). Data showed that singleton as well as multiple pregnancies resulting from IVF have increased rates of preterm birth compared with naturally conceived pregnancies (45). The most plausible explanation seems to be a more liberal use of elective preterm birth. In any case, the most appropriate endpoint after an ART cycle should also include preterm or term birth as a measure of success. In a recent retrospective population-based study, it was found that during the period 1987–2010, there was a nearly two-fold increase in twins, including a three-fold increase of twins and a 27-fold increase in preterm twin births following ART cycles (46).

Finally, the patient with an IMP should also be considered in evolutionary terms. Innumerable studies have shown that, over the millennia, evolutionary forces selected a female prototype for spontaneous twins. Black, fertile, older, taller, and more heavily built women are more likely to have twins, and the outcome is likely better than in women with other characteristics. Thus, the fact that ART involves no selection (except fertility), and certainly no selection for motherhood of multiples, makes the IMP in many ways an iatrogenic contra-evolutionary phenomenon.

THE PHYSICIAN

Three types of physicians comprise the third part of the IMP triangle: those involved in ART, those caring for maternal–fetal issues, and the pediatricians. Each is in charge of a different phase.

The reproduction phase

Since there seems to be a direct relation between the number of transferred embryos and the success rate of ART on the one hand and the IMP rate on the other hand, there seems to be an inherent conflict in the reproduction phase. An idea about the anticipated rates of IMP comes from centers in which all available embryos were transferred and MFPR is not used (Figure 67.5) (47). Before the implementation of the 2004 Italian Reproduction Law (48), the Reggio Emilia (Italy) Center for Reproductive

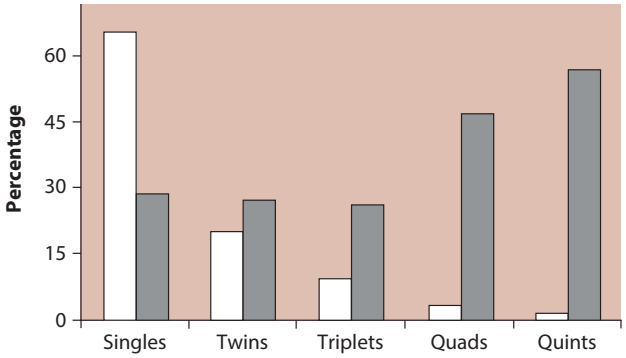

Figure 67.5 Spontaneous loss in iatrogenic multiple pregnancies when all available embryos are transferred and natural multi fetal pregnancy reduction is not used. Light bars: percentage of clinical pregnancies at 35 days post-transfer; dark bars: percentage of disintegrated gestational sacs. (Adapted from La Sala GB et al. *Twin Res* 1999; 2: S6.)

Medicine observed that 34.6% of the clinical pregnancies were multiples, comprising 20% twins and 14.6% HOMPs (47). Interestingly, implementation of the Italian Reproductive Law, which limited the number of fertilized oocytes to three but obliged transfer of all embryos, did not significantly change the incidence of multiples and somewhat improved the overall outcome (48). Some concern exists, however, regarding the group of patients aged >38 years.

Ethical, legal, religious, and technical (i.e., availability of cryopreservation) constraints that obviate selection and/or disposal of surplus embryos are the easy approaches to deciding on the number of embryos that should be transferred. The hard way is careful analysis of success (live birth) versus failure (IMP) rates using selected embryos. Genetic and biochemical markers would supplement morphological criteria, as normal-appearing embryos may be genetically abnormal. Preimplantation genetic studies may also replace invasive procedures during pregnancy following ART. For the time being, the first step has already been done by implementing elective single ET in several countries without significantly reducing outcomes.

Many of the recommendations have been based on ET without specifying their quality and their implantation potential. In the meantime, it has become possible to culture embryos to the blastocyst stage, selecting the fittest embryos for transfer and synchronizing the embryonic with the endometrial stages. Blastocyst transfer has been associated with a much improved implantation rate compared with that of three-day embryos. It is expected that the high "take-home baby" rate following the excellent implantation rates would lead to transfer of one or two blastocysts only, with a concomitant reduction of the IMP rate. However, not all embryos will become blastocysts, and it is unknown which dividing embryo will become a blastocyst *in vitro*. Thus, physicians may not wait for the five-day stage and will first transfer three-day embryos

and then, when blastocysts are successfully cultured, will transfer additional blastocysts, generating iatrogenic superfecundations.

To date, there are no data regarding the consequences of such protocols. Logically, mixed-stage ETs will necessarily increase the chance of IMPs by adding the successful implantation of the five-day to that of the three-day embryo(s). In addition, we do not know the influence of co-implantation at different embryonic ages on the risk of zygotic splitting. We, among others, have noticed some bizarre complex chorionicity arrangements that have never been seen with the usual IVF–ET protocols (unpublished data) (Figure 67.6).

It is therefore reasonable to conclude that demands from infertile couples and fertility clinics to maximize success rates conflict with the need to reduce the number of IMPs. In many centers, IMPs are considered a complication of ART and not a success.

The pregnancy phase

Once pregnant, the woman is not infertile anymore and there should be no difference in the management of spontaneous as compared to iatrogenic pregnancies. However, the past reproductive history continues to follow the patient, albeit her pregnancy may be absolutely normal. When an IMP results, the designation of "premium gestation" seems appropriate, and most reproduction experts may refer the patient to a clinician involved in maternal–fetal medicine (MFM) within a high-risk pregnancy clinic.

Couples frequently create a special attitude toward the "producer" and may feel abandoned when referred to another physician who takes over. Quite often, the optimism involved in infertility treatment may change to pessimism or even to criticism (49). Then, the unprepared couples may consider MFPR or risky interventions

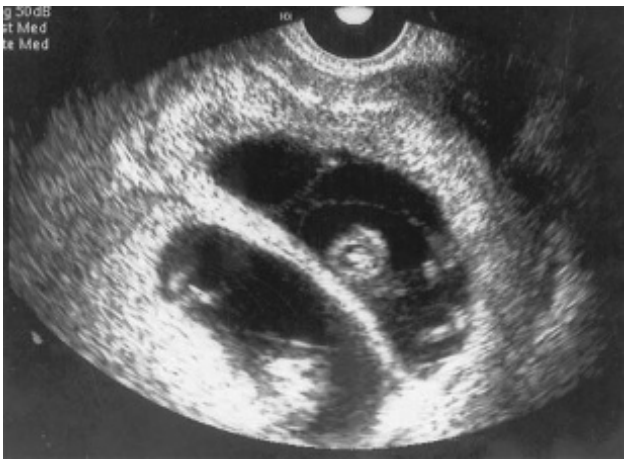

Figure 67.6 Complex chorionicity. Sonographic image showing a seven-week quadruplet pregnancy following sequential transfer of two embryos and one blastocyst. This bichorionic quadruplet pregnancy comprises monochorionic triamniotic triplets (upper sac) and a singleton (lower sac). (Image courtesy of B. Caspi, MD.)

as hostile suggestions. It follows that the dissociation between the reproductive and the MFM physician is by no means simple for any of the parties involved.

It is not yet accepted who should treat the IMP. Obviously, many subspecialties are involved; for example, the sonographer who makes the diagnosis may not be the one who will carry out the MFPR, and both may not take care of the pre-eclamptic patient. This complicated pregnancy follow-up is therefore never a one-man show, and well-orchestrated teamwork is encouraged. Indeed, it has been shown that special multiple pregnancy clinics do have better results.

The extremely varied spectrum of IMPs is superimposed on the special patient–physician relationship. It is beyond the scope of this chapter to discuss in detail follow-up protocols tailored for the diverse presentations of IMP. A 32-year-old patient with premature ovarian failure and a 48-year-old perimenopausal woman may undergo similar egg or embryo donations, but are expected to have different age-related obstetric risks. Likewise, 20-year-old and 40-year-old women may need similar ICSI techniques for severe oligospermia, but differ in respect of anticipated age-related pregnancy complications.

The obligations for the fetus as a patient in a multiple pregnancy are quite complicated (50). In addition to the physician–mother–fetus relationship, there are feto–fetal relations that must be contemplated. The simplest example is a preterm multiple pregnancy in which fetal distress is suspected in one fetus. The obstetrician is faced with the dilemma of salvaging one fetus by conferring risks of prematurity on the non-distressed fetus. A more complicated example is the consideration of MFPR in a BC triplet pregnancy (i.e., MC twins plus a singleton). Obviously, a three to two reduction will end with an MC twin gestation in which MC-specific complications (e.g., TTTS) present additional risk. On the other hand, reducing the twins will increase the risk of losing the entire pregnancy. A third example is a single sac, remote from term, with rupture of the membranes in a triplet pregnancy. Should a delayed-interval delivery be performed (increasing the risk of amnionitis) or should the whole pregnancy be terminated?

It seems that there is never a dull moment in caring for the mother with multiples, exemplified by conflicts between maternal condition and continuation of pregnancy. The lack of effective prophylactic measures against preterm labor and the risks associated with tocolysis are good examples of how the physiologic adaptation for a multiple gestation may complicate treatment with β-mimetic drugs or with $MgSO_4$. Thus, the risk of arresting preterm labor (to the mother) may be as significant as the risk (to the neonate) of delivering premature multiples.

It is beyond the scope of this chapter to describe the plethora of inefficient methods to reduce the preterm birth rate in multiple pregnancies. This pessimistic realization was reached by trying to carry multiple pregnancies to term (by singleton standards), whereas medicine is apparently unable to change the inherent inadequacy of

the uteroplacental unit to accommodate and nurture multiples that long. In this respect, two points should be made. First, "term" in singletons is different from in twins or in HOMPs. Thus, it seems futile to aim for 38 weeks' gestation in multiples just to conclude that this target is unattainable. Second, it follows that a realistic gestational age based on related survival and morbidity rates should be set. For example, obstetricians should aim for 30 weeks' gestation if their neonatal service provides good outcomes for neonates at this age. Thus, it seems reasonable to suggest that if prematurity in multiples is not preventable, efforts should be made to prevent extreme prematurity.

Finally, a time comes when the obstetrician and the patient must consider the mode of delivery. There is little doubt that a planned (daytime), elective cesarean delivery offers a simple solution in terms of required personnel and safety to mother and neonates. This seems to be intuitively true for HOMPs and for small twins, although there are no prospective studies to support this assumption. For twins weighing at least 1500 g each, either route of delivery seems to be appropriate, irrespective of fetal presentation (51). However, as mentioned above, IMPs are frequently considered as "premium," high-risk pregnancies, and many will follow the dictum that "no high-risk pregnancy should end with a high-risk delivery" and opt for an elective abdominal birth.

The neonatal phase

There is no significant difference between treating three preterm singletons and a preterm triplet pregnancy, as each of these neonates deserves its own special care. However, the epidemic dimensions of IMP create consequential logistical problems that ideally should be separated from the purely medical problems. Regrettably, advances in ART have been much faster than the preparation of sufficient cribs in the neonatal intensive care unit (NICU). As a result, overproduction of preterm neonates overwhelms the capacity of many NICUs, leading to medical problems associated with overcrowded stations.

A Canadian study compared the preterm birth rates in two three-year periods, 1981–1983 and 1992–1994 (52). The preterm birth rate increased by 9% (from 6.3% to 6.8%). Importantly, the rate of preterm births among live births resulting from multiples increased by 25% compared with 5% in singletons, confirming that the increase in preterm births is largely attributable to the increase in multiple birth rates.

HOMP births are at much greater risk than single births. An NCHS report on the final 1996 birth statistics for the U.S.A. found that infant mortality rates are 12-times higher for triplets than for singletons, triplets are 12-times more likely to die within the first year of life, the average birth weight of a triplet baby is half that of a singleton, and the gestational duration is, on average, seven weeks shorter. For 1995, 92% of triplets were preterm compared with about 10% of births in single deliveries.

Delivery of a multiple pregnancy should be a carefully planned event. A minimal neonatal team for a triplet delivery may include as many as 10 persons, including physicians, assistants, and a supervisor. Obviously, chaos prevails unless teamwork is harmonized. Maternal ("transport *in utero*") or neonatal transportation should be available if the expected number of neonates exceeds the number of available NICU cribs.

Logistic considerations do not end at delivery. Once at the nursery, all the multiples must be given equal opportunity to bond with their parents and, perhaps, according to psychological view, to continue their intrauterine contacts with their siblings. For instance, there is increasing evidence that co-bedding of twins in the NICU improves thermoregulation, feeding, and sleeping parameters (53). Indeed, the special and unique interaction between multiples during childhood and beyond seems to reflect the unique relationship that exists between fetuses that grow together *in utero* (Appendix).

Figure 67.7 shows the mortality rates of twins, triplets, and higher-order multiples in England and Wales in 1993 relative to singletons, demonstrating the much increased incidence of stillbirth, perinatal, neonatal, and infant deaths in multiple pregnancy (54). Thus, parents of a multiple pregnancy are more likely to experience bereavement than those with singletons. The care that parents should receive when all fetuses/babies die is not different from that when a singleton dies. When one baby of a multiple birth dies, the loss is frequently underestimated; however, the loss of parents that are left "with something" is no less painful.

The time spent in the nursery may be the only opportunity for the parents to prepare for the future. At home, mothers may find the reality of coping with their multiples more demanding than they had expected. Needless to say, professional help is needed during infancy

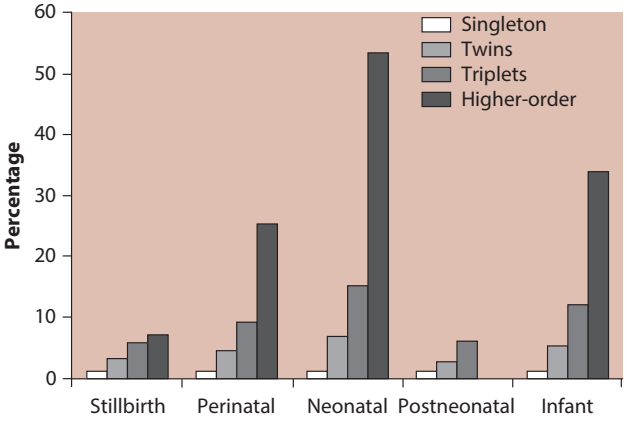

Figure 67.7 Mortality rates of twins, triplets, and higher-order multiples in England and Wales in 1993 relative to singletons. A much increased incidence of stillbirth, perinatal, neonatal, and infant deaths is shown in multiple pregnancy. (Adapted from Dunn A, MacFarlane A. *Arch Dis Child* 1996; 75: F10–9.)

and childhood to the same extent that it had been needed before and during pregnancy.

Finally, it is well accepted that even perfectly normal multiples are a significant financial burden for every family.

Many studies have estimated the expenses involved in IMP. Given that the costs involved in ART are similar to conceptions ending with a singleton, and given that the costs of pregnancy surveillance of multiples are moderately increased as compared with singletons, the major financial impact of IMP evolves from raising premature infants in the expensive environment of the NICU. No mathematical skills are needed to establish the number of NICU days per IMP and to multiply the product by the daily cost of NICU hospitalization. Moreover, lifelong morbidity, which is significantly associated with preterm birth, has further implications on the expenses involved in caring for handicapped children. Thus, from a financial perspective, IMP must be considered as a syndrome of an affluent society.

EPILOGUE: REDEFINING SUCCESS

Every day, there are numerous healthy multiples delivered following ART conceptions. Almost every proud reproductive center documents this success in pictures of smiling parents, cute babies, and grinning physicians. The media love it as well, and give primetime priority for items related to HOMP births. As a consequence, infertile couples exposed to these encouraging results are bound to push ART to its available limits, irrespective of the untoward outcomes of a multiple pregnancy.

As stated previously, and until proven otherwise, the human female is programmed by nature to have one child at a time. Consequently, success should have only one meaning: a "take-home baby" rate of one infant per pregnancy. Thus, there is an inherent absurdity in considering a HOMP in need of MFPR as a successful outcome, and it is likewise irrational to consider the delivery of triplets at 29 weeks' gestation as a successful event. Obviously, producing a three- to six-fold increased risk for a lifelong handicap such as CP cannot be considered successful.

Two of the several solutions proposed in order to overcome the epidemic of IMP are relevant to ART. First, the dissociation between members of the "production line" should be minimal. Thus, both reproductive experts and their patients should have an accurate perspective of the potential obstetric, neonatal, and lifelong complications associated with IMPs. Second, the current changing trends from quantity to quality in ART, by transferring fewer but higher-quality embryos or blastocysts, may be the light at the end of the tunnel.

One should consider the international consensus statement on the perinatal care of multiples (Appendix), where many aspects related to ART are discussed. In any case, the apocalyptic views expressed in this chapter will remain pertinent as long as demands for better pregnancy rates by couples undergoing ART are accepted by overzealous reproduction centers without a clear definition of what should be considered successful.

APPENDIX

Recommendations and guidelines for perinatal practice*

The Istanbul international consensus statement on the perinatal care of multiple Pregnancy

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Abstract

The purpose of this document is to expand the 1995 International Society of Twin Studies/Council of Multiple Birth Organisations (ISTS/COMBO) Declaration of Rights that was initially produced to promote awareness of the special needs of multiple birth infants, children, and adults. It addresses the clinical and ethical dimensions of perinatal care of multiple pregnancies.

The *ad hoc* committee was chaired by Isaac Blickstein. The following individuals were present (in alphabetical order): Birgit Arabin (Zwolle, Netherlands/Berlin, Germany), Isaac Blickstein (Rehovot, Israel), Frank A. Chervenak (New York, U.S.A.), Zehra Nese Kavak (Istanbul, Turkey), Louis G. Keith (Chicago, U.S.A), Eric S. Shinwell (Rehovot, Israel), and Yves Ville (Paris, France). Secretary of the meeting was Alin Basgul (Istanbul, Turkey).

This statement was endorsed by the International Society of Twin Studies (Ghent, Belgium, June 2007) and by the World Association of Perinatal Medicine (Florence, Italy, September 2007).

Consensus statement

1. Multiples and their families, as with any other individuals, have a right to full protection under the law and freedom from discrimination of any kind.
2. Pregnant women and their multiples have a right to be cared for by professionals who are knowledgeable

* Constructed by an *ad hoc* committee convened in Istanbul, January 26, 2007. Coordinator of WAPM Multiple Pregnancies Working Group: Isaac Blickstein. *J. Perinat. Med.* 2007; 35: 465–7. Reproduced by permission.

regarding the management of multiple gestation and/or the lifelong special needs of multiples.

3. Individuals or couples seeking information and/or treatment for infertility have a right to full disclosure about clinically relevant information that might influence the conception of multiples, the associated risks, and the medically reasonable management alternatives for them. This disclosure should be specific to each potential intervention. Thus, if successive interventions are considered, multiple disclosures should be provided and consent obtained for each intervention.
4. When infertility treatment is contemplated, the *a priori* risks and consequences of having a multiple pregnancy secondary to each infertility treatment should be discussed. In settings where iatrogenic multiple gestations may result, the potential need for MFPR and its associated risks should be discussed.
5. Given the increased risk of any multiple pregnancy:
   a. Infertility treatment should intend to prevent multiple pregnancies, in particular high-order multiples. This implies that a high-order multiple gestation after infertility treatment should be considered as a complication.
   b. Infertility services should disclose their number of multiple pregnancies, both intentional and unintentional.
   c. The economical justification of choosing a multiple pregnancy, especially HOMP, should be discouraged.
   d. The use of a multiple pregnancy as a potential cohort from which fetus of either sex can be selected is discouraged.
   e. In the special case of inherited disease, pre-gestational diagnosis to select embryos for transfer should be preferred to producing a multiple pregnancy in order to reduce the affected embryos.
6. MFPR should be considered as a destructive measure for reduced fetuses and a therapeutic measure for the remaining fetuses. It may also be traumatic for one or both parents. Its only role is to potentially promote a better outcome for the remaining embryo(s). It follows that:
   a. The decision about MFPR is ultimately the pregnant woman's to make. The pregnant woman should be provided with reliable information about institutional rather than national success rates of MFPR. The patient's beliefs and values are determinative in the decision-making process regarding whether MFPR is to be performed and, if so, the number of embryos to be reduced.
   b. Diagnostic evaluation of fetuses before MFPR is appropriate, as the resulting information is relevant to the pregnant woman's decision making.
   c. MFPR should be considered as a relevant antenatal event and registries should be encouraged so that outcome can be appreciated.
7. Selective reduction of an anomalous fetus should be performed in a manner as to minimize the potential danger to the remaining fetus(es), taking chorionicity into consideration:
   a. Screening, diagnosis, and selective reduction should be performed at a timing to optimize the outcome for the remaining fetus(es).
   b. Selective reduction should be considered as a relevant antenatal event and registries should be encouraged so that outcomes can be appreciated.
8. A distinction must be made between a multiple pregnancy and a multiple birth. For epidemiological purposes, singleton births that started as a multiple pregnancy should be recorded in order to properly account for spontaneous and iatrogenic embryonic and fetal loss(es).
9. Ultrasound technology is critical for antenatal care in all multiple pregnancies. Chorionicity should be established by ultrasound as accurately and as early as possible in all multiple pregnancies. Information about chorionicity should be provided to the expectant mother along with its clinical significance. When this information is lacking, careful postpartum placental examination should be performed.
10. Zygosity determination should be a prerogative of the parents or of the multiples and not of the care providers (except for clearly defined research objectives in which informed consent has been given). Zygosity should be respected as any other human trait and deserves the same privacy rules. Involvement in registries of MZ twins should be absolutely voluntary on the part of the multiples.
11. Complex cases associated with MC placentation, such as TTTS, twin reversed arterial perfusion sequence, and severe discordance, should be evaluated and treated in specialized centers based on scientific and ethical considerations. In the absence of the possibility of referral, consultation should be obtained requesting the best potential therapy in the local setting.
12. Following single perinatal demise within a set of multiples, parents may consider informing the survivor(s) that he/she/they were a sib of a multiple pregnancy.
13. Whenever the clinical circumstance of one twin jeopardizes the other, care should be exercised to select a management plan that would optimize the outcome of both fetuses. The pregnant woman should be involved in such decision making.
14. Fetal surveillance before and during labor should be carried out on all fetuses. It follows that each fetus should be adequately and appropriately monitored.
15. Delivery considerations should include the welfare of all fetuses. Mode of delivery should be based on medical considerations pertaining to each fetus, as well as on maternal health and preferences.
16. Delivery of multiples should ideally take place:
   a. In a center that is equipped with neonatal intensive care facilities available simultaneously for each infant.
   b. Where a medical care provider certified in neonatal resuscitation is present for each neonate.

c. With facilities for a high-risk delivery (such as availability of an experienced obstetrician, 24-hour anesthesia coverage, blood availability, etc.). If this is not possible, *in utero* transfer may be preferable to postpartum transfer.

17. Governments and private payers should be aware of the financial costs of multiple pregnancy and birth and the future upbringing of the multiples. Whenever possible, direct financial aid should be supplied to parents in proportion to plurality.
18. Any research involving multiples must be conducted using informed consent of the participants or their parents and must comply with accepted international codes of ethics and scientific standards for conducting human subject research.
19. In the instance when one or more of a set of multiples manifests a physical and/or mental handicap, management plans should respect the special needs of the handicapped member(s) in the setting of a multiple pregnancy while also giving attention to the special needs of the non-handicapped sib(s).
20. Public policy should support a set of multiples remaining together in foster care and adoptive families.

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REFERENCES

1. Blickstein I, Verhoeven HC, Keith LG. Zygotic splitting after assisted reproduction. *N Engl J Med* 1999; 340: 738–9.
2. Blickstein I, Jones C, Keith LG. Zygotic-splitting rates after single-embryo transfers in *in vitro* fertilization. *N Engl J Med* 2003; 348: 2366–7.
3. Gielen M, Lindsey PJ, Derom C et al. Modeling genetic and environmental factors to increase heritability and ease the identification of candidate genes for birth weight: A twin study. *Behav Genet* 2008; 38: 44–54.
4. Templeton A, Morris JK. Reducing the risk of multiple births by transfer of two embryos after *in vitro* fertilization. *N Engl J Med* 1998; 339: 573–7.
5. Blickstein I. Perinatal implications of iatrogenic multiple pregnancies. In: *4th World Congress of Perinatal Medicine*. Voto LS, Margulies M, Cosmi EV (eds). Bologna, Italy: Monduzzi Editore, 1999, pp. 167–72.
6. Leroy F. *Les Jumeaux dans Tous Leurs Etats*. Brussels, Belgium: De Boeck-Wesmael, 1995.
7. Blickstein I, Keith LG, eds. *Iatrogenic Multiple Pregnancy: Clinical Implications*. Lancaster, U.K.: Parthenon, 2000.
8. Blickstein I, Keith LG, eds. *Multiple Pregnancy*, 2nd edn. Oxford, U.K.: Taylor & Francis, 2005.
9. Blickstein I, Smith-Levitin M. Twinning and twins. In: *Current Perspectives on the Fetus as a Patient*. Chervenak FA, Kurjak A (eds). Lancaster, U.K.: Parthenon, 1996, pp. 507–25.
10. Blickstein I, Smith-Levitin M. Multifetal pregnancy. In: *Fetal Disorders: Diagnosis and Management*. Petrikovsky BM (ed.). New York, NY: John Wiley and Sons, 1998, pp. 223–47.
11. Blickstein I, Goldman RD, Smith-Levitin M et al. The relation between inter-twin birth weight discordance and total twin birth weight. *Obstet Gynecol* 1999; 93: 113–6.
12. Palermo GD, Cohen J, Alikani M, Adler A, Rosenwaks Z. Intracytoplasmic sperm injection: A novel treatment for all forms of male factor infertility. *Fertil Steril* 1995; 63: 1231–40.
13. Gavriil P, Jauniaux E, Leroy F. Pathologic examination of placentas from singleton and twin pregnancies obtained after *in vitro* fertilization and embryo transfer. *Pediatr Pathol* 1993; 13: 453–62.
14. Pharoah PO, Cooke RW. A hypothesis for the aetiology of spastic cerebral palsy—The vanishing twin. *Dev Med Child Neurol* 1997; 39: 292–6.
15. Blickstein I. Reflections on the hypothesis for the etiology of spastic cerebral palsy caused by the "vanishing twin" syndrome. *Dev Med Child Neurol* 1998; 40: 358.
16. Matias A, La Sala GB, Blickstein I. Early loss rates of entire pregnancies after assisted reproduction are lower in twin than in singleton pregnancies. *Fertil Steril* 2007; 88: 1452–4.
17. Blickstein I. Do multiple gestations raise the risk of cerebral palsy? *Clin Perinatol* 2004; 31: 395–408.
18. Pharoah PO, Cooke T. Cerebral palsy and multiple births. *Arch Dis Child Fetal Neonatal Ed* 1996; 75: F174.
19. Blickstein I, Weissman A. Estimating the risk of cerebral palsy after assisted reproduction. *N Engl J Med* 1999; 341: 1313–4.
20. Blickstein I, Keith LG. On the possible cause of monozygotic twinning: Lessons from the 9-banded armadillo and from assisted reproduction. *Twin Res Hum Genet* 2007; 10: 394–9.
21. Derom C, Leroy F, Vlietinck R, Fryns JP, Derom R. High frequency of iatrogenic monozygotic twins with administration of clomiphene citrate and a change in chorionicity. *Fertil Steril* 2006; 85: 755–7.
22. Simões T, Queirós A, Marujo AT, Valdoleiros S, Silva P, Blickstein I. Outcome of monochorionic twins conceived by assisted reproduction. *Fertil Steril* 2015; 104: 629-32.
23. Blickstein I. Should the reduced embryos be considered in outcome calculations of multifetal pregnancy reduction? *Am J Obstet Gynecol* 1994; 171: 866–7.
24. Meyers C, Adam R, Dungan J, Prenger V. Aneuploidy in twin gestations: When is maternal age advanced? *Obstet Gynecol* 1997; 89: 248–51.

25. Sebire NJ, Snijders RJ, Hughes K, Sepulveda W, Nicolaides KH. Screening for trisomy 21 in twin pregnancies by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. *Br J Obstet Gynaecol* 1996; 103: 999–1003.
26. Maymon R, Jauniaux E, Holmes A et al. Nuchal translucency measurement and pregnancy outcome after assisted conception versus spontaneously conceived twins. *Hum Reprod* 2001; 16: 1999–2004.
27. Baor L, Blickstein I. En route to an 'instant family': Psychosocial considerations. *Obstet Gynecol Clin North Am* 2005; 32: 127–39.
28. Goldfarb J, Kinzer DJ, Boyle M, Kurit D. Attitudes of *in vitro* fertilization and intrauterine insemination couples toward multiple gestation pregnancy and multifetal pregnancy reduction. *Fertil Steril* 1996; 65: 815–20.
29. Laruelle C, Englert Y. Psychological study of *in vitro* fertilization–embryo transfer participants' attitudes toward the destiny of their supernumerary embryos. *Fertil Steril* 1995; 63: 1047–50.
30. Berkowitz RL, Lynch L, Stone J, Alvarez M. The current status of multifetal pregnancy reduction. *Am J Obstet Gynecol* 1996; 174: 1265–72.
31. Garel M, Stark C, Blondel B et al. Psychological reactions after multifetal pregnancy reduction: A 2-year follow-up study. *Hum Reprod* 1997; 12: 617–22.
32. Garel M, Salobir C, Blondel B. Psychological consequences of having triplets: A 4-year follow-up study. *Fertil Steril* 1997; 67: 1162–5.
33. Akerman BA, Hovmolle M, Thomassen PA. The challenges of expecting, delivering and rearing triplets. *Acta Genet Med Gemellol Roma* 1997; 46: 81–6.
34. Blickstein I. Maternal mortality in twin gestations. *J Reprod Med* 1997; 42: 680–4.
35. Conde-Agudelo A, Belizan J. Maternal mortality and morbidity associated with multiple pregnancy. *Twin Res* 1999; 2: S3.
36. Malone FD, Kaufman GE, Chelmow D et al. Maternal morbidity associated with triplet pregnancy. *Am J Perinatol* 1998; 15: 73–7.
37. Albrecht JL, Tomich PG. The maternal and neonatal outcome of triplet gestations. *Am J Obstet Gynecol* 1996; 174: 1551–6.
38. Skupski DW, Nelson S, Kowalik A et al. Multiple gestations from *in vitro* fertilization: Successful implantation alone is not associated with subsequent preeclampsia. *Am J Obstet Gynecol* 1996; 175: 1029–32.
39. Sivan E, Maman E, Homko CJ et al. Impact of fetal reduction on the incidence of gestational diabetes. *Obstet Gynecol* 2002; 99: 91–4.
40. Simões T, Queirós A, Correia L et al. Gestational diabetes mellitus complicating twin pregnancies. *J Perinat Med* 2011; 39: 437–40.
41. Blickstein I. Motherhood at or beyond the edge of reproductive age. *Int J Fertil Womens Med* 2003; 48: 17–24.
42. Keith LG, Goldman RD, Breborowicz G, Blickstein I. Triplet pregnancies in women aged 40 or older: A matched control study. *J Reprod Med* 2004; 49: 683–8.
43. Sauer MV, Paulson RJ, Lobo RA. Oocyte donation to women of advanced reproductive age: Pregnancy results and obstetrical outcomes in patients 45 years and older. *Hum Reprod* 1996; 11: 2540–3.
44. Simini B. Italian surrogate twins. *Lancet* 1997; 350: 1307.
45. Blickstein I. Does assisted reproduction technology, per se, increase the risk of preterm birth? *BJOG* 2006; 113(Suppl 3): 68–71.
46. Tul N, Lucovnik M, Verdenik I, Druskovic M, Novak Z, Blickstein I. The contribution of twins conceived by assisted reproduction technology to the very preterm birth rate: A population-based study. *Eur J Obstet Gynecol Reprod Biol* 2013; 171: 311–3.
47. La Sala GB, Montanari R, Cantarelli M et al. Iatrogenic multifetal pregnancies and SPIER. *Twin Res* 1999; 2: S6.
48. La Sala GB, Vilani MT, Nicoli A et al. The effect of legislation on outcomes of assisted reproduction technology: Lessons from the 2004 Italian law. *Fertil Steril* 2008; 89: 854–9.
49. Blickstein I. Litigation in multiple pregnancy and birth. *Clin Perinatol* 2007; 34: 319–27.
50. Chervenak FA, McCullough LB. *Ethics in Obstetrics and Gynecology*. New York, NY: Oxford University Press, 1994.
51. Blickstein I. Cesarean section for all twins? *J Perinat Med* 2000; 28: 169–74.
52. Joseph KS, Kramer MS, Marcoux S et al. Determinants of preterm birth rate in Canada from 1981 through 1983 and from 1992 through 1994. *N Engl J Med* 1998; 339: 1434–9.
53. Mazela JL, Gdzinowski J. Co-bedding twins and multiples—Is there strong clinical evidence? *Twin Res* 1999; 2: S17.
54. Dunn A, MacFarlane A. Recent trends in the incidence of multiple births and associated mortality in England and Wales. *Arch Dis Child* 1996; 75: F10–9.

Egg and embryo donation

68

MARK V. SAUER and CATHA FISCHER

INTRODUCTION

Human egg (oocyte) and embryo donation was first introduced in 1983 and has evolved over the past three decades into a relatively common procedure that addresses a variety of reproductive disorders. This method has provided key insights into the physiology and pathophysiology of reproduction and, like other assisted reproduction technologies (ARTs), has engendered its share of controversy. Furthermore, techniques introduced by egg donation, such as schemes for adequate hormonal preparation of the uterus for synchronizing embryos with a receptive endometrium, have been successfully applied to other fertility therapies, including the management of patients with cryopreserved embryos for transfer and those requiring *in vitro* maturation of immature oocytes.

The first report of a successful egg donation in a mammalian species involved rabbits. Heape in 1890 described the transfer of rabbit embryos from the uterus of a donor to the uterus of a synchronized recipient, followed by the delivery of healthy offspring (1). During the 1970s, mammalian embryo donation was applied to cattle to improve the reproductive efficiency of prize animals. By 1990, almost 19,000 calves were born annually in the U.S.A. as a result of embryo transfer procedures (2).

In 2007, approximately 40,000 calves, representing 11.5% of the total registered Angus cattle born that year, were a result of embryo transfer (3).

The vast majority of mammalian egg donations resulted from embryos fertilized *in vivo*, recovered from the donor by uterine lavage, and then transferred to the recipient uterus. Using a modification of this technique, in 1983, researchers at the University of California, Los Angeles fertilized an oocyte *in vivo* after the artificial insemination of a human donor and then transferred the recovered embryo into a synchronized recipient (4). A total of 14 insemination cycles resulted in two ongoing pregnancies (5). In 1984, the first delivery of a healthy male infant was reported (6).

During this same time period, researchers at Monash University in Melbourne began transferring embryos to infertile recipients as a result of eggs fertilized *in vitro* from donated oocytes obtained laparoscopically from infertile women (7). In 1984, they also reported the first live birth following egg donation and *in vitro* fertilization (IVF) (8). Synchronization of the recipient and donor was achieved using oral estradiol valerate and intravaginal progesterone pessaries prescribed to the functionally agonadal recipient.

Donor uterine lavage was popular in the early 1980s since it was far less invasive than laparoscopy, but by 1987, uterine lavage was discontinued in humans because of the fear of human immunodeficiency virus transmission and the inability to prevent occasional retained pregnancies in the embryo donors. Furthermore, around this time, the introduction of transvaginal oocyte aspiration using ultrasound guidance enabled oocyte donation to be performed within an office setting, greatly reducing its inconvenience, improving its safety, and lessening its cost.

The popularity of egg and embryo donation is evidenced by the rapidly increasing demand for services. In the U.S.A., 19,847 procedures involving fresh or frozen embryos procured through oocyte donation were reported to the Centers for Disease Control and Prevention in 2012, four times the number reported in 1996 (9). This increase is largely due to the rising percentage of women who remain childless past the age of 40 years, a number that has sharply increased over the past 30 years (10). Many women are marrying later, or are pursuing education and vocation and deliberately delaying childbearing (11). Unfortunately, there is a natural decline in fertility associated with advancing age, and many healthy women later experience difficulties as a result of normal aging.

INDICATIONS FOR EGG AND EMBRYO DONATION

The indications for egg and embryo donation have expanded since its inception. Originally envisioned as a fertility treatment for women with premature ovarian insufficiency (POI) (12), today women with many other reproductive disorders are considered prime candidates for therapy (Table 68.1).

Non-iatrogenic POI, defined as women <40 years old with persistent amenorrhea and elevated gonadotropins, affects approximately 1% of the female population (13). The majority of cases are idiopathic, but about 20% are suspected of being autoimmune in nature or the result of concomitant glandular autoimmune disease (14). Thus, it is important to ensure that clinical or subclinical failure of the thyroid, parathyroid, and adrenal glands does not coexist, as well as diabetes mellitus and myasthenia gravis. Any of these conditions may adversely affect pregnancy outcome as well as impact upon the general health and well-being of the patient. If POI occurs at <30 years old, a karyotype should also be requested to ascertain the presence of Y-chromosome mosaicism. Patients discovered to be mosaic are at risk of gonadal tumors and require extirpation of the abnormal gonad (15). In addition, a bone density evaluation is helpful to identify patients with osteopenia or osteoporosis, which may be present despite hormone-replacement therapy (16). Turner syndrome is the most common gonadal dysgenesis in women, with a prevalence

Table 68.1 Indications for oocyte donation

Premature ovarian failure
Gonadal dysgenesis
Repeat *in vitro* fertilization failure
Natural menopause
Inheritable disorders
Same-sex couples

of 1 in 2000 live-born females. Both spontaneous puberty and spontaneous pregnancy are relatively rare in these patients, occurring in less than 5% of affected individuals (17). Other rare conditions associated with POI include congenital thymic aplasia (e.g., DiGeorge syndrome) (18), galactosemia (19), and ataxia-telangiectasia (20), all of which require a more thorough and specific evaluation.

A common cause of POI, with the increased use of genetic screening, is fragile X pre-mutation. Pre-mutation of more than 55 but less than 200 CGG repeats is not associated with fragile X syndrome; however, this has been shown to be associated with POI. Approximately 15% of women who have this fragile X pre-mutation will suffer from POI (21).

Chemotherapy and radiation treatments for cancer may also lead to POI. Gonadotoxicity is age and dose dependent, with younger patients being more resistant to damage (22,23). Removal of the ovaries is often required for treatment of malignancies, but surgical castration more commonly results from non-cancerous conditions, including infection, torsion, or overly aggressive removal of intra-ovarian lesions (e.g., cystic teratomas and endometriomas).

Repetitive failure at IVF is common when a poor ovarian response to gonadotropins occurs. Occasionally, patients are identified as poor candidates for IVF treatment prior to initiating care, thus sparing them the expense and psychological distress of multiple failed cycles. The first consideration is the age of the patient. It has long been known that natural fertility decreases with age, and this is also true with IVF (Figure 68.1) (9). Many IVF centers have a maximum age limit beyond which they will not perform IVF without oocyte donation. Women of advanced reproductive age have far greater success with donated oocytes (24). Ovarian reserve is evaluated with serum follicle-stimulating hormone (FSH) levels on day 2 or 3 of the menstrual cycle (25). Values >15 mIU/mL are prognostic for a greatly reduced IVF success rate. Another useful serum marker is day-2 or -3 estradiol (26). Values >45 pg/mL are predictive of lower pregnancy rates and, if >75 pg/mL, the attempts usually end in failure. It is important that each laboratory determines the threshold values that are useful for their program.

Anti-Mullerian hormone (AMH) is produced in the granulosa cells from preantral and small antral follicles and serum levels are measurable and reflective of ovarian reserve. Higher levels of AMH are associated with greater numbers of retrieved oocytes in women undergoing IVF, while low levels appear to be reliable markers for diminished ovarian reserve (27). Thus, AMH testing may identify women at risk for either extreme (hypo- or hyper-) in ovarian responsiveness. Other tests are extant to assess ovarian reserve, but are more cumbersome than day-3 serum FSH and estradiol. The clomiphene challenge test measures serum FSH, luteinizing hormone (LH), and estradiol at baseline and again after five days (days 5–9) of 100 mg clomiphene citrate (28). Serum FSH values >15 mIU/mL post-clomiphene are predictive of IVF failure. Day-2 or -3 serum inhibin B may also define ovarian reserve (29), but the commercially available assay is currently far more complex and time consuming than assays for FSH, estradiol, and AMH and not readily available.

Antral follicle count is another marker of ovarian reserve. Antral follicles are the follicles measuring between 2 and 10 mm during the early follicular phase. A low antral follicle count defined as between 4 and 10 in total is associated with poor ovarian reserve. A low antral follicle count, while suggestive of poor ovarian reserve, is a poor predictor of oocyte quality, IVF success, and pregnancy outcome (30).

In certain cases, ovarian stimulation is adequate, but fertilization rates are poor and often oocyte quality is marginal. Intracytoplasmic sperm injection (ICSI) may or may not be helpful, but if fertilization failure is persistent, then oocyte donation is indicated. Similarly, successful fertilization may be present, but implantation still might not occur. Assisted hatching may be helpful in these cases. Both ICSI and assisted hatching are discussed in detail in other chapters, but the belief is that recurrent implantation failure is often secondary to poor gametes and may be overcome by oocyte donation. Less clear is the patient with recurrent pregnancy loss, although at least one report suggests that oocyte donation is effective in these cases as well (31). Finally, in rare instances, IVF failure may be due to ovaries that are inaccessible to either transvaginal or laparoscopic retrieval, and oocytes can be provided only through donation.

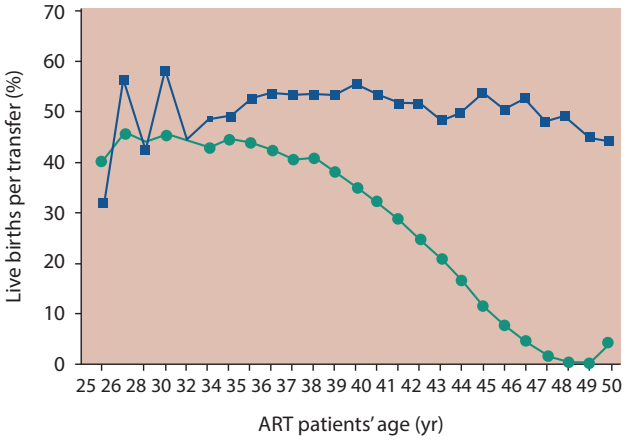

Figure 68.1 Live births per transfer for ART cycles using fresh embryos from own and donor eggs, by ART patient's age, 2012 (9) (squares, donor eggs; circles, non-donor eggs). *Abbreviation*: ART, assisted reproduction technology.

Although controversial, oocyte donation to treat infertility in women with physiological menopause is very effective (24). The Ethics Committee of the American Society for Reproductive Medicine (ASRM) stated that because of the physical and psychological risks involved (to both mother and child), oocyte donation in postmenopausal women should be discouraged (32). However, data on pregnancy outcomes in these women, albeit after careful medical and psychological screening, do not reveal any unreasonable risks (33). Some have argued that postmenopausal pregnancy is "unnatural," but the same may be said of most ARTs. Furthermore, denying healthy older women donated oocytes while allowing older men complete access to reproductive care is considered by many to be both prejudicial and sexist (34).

Less controversial is the use of egg donation for inheritable conditions such as X-linked or autosomal traits and chromosomal translocations (35). However, with progress in preimplantation diagnosis, this reason for choosing egg donation has decreased (36).

Individuals in same-sex relationships are increasingly seeking fertility care to have children. For gay males, and in the cases of some lesbian couples, the use of oocyte donation and gestational carriers is required (37).

Table 68.2 Suggested medical screening of oocyte recipient(s)

Oocyte recipient	Male partner
Complete blood count with platelets	Blood Rh and type
Blood Rh and type	Hepatitis screen
Serum electrolytes, liver, and kidney function	VDRL
Sensitive TSH	HIV-1, HTLV-1
Rubella and hepatitis screen	Semen analysis and culture
VDRL	
HIV-1, HTLV-1	
Urinalysis and culture	
Cervical cultures for gonorrhea and chlamydia	
Pap smear	
Transvaginal ultrasound	
Uterine cavity evaluation (sonohysterogram, diagnostic hysteroscopy, or hysterosalpingogram)	
Electrocardiogram	
Chest X-ray	
Mammogram	
Hemoglobin A1C	

Abbreviations: HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; TSH, thyroid-stimulating hormone; VDRL, Venereal Disease Research Laboratory.

The Ethics Committee of the ASRM has issued guidelines that call for a non-discriminatory policy in treating same-sex couples requesting fertility assistance (38). As a result, a rising number of requests for oocyte donation services is coming from this population of patients.

RECIPIENT SCREENING

In addition to a complete history and physical examination, the suggested medical screening for recipients is shown in Table 68.2. Most of the tests are requisite standards for expectant mothers and IVF candidates. Patients of advanced maternal age are at higher risk for certain conditions such as diabetes mellitus, hypertension, and heart disease and therefore require additional testing focused on these disorders. Other recipients may warrant more comprehensive evaluations, such as a karyotype and autoimmune screen in patients with POI, or screening for anomalies of the aorta and urological system in patients with gonadal dysgenesis.

Psychological screening of recipient couples is also recommended. The stress that infertility places on relationships is well known (39). Furthermore, with respect to oocyte donation, the resulting child will not be genetically related to the mother. Most couples reconcile themselves to this, and research has shown that the desire to be parents is more important for positive parenting than a genetic link with the child (40). However, it remains important to address any grief, anxiety, and depression directly with the couple prior to proceeding. The role of the mental healthcare professional is usually one of support and guidance for the couple struggling with these issues. Occasionally, a couple is found to have greatly disparate ideas of what the pregnancy will accomplish. A pregnancy conceived merely to salvage a marriage or relationship is best deferred until the couple resolves their differences.

The presence of endometriosis does not affect the pregnancy rate of patients undergoing oocyte donation (41). However, a hydrosalpinx is probably deleterious, and surgical treatment to relieve the obstruction (tuboplasty) or remove the damaged tube (salpingectomy) is recommended (42). Recipients should have a normal uterine cavity free of adhesions, space-occupying lesions, and pathology. This is best assessed by a pre-cycle sonohysterogram or diagnostic hysteroscopy.

A mock endometrial preparation cycle and timed endometrial biopsy is performed in many programs to ensure that an adequate response to endometrial priming is present. Glandular/stroma dyssynchrony is often found during endometrial stimulation (43), but apparently this does not adversely affect pregnancy rates (44). Other studies have evaluated endometrial thickness as a predictor of success with oocyte donation (44–47). An endometrium of <6 mm is associated with poor outcome. Another study showed that all endometrial biopsies were in phase if the thickness was >7 mm (48). A recent Cochrane review failed to confirm any one particular protocol for optimizing endometrial preparation with regards to pregnancy rate in a retrospective analysis of 22 randomized controlled clinical trials (49). The great majority of women will have adequate responses

to hormone replacement and, therefore, at Columbia University, we have chosen to forgo the mock cycle except in women in whom a poor response is anticipated, such as patients with prior pelvic radiation (50). Should a recipient have a thin endometrium (<7 mm) on a previous attempt at egg donation or during a mock cycle, a trial of low-dose aspirin (81 mg daily) given at the time of the transfer cycle may increase pregnancy rates. Weckstein et al. found that in women with a previous endometrial thickness of <8 mm, the addition of low-dose aspirin increased the implantation rate from 9% in the untreated group to 24% in the treated group, despite a lack of increased endometrial thickness (51).

It has been argued that women of advanced reproductive age may demonstrate a higher percentage of out-of-phase biopsies (52), but both the biopsy and pregnancy outcomes may be corrected with appropriate doses of progesterone (53). Notably, older women can expect pregnancy rates with oocyte donation comparable to younger recipients (54–56), whether or not mock cycles are performed.

OOCYTE DONOR RECRUITMENT

Perhaps the greatest obstacle to performing oocyte donation is the recruitment of suitable donors (57). Historically, donor eggs were obtained from women undergoing IVF with "excess oocytes." Many of these patients had ovarian abnormalities underlying their own infertility, making them imperfect donors. Furthermore, with the advent of increasingly successful embryo cryopreservation and the use of "softer" stimulation protocols, "extra oocytes" have become scarce. Obvious sources for oocytes are women undergoing tubal sterilization who might be willing to be hyperstimulated. However, very few of these women are eligible, since most are not willing to undergo fertility drug treatment, and many are >35 years old (58). Known designated donors are yet another option. Typically, a family member (e.g., sister or niece) or very close friend is selected. The final sources of donors are women recruited from the general population at large, most often through advertisement.

There has been a long-standing debate as to whether it is ethical to pay oocyte donors for their eggs, and if so, how much. Areas of contention include the selling of body parts and exaggerated incentives that may represent an enticement for a procedure that carries risk and no direct medical benefit to the donor. For this reason, many countries do not permit oocyte donation (e.g., Germany, Norway, and Sweden) (59). Other countries allow only IVF patients with excess oocytes to donate. Israel, Great Britain, and Canada allow anonymous oocyte donation, but strongly discourage payment to the donor, except for verified expenses. The U.S.A. has no current regulation on payments to donors. The payments are construed as reimbursement for time and inconvenience (60), and indeed, without payment, it is doubtful that any country will recruit sufficient donors to meet demand (61). The appropriate amount of payment remains hotly debated (62).

Another area of controversy focuses on anonymity and identity disclosure. Most donors express a strong desire not to be identified by the children. In exchange for anonymity, they willingly forfeit all legal obligations as parents. There is, however, an opposing view that, similar to adopted children, offspring of egg donation should have the same right to ultimately identify their genetic mother (63). As a result, the U.K. now mandates that donor identity be revealed to a child resulting from egg donation once he or she reaches the age of 18 years. In the U.S.A., there is little historic precedent for such a change in public policy, but should such legislation be enacted, a deleterious effect on donor recruitment can be expected (64).

An alternative to cycle-synced egg donation is the "egg bank." In the U.S.A., there are currently over 3000 oocytes from 294 donors stored in commercial egg banks (65). For recipients, the appeal of using an egg bank is great. Egg banks remove wait times, allow for longer quarantine periods permitting a longer infectious disease screen, and, in many cases, offer more options in terms of donors. For clinicians, egg banks may reduce the amount of discarded embryos after donor cycles. Given that commercial egg banks are still relatively new, data are not widely available about pregnancy rates. The preliminary data suggest that success rates are similar to those of major IVF centers (65).

OOCYTE DONOR SCREENING

Oocyte donors need to provide full and comprehensive informed consent. The risks of participating in oocyte donation are few, and are basically no different from those of standard IVF. Controlled ovarian stimulation entails both known and theoretical risks. The risk of severe ovarian hyperstimulation syndrome (OHSS) is reported in approximately 1% of cases, although donors may be at less risk of severe OHSS compared with patients undergoing IVF, since pregnancy does not occur in the donor and moderate cases of OHSS are therefore not exacerbated (66). Using gonadotropin-releasing hormone (GnRH) agonist to trigger final oocyte maturation has been shown to significantly reduce the occurrence of OHSS compared to using human chorionic gonadotropin and represents a valid alternative for hyperstimulated egg donors to further reduce morbidity (67). In addition to a complete medical history and physical examination, the suggested medical screening of oocyte donors is shown in Table 68.3. Of utmost importance is the screening for infectious diseases. Unlike sperm, which are amenable to cryopreservation, oocytes have traditionally not been frozen for subsequent use. In sperm donation, cryopreservation allows a quarantine period and follow-up testing for infectious diseases. With respect to current practice, egg cryopreservation has not been universally adapted to oocyte donors. Transvaginal ultrasound examination is performed to detect pelvic pathology and determine ovarian morphology.

It is preferable that oocyte donors be under 30 years old, as younger donors appear to have higher pregnancy rates (54,68). Pregnancy rates of donors >30 years old are still acceptable, however, and other traits and characteristics (e.g., a close physical match to the recipient or advanced educational degree) may make a particular older donor desirable to a recipient. The prior fertility history of the

Table 68.3 Suggested medical screening of oocyte donors

Complete blood count with platelets
Blood type
Hepatitis screen
VDRL, HIV-1, HTLV-1
Cervical cultures for gonorrhea and chlamydia
Pap smear
Transvaginal ultrasound of pelvis
Appropriate genetic tests

Abbreviations: HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; VDRL, Venereal Disease Research Laboratory.

donor does not appear to affect pregnancy outcomes (66,68). The concept of a "proven" donor is a popular myth, and lacks evidence-based support.

Psychological evaluation by a licensed mental health practitioner is recommended for anonymous donors and is mandatory for known donors. Screening should focus on their motivation to donate, as well as their financial status to ensure that their participation is not overly influenced by monetary enticement. An assessment of coping skills and lifestyle is important to predict the donor's ability to participate in a lengthy and complicated process.

Occasionally, a history of psychiatric illness or drug and/or alcohol use in the donor or her family is elicited. These behaviors may have a genetic etiology and as such would exclude the potential donor from participation. Genetic screening begins with a detailed history of the potential donor and her family. A sample history form is presented in Table 68.4 (69). The presence of any of the disorders should exclude her from participating. Donors should be <35 years old to reduce the risk of aneuploidy in the offspring. Exceptions can be made in circumstances such as sister-to-sister donation where the benefits of a shared genetic background may balance the known risks (which can be largely discovered by amniocentesis). A donor should not have any major Mendelian disorder. These include cystic fibrosis in whites, a sickle cell anemia test for blacks, and a complete blood count and mean corpuscular volume followed by hemoglobin electrophoresis in abnormal results for people of Mediterranean and Chinese ancestry to assess the risk of β-thalassemia, and in people of Southeast Asian ancestry for α-thalassemia. Jews of eastern European ancestry should be screened for Tay–Sachs disease, Gaucher disease, mucolipidosis IV, Niemann–Pick disease, Bloom syndrome, familial dysautonomia, Fanconi anemia, fragile X syndrome, and Canavan disease. A donor should not have any major malformation of complex cause, such as spina bifida or heart malformation. A donor should not carry a known karyotypic abnormality that may result in chromosomally unbalanced gametes. If a donor is a member of a high-risk group, then the donor must be screened for carrier status (70). It is important to inform the recipient couples that even with appropriate screening, 2%–3% of babies are born with a major or minor malformation, and many genetic disorders cannot be detected or prevented with current testing methodology (71).

Guidelines for gamete and embryo donation have been periodically published and recently updated in an attempt to standardize screening policies and to incorporate recent regulations from the U.S. Food and Drug Administration (FDA) (70).

ENDOMETRIAL STIMULATION AND SYNCHRONIZATION

Endometrial preparation of the recipient is modeled on the natural menstrual cycle, using estrogen and progesterone (24). The initial estrogenic phase is most often maintained using either daily oral estradiol 4–8 mg or transdermal estrogen 0.2–0.4 mg. The initial results of oocyte donation cycles were significantly better than typically seen after standard IVF. The apparent detrimental effect of standard IVF on embryo implantation was felt to be secondary to the supraphysiologic concentrations of estrogen attained after controlled ovarian stimulation (72,73). Transdermal estrogen adequately prepares the endometrium with overall lower serum concentrations of estrogens because of the lack of hepatic first-pass effect. However, the higher concentrations of serum estrogens noted following oral administration are of questionable clinical significance. Krasnow et al. found estradiol concentrations to be 10-fold higher in the oral estrogen group and noted a higher rate of out-of-phase endometrial biopsies (74). Others, however, have shown no detriment with high levels of estrogen (75). Most programs continue to prescribe oral estradiol due to its ease of administration, lack of side effects, and long history of clinical success.

The length of estrogenic exposure may vary widely with little apparent clinical effect, again mimicking the variable follicular phase found in natural menstrual cycles. Anywhere from 6 to 38 days of prescribed estrogen prior to progesterone appears adequate (43,76,77). Most programs prescribe at least 12–14 days of estrogen before initiating progesterone, but studies report that if it is necessary to prolong this period, perhaps because of a slow stimulation of the oocyte donor, no adverse effects are expected.

Synchronization of the recipient and donor is relatively easy to accomplish. The recipient begins estrogen several days prior to beginning ovarian stimulation in the donor to provide approximately 14 days of estradiol prior to progesterone administration. Ovulating recipients typically receive GnRH agonist for downregulation as in standard IVF cycles (e.g., 1 mg leuprolide acetate daily until suppressed, then 0.5 mg daily thereafter) to render them functionally agonadal. Alternatively, ovulating recipients are started on oral estrogen at the beginning of their menstrual cycle and maintained on estrogen, and a GnRH antagonist is used to block the LH surge, until the day of the donor's oocyte retrieval when progesterone is begun (Figure 68.2) (76).

The timing of progesterone administration is more stringent. Navot et al. reported that the optimal time for embryo transfer was two to four days after progesterone

Table 68.4 Genetic screening form given to oocyte donors

Pregnancy history: Please list all the times you have been pregnant and the outcomes.

Family ethnic background: Please indicate all relevant information in the following tables. When the requested information is unknown, please say so. If comments are needed, please make them. Remember that we are interested in your genetic background. If any relevant family member is adopted, please say so.

Relation	Age if living	Age at death	Cause of death
Grandfather (paternal)			
Grandmother (paternal)			
Grandfather (maternal)			
Grandmother (maternal)			
Father			
Mother			
Brothers			
Sisters			

Family genetic history

Familial conditions	Self	Mother	Father	Siblings	Comments
High blood pressure					
Heart disease					
Deafness					
Blindness					
Severe arthritis					
Juvenile diabetes					
Alcoholism					
Schizophrenia					
Depression or mania					
Epilepsy					
Alzheimer's disease					
Other (specify)					
Malformations					
Cleft lip or palate					
Heart defect					
Clubfoot					
Spina bifida					
Other (specify)					
Mendelian disorders					
Color blindness					
Cystic fibrosis					
Hemophilia					
Muscular dystrophy					
Sickle cell anemia					
Huntington's disease					
Polycystic kidneys					
Glaucoma					
Tay–Sachs disease					

Please take the time to explain any other problems or conditions in your family history that you feel could pertain to the health of future generations.

Source: From Maxwell K et al. *Fertil Steril* 2008; 90: 2165–71.

initiation for embryos at the 2- to 12-cell stage (78). This corresponds to days 17–19 of the recipient's cycle, with day 15 defined as the day of progesterone initiation. No pregnancies were observed before two days or after four days of progesterone administration. These findings were confirmed by Prapas et al., who further delineated the optimal time for transfer of four- to eight-cell embryos to days 18 and 19 (79).

The dose of progesterone is typically 100 mg intramuscularly daily or 100–600 mg transvaginally daily. Many groups prefer the transvaginal approach because lower serum concentrations of progesterone are required to achieve target organ effect. Serum levels are low in these patients, but local tissue levels are high probably because of the absence of the hepatic first-pass effect on clearance. As with estrogen, however, it has not been resolved as to whether the mode of delivery of progesterone or its dose is of clinical significance. Most groups continue estrogen support through the progestational period, although at least one study has shown that continued estrogen use is not actually required (80).

Progesterone (and estrogen) administration can be discontinued once the placenta has established adequate steroidogenesis. Devroey et al. estimated this to occur at seven to nine weeks of gestation (81), while others have advanced this to the fifth week (82). Clinically, we begin weekly monitoring of serum progesterone concentrations 10 weeks after embryo transfer when a serum level of ≥30 ng/mL is typically attained. At that point, prescribing exogenous steroids is superfluous.

CLINICAL AND OBSTETRIC OUTCOMES

Recipients of donated eggs experience implantation and pregnancy rates similar to those normally seen in young women undergoing IVF. Thus, the ASRM recommends that no more than two high-quality embryos be transferred to patients in order to lessen the risk of multiple gestation. Oocyte donation has always been associated with the highest success rate among ARTs, and presently more than 50% of embryo transfers result in live births (9). Occasionally, preimplantation genetic screening (PGS) has also been used in an attempt to select better embryos for transfer, since nearly half of biopsied normal-appearing embryos selected from donors in their mid-20s were aneuploid (83). In this study, transferring the PGS-selected embryos improved delivery rates and lowered the miscarriage rate of recipients, although at an additional fiscal cost to the patient. Egg donation has been applied to treat infertility in women of advanced reproductive age (>45 years old) since 1990 and has soared in popularity as a result of its ability to reverse the inevitable loss of fertility in women approaching menopause (84). However, as demonstrated by a large retrospective review of 3089 cycles from Valencia, recipients >45 years old had lower pregnancy rates (49% vs. 44%), lower implantation rates (21% vs. 17%), and higher miscarriage rates (17% vs. 23%) than

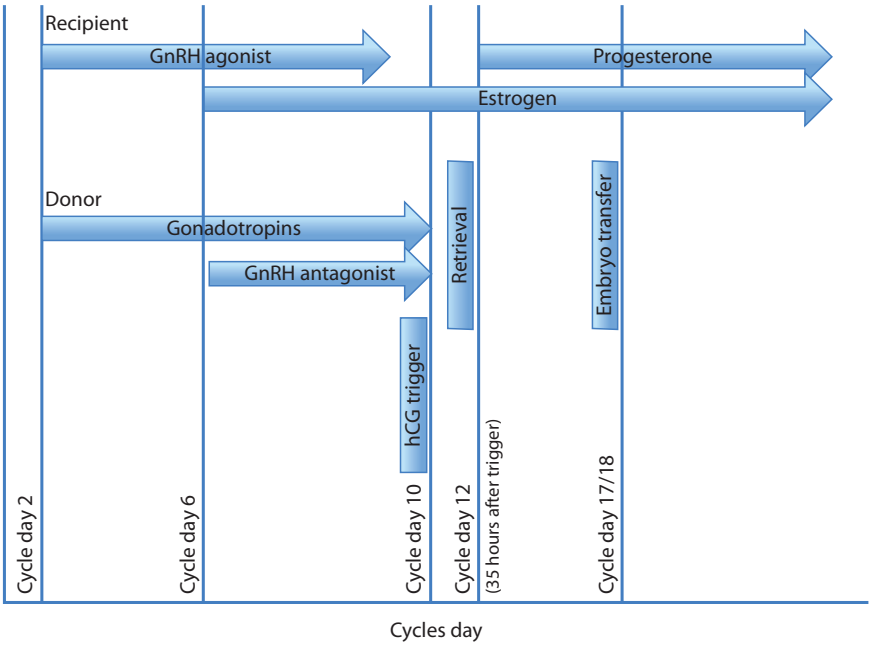

Figure 68.2 Schematic representation of cycle synchronization using a GnRH agonist in both donor and recipient. GnRH agonists are used to down-regulate the pituitary of recipients with evidence of ovarian activity prior to beginning oral estradiol. Oral estradiol is prescribed to the recipient four to five days in advance of the donor starting gonadotropin injections. Progesterone is administered starting the day after hCG injection in the donor, and one day prior to aspirating oocytes. Embryo transfer is performed three days following oocyte retrieval. Serum pregnancy testing occurs 12 days post-transfer. Pregnant patients are maintained on estradiol and progesterone through to 12 weeks of gestational age. *Abbreviations*: GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin.

younger recipients (85). This trend is similarly apparent in reviewing the CDC/SART data from 2012 (Figure 68.3). Therefore, the ability to totally restore uterine receptivity using estradiol and progesterone replacement remains uncertain in the presence of advancing reproductive age.

Several groups have evaluated the obstetric outcomes of pregnancies following oocyte donation and concluded that results are favorable (33,86–89). Common to all reports, however, were increases in the incidence of gestational hypertension and delivery by cesarean section. Soderstrom-Anttila et al. compared 51 oocyte donation deliveries to 97 IVF deliveries and noted a higher rate of pregnancy induced hypertension (PIH) (31% vs. 14%) and cesarean section (57% vs. 37%) with oocyte donation (88). Gestational hypertension was evaluated by the study of 72 pregnancies from donated gametes with age- and parity-matched controls (90). Pre-eclampsia was noted to be much higher in the donated gamete group (18.1% vs. 1.4%), suggesting an autoimmune component to the disorder. The increased risk of gestational hypertension appears to occur in younger recipients (<35 years old) as well (91). Two other studies evaluated older oocyte donation patients and found most complications, such as gestational diabetes and preterm labor, were associated with multiple pregnancies (33,86). Another clinical trial showed that 59 children of oocyte donation aged six months to four years had growth and development comparable to children from IVF and the general population (92). A review of pregnancy outcomes of 45 women >50 years old who delivered babies following egg donation at the University of Southern California demonstrated an increase in obstetric complications, with pre-eclampsia occurring in 35%, gestational diabetes in 20%, and multiple births in 35% (93). Antinori et al. described a 12-year experience with peri- and post-menopausal women aged between 45 and 63 years in which 2729 women were screened (94). Only 42% of these women were suitable candidates, as the majority were deemed too high risk for pregnancy due to underlying medical conditions. Overall, 1288 recipient cycles resulted in pregnancy in 38% of transfer events, with 28% delivering per transfer. Antenatal complications were common (23.6%) in the ongoing pregnancies and included gestational hypertension, gestational diabetes, and preterm labor. A recent review of 101 consecutive pregnancies in women aged 50 years and older at Columbia University also noted an increased incidence of hypertensive disorders, gestational diabetes, premature rupture of the membranes, preterm labor, and abnormal placentation, similar to rates also seen in younger women (<42 years) undergoing egg donation (95). In summary, oocyte donation pregnancies should be considered high risk. However, in well-screened patients, the complications are manageable and parents can reasonably expect healthy children.

Additional factors that may affect donor IVF (DIVF) outcomes include male age, racial differences (particularly in the black population), and advanced maternal age. In DIVF cycles, advanced paternal age, usually quantified as >50 year old, has been linked to adverse pregnancy outcomes. Frattarelli et al. examined 1023 infertile couples undergoing oocyte donation cycles. Amongst this group, there was a significant increase in pregnancy loss, decrease in live birth rate, and decrease in blastocyst formation rate if the men were >50 years old (96). Race, particularly black race, can lead to poorer reproductive outcomes in DIVF cycles. In a large retrospective analysis of 1012 couples undergoing DIVF cycles, the pregnancy rates for black and Caucasian couples were significantly different. Black couples conceived at a rate of 24.6% compared to Caucasian couples at 36.8%. After adjusting for confounding variables, black race was identified as an independent risk factor for not achieving

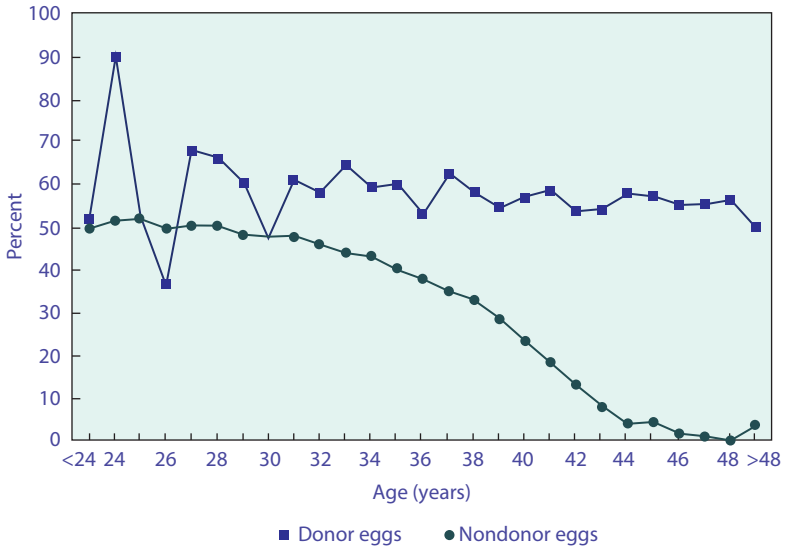

Figure 68.3 Live births per transfer and singleton live births per transfer for assisted reproduction technology cycles using fresh embryos from donor eggs, by assisted reproduction technology partner's age, 2012 (9) (squares, donor eggs; circles, non-donor eggs).

an ongoing pregnancy (97). There is some concern that endometrial receptively declines with advancing maternal age. Yen et al. published a retrospective cohort evaluation of 27,959 fresh DIVF cycles comparing implantation, clinical pregnancy, and live birth rates among recipient age groups. They found that all of these outcomes were significantly decreased in recipients older than 45 years. Moreover, rates were significantly worse in the >50 years old group compared to the 45–49 years old group (98).

EMBRYO DONATION

Embryo donation has become more common as social attitudes toward single women and assisted reproduction have relaxed and the enhanced efficiency of cryopreservation has led to the banking of a large number of human embryos. The deliberate use of donor gametes, utilizing both sperm and egg, was described in 1995 as a means of "preimplantation adoption" (99). A programmed approach for creating embryos using donor gametes in single women of advanced reproductive age was suggested again in 1999 as a highly efficient and cost-effective means of establishing pregnancy (100). More often, donated embryos are obtained from couples who have successfully conceived through IVF and now wish to give their cryopreserved supernumerary embryos to clinical programs for use in infertile women (101). Interestingly, couples and women who did not use frozen embryos after pregnancy with donor gametes were more likely to donate them for use in other women than women who had embryos banked following standard IVF with their own eggs. Original guidelines for embryo donation were published by the ASRM in 1998 (102). Recommendations include that the embryos undergo a minimum of six months of quarantine and that all donors are retested for infectious diseases prior to their use. Proper documentation of chain of custody of donated embryos and witnessed written relinquishment of embryos is also suggested. Although the program may charge professional fees for the service, embryos cannot be "sold," and donors cannot receive compensation.

FUTURE DIRECTIONS

The next frontier in oocyte donation may include the use of enucleated donor oocytes, which would allow recipients to use their own genetic material. This has already been successful done in humans (103). This concept of three-parent IVF would potentially allow for eradication of mitochondrial diseases. By removing the nuclear DNA from an oocyte with abnormal mitochondrial DNA (mtDNA) into a fertilized donor oocyte, the embryo would have nuclear DNA from each parent and mtDNA from a donor. This is in the early stages of investigation. As a last note, we should mention that there is no consensus among providers who have DIVF programs as to what an evidence-based approach to management of oocyte donors and recipients is (104). Given the controversies inherent to gamete donation, a closer examination of practices would be beneficial in the future to standardize our care.

REFERENCES

1. Heape W. Preliminary note on the transplantation and growth of mammalian ova in a foster mother. *Proc R Sc London* 1890; 48: 457.
2. Hasler JF. Current status and potential of embryo transfer and reproductive technology in dairy cattle. *J Dairy Sci* 1992; 75: 2857–79.
3. Grimes JF. *Utilization of Embryo Transfer in Beef Cattle. Fact sheet Agriculture and Natural Resources ANR-17-08*. The Ohio State University Extension, 2008. Available from: http://ohioline.osu.edu
4. Buster JE, Bustillo M, Thorneycroft I et al. Nonsurgical transfer of an *in-vivo* fertilised donated ovum to an infertility patient. *Lancet* 1983; 1: 816–7.
5. Buster JE, Bustillo M, Thorneycroft IH et al. Nonsurgical transfer of *in vivo* fertilised donated ova to five infertile women: Report of two pregnancies. *Lancet* 1983; 2: 223–4.
6. Bustillo M, Buster JE, Cohen SW et al. Delivery of a healthy infant following nonsurgical ovum transfer. *JAMA* 1984; 251: 889.
7. Trounson A, Leeton J, Besanko M et al. Pregnancy established in an infertile patient after transfer of a donated embryo fertilised *in vitro*. *Br Med J (Clin Res Ed)* 1983; 286: 835–8.
8. Lutjen P, Trounson A, Leeton J, et al. The establishment and maintenance of pregnancy using *in vitro* fertilization and embryo donation in a patient with primary ovarian failure. *Nature* 1984; 307: 174–5.
9. Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2010 *Assisted Reproductive Technology Fertility Clinic Success Rates Report*. Atlanta: U.S. Department of Health and Human Services; 2012.
10. Ventura SJ. First births to older mothers, 1970–86. *Am J Public Health* 1989; 79: 1675–7.
11. Hollander D, Breen JL. Pregnancy in the older gravida: How old is old? *Obstet Gynecol Surv* 1990; 45: 106–12.
12. Bustillo M, Buster JE, Cohen SW et al. Nonsurgical ovum transfer as a treatment in infertile women. preliminary experience. *JAMA* 1984; 251: 1171–3.
13. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. *Obstet Gynecol* 1986; 67: 604–6.
14. Coulam CB. Premature gonadal failure. *Fertil Steril* 1982; 38: 645–55.
15. Manuel M, Katayama PK, Jones HW, Jr. The age of occurrence of gonadal tumors in intersex patients with a Y chromosome. *Am J Obstet Gynecol* 1976; 124: 293–300.
16. Cohen MA, Chang PL, Lindheim SR, Sauer MV. Diminished bone density in menopausal women undergoing ovum donation. *Annual Meeting of the American Society for Reproductive Medicine*, San Francisco, CA, 1998 (abstract).

17. Chevalier N, Letur H, Lelannou D et al. Maternal-Fetal cardiovascular complication in turner syndrome after oocyte donation: Insufficient pre-pregnancy screening and pregnancy follow-up are associated with poor outcome. *J Clin Endocrinol Metab* 2011; 96: E260–7.
18. Moncayo R, Moncayo HE. Autoimmunity and the ovary. *Immunol Today* 1992; 13: 255–8.
19. Kaufman FR, Donnell GN, Roe TF, Kogut MD. Gonadal function in patients with galactosaemia. *J Inherit Metab Dis* 1986; 9: 140–6.
20. Christin-Maitre S, Vasseur C, Portnoi MF, Bouchard P. Genes and premature ovarian failure. *Mol Cell Endocrinol* 1998; 145: 75–80.
21. Sherman SL. Premature ovarian failure in the fragile X syndrome. *Am J Med Genet* 2000; 97: 189–94.
22. Byrne J, Mulvihill JJ, Myers MH et al. Effects of treatment on fertility in long-term survivors of childhood or adolescent cancer. *N Engl J Med* 1987; 317: 1315–21.
23. Gradishar WJ, Schilsky RL. Ovarian function following radiation and chemotherapy for cancer. *Semin Oncol* 1989; 16: 425–36.
24. Sauer MV, Paulson RJ, Lobo RA. A preliminary report on oocyte donation extending reproductive potential to women over 40. *N Engl J Med* 1990; 323: 1157–60.
25. Scott RT, Toner JP, Muasher SJ et al. Follicle-stimulating hormone levels on cycle day 3 are predictive of *in vitro* fertilization outcome. *Fertil Steril* 1989; 51: 651–4.
26. Licciardi FL, Liu HC, Rosenwaks Z. Day 3 estradiol serum concentrations as prognosticators of ovarian stimulation response and pregnancy outcome in patients undergoing *in vitro* fertilization. *Fertil Steril* 1995; 64: 991–4.
27. LaMarca A, Sighinolfi G, Radi D et al. Anti-Mullerian hormone as a predictive marker in assisted reproductive technology. *Hum Reprod Update* 2010; 16: 113–30.
28. Navot D, Rosenwaks Z, Margalioth EJ. Prognostic assessment of female fecundity. *Lancet* 1987; 2: 645–7.
29. Seifer DB, Lambert-Messerlian G, Hogan JW et al. Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. *Fertil Steril* 1997; 67: 110–4.
30. Hsu A, Arny M, Knee AB, Bell C, Cook E, Novak AL, Grow DR. Antral follicle count in clinical practice: Analyzing clinical relevance. *Fertil Steril* 2011; 95: 474.
31. Remohi J, Gallardo E, Levy M et al. Oocyte donation in women with recurrent pregnancy loss. *Hum Reprod* 1996; 11: 2048–51.
32. Ethics Committee of the American Society for Reproductive Medicine. Oocyte or embryo donation to women of advanced age: A committee opinion. *Fertil Steril* 2013; 100: 337–40.
33. Sauer MV, Paulson RJ, Lobo RA. Oocyte donation to women of advanced reproductive age: Pregnancy results and obstetrical outcomes in patients 45 years and older. *Hum Reprod* 1996; 11: 2540–3.
34. Paulson RJ, Sauer MV. Pregnancies in postmenopausal women. Oocyte donation to women of advanced reproductive age: 'How old is too old?' *Hum Reprod* 1994; 9: 571–2.
35. Van Voorhis BJ, Williamson RA, Gerard JL et al. Use of oocytes from anonymous, matched, fertile donors for prevention of heritable genetic diseases. *J Med Genet* 1992; 29: 398–9.
36. Munné S, Chen S, Colls P et al. Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos. *Reprod Biomed Online* 2007; 14: 628–34.
37. Greenfield DA, Seli E. Gay men choosing parenthood through assisted reproduction: Medical and psychosocial considerations. *Fertil Steril* 2011; 95: 225–9.
38. Ethics Committee of the American Society for Reproductive Medicine. Access to fertility treatment by gays, lesbians, and unmarried persons. *Fertil Steril* 2006; 86: 1333–5.
39. Burns L. An overview of the psychology of infertility. *Infertil Reprod Med Clin N Am* 1993; 3: 433–54.
40. Golombok S, Cook R, Bish A, Murray C. Families created by the new reproductive technologies: Quality of parenting and social and emotional development of the children. *Child Dev* 1995; 66: 285–98.
41. Bustillo M, Krysa LW, Coulam CB. Uterine receptivity in an oocyte donation programme. *Hum Reprod* 1995; 10: 442–5.
42. Cohen MA, Lindheim SR, Sauer MV. Hydrosalpinges adversely affect implantation in donor oocyte cycles. *Hum Reprod* 1999; 14: 1087–9.
43. Navot D, Anderson TL, Droesch K et al. Hormonal manipulation of endometrial maturation. *J Clin Endocrinol Metab* 1989; 68: 801–7.
44. Navot D, Bergh PA, Williams M et al. An insight into early reproductive processes through the *in vivo* model of ovum donation. *J Clin Endocrinol Metab* 1991; 72: 408–14.
45. Abdalla HI, Brooks AA, Johnson MR et al. Endometrial thickness: A predictor of implantation in ovum recipients? *Hum Reprod* 1994; 9: 363–5.
46. Antinori S, Versaci C, Gholami GH et al. Oocyte donation in menopausal women. *Hum Reprod* 1993; 8: 1487–90.
47. Shapiro H, Cowell C, Casper RF. The use of vaginal ultrasound for monitoring endometrial preparation in a donor oocyte program. *Fertil Steril* 1993; 59: 1055–8.
48. Hofmann GE, Thie J, Scott RT, Jr., Navot D. Endometrial thickness is predictive of histologic endometrial maturation in women undergoing hormone replacement for ovum donation. *Fertil Steril* 1996; 66: 380–3.

49. Glujovsky D, Pesce R, Fiszbajn G et al. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. *Cochrane Database Syst Rev* 2010: 1: CD006359.
50. Li TC, Dockery P, Ramsewak SS et al. The variation of endometrial response to a standard hormone replacement therapy in women with premature ovarian failure. An ultrasonographic and histological study. *Br J Obstet Gynaecol* 1991; 98: 656–61.
51. Weckstein LN, Jacobson A, Galen D et al. Low-dose aspirin for oocyte donation recipients with a thin endometrium: Prospective, randomized study. *Fertil Steril* 1997; 68: 927–30.
52. Potter DA, Witz CA, Burns WN et al. Endometrial biopsy during hormone replacement cycle in donor oocyte recipients before *in vitro* fertilization–embryo transfer. *Fertil Steril* 1998; 70: 219–21.
53. Meldrum D. Female reproductive aging—Ovarian and uterine factors. *Fertil Steril* 1993; 59: 1–5.
54. Balmaceda JP, Bernardini L, Ciuffardi I et al. Oocyte donation in humans: A model to study the effect of age on embryo implantation rate. *Hum Reprod* 1994; 9: 2160–3.
55. Abdalla HI, Wren ME, Thomas A, Korea L. Age of the uterus does not affect pregnancy or implantation rates; a study of egg donation in women of different ages sharing oocytes from the same donor. *Hum Reprod* 1997; 12: 827–9.
56. Stolwijk AM, Zielhuis GA, Sauer MV, Hamilton CJ, Paulson RJ. The impact of the woman's age on the success of standard and donor *in vitro* fertilization. *Fertil Steril* 1997; 67: 702–10.
57. Marina S, Exposito R, Marina F et al. Oocyte donor selection from 554 candidates. *Hum Reprod* 1999; 14: 2770–6.
58. Feinman M, Barad D, Szigetvari I, Kaali SG. Availability of donated oocytes from an ambulatory sterilization program. *J Reprod Med* 1989; 34: 441–3.
59. Gunning JH. Oocyte donation: The legislative framework in Western Europe. *Hum Reprod* 1998; 13(Suppl 2): 98–104.
60. Sauer MV. Reproductive prohibition: Restricting donor payment will lead to medical tourism. *Hum Reprod* 1997; 12: 1844–5.
61. McLaughlin EA, Day J, Harrison S et al. Recruitment of gamete donors and payment of expenses. *Hum Reprod* 1998; 13: 1130–2.
62. Sauer MV. Indecent proposal: $5,000 is not "reasonable compensation" for oocyte donors. *Fertil Steril* 1999; 71: 7–10.
63. Annas GJ. The shadowlands—Secrets, lies, and assisted reproduction. *N Engl JMed* 1998; 339: 935–9.
64. Sauer MV. Further HFEA restrictions on egg donation in the UK: Two strikes and you're out! *Reprod Biomed Online* 2005; 10: 431–3.
65. Quaas, A, Melamed A, Chung K et al. Egg banking in the United States: Current status of commercially available cryopreserved oocytes. *Fertil Steril* 2013;99;827–31.
66. Maxwell K, Cholst IN, Rosenwaks Z. The incidence of both serious and minor complications in young women undergoing oocyte donation. *Fertil Steril* 2008; 90: 2165–71.
67. Humaidan P, Kol S, Papanikolaou EG. GnRH agonist for triggering of final oocyte maturation: Time for a change of practice? *Hum Reprod Update* 2011; 17: 510–24.
68. Cohen MA, Lindheim SR, Sauer MV. Donor age is paramount to success in oocyte donation. *Hum Reprod* 1999; 14: 2755–8.
69. Brown S. Genetic aspects of donor selection. In: *Principles of Oocyte and Embryo Donation*. Sauer MV (ed.). New York, NY: Springer-Verlag, 1998, pp. 53–63.
70. Practice Committee of American Society for Reproductive Medicine, Practice Committee of Society for Assisted Reproductive Technology. Recommendations for gamete and embryo donation: A committee opinion. *Fertil Steril* 2013; 99: 47–62.
71. Baird PA, Anderson TW, Newcombe HB, Lowry RB. Genetic disorders in children and young adults: A population study. *Am J Hum Genet* 1988; 42: 677–93.
72. Edwards RG. Why are agonadal and post-amenorrhoeic women so fertile after oocyte donation? *Hum Reprod* 1992; 7: 733–4.
73. Check JH, Nowroozi K, Chase J, Nazari A, Braithwaite C. Comparison of pregnancy rates following *in vitro* fertilization–embryo transfer between the donors and the recipients in a donor oocyte program. *J Assist Reprod Genet* 1992; 9: 248–50.
74. Krasnow JS, Lessey BA, Naus G et al. Comparison of transdermal versus oral estradiol on endometrial receptivity. *Fertil Steril* 1996; 65: 332–6.
75. de Ziegler D. Hormonal control of endometrial receptivity. *Hum Reprod* 1995; 10: 4–7.
76. Serhal PF, Craft IL. Ovum donation—A simplified approach. *Fertil Steril* 1987; 48: 265–9.
77. Younis JS, Mordel N, Ligovetzky G et al. The effect of a prolonged artificial follicular phase on endometrial development in an oocyte donation program. *J In Vitro Fert Embryo Transf* 1991; 8: 84–8.
78. Navot D, Scott RT, Droesch K et al. The window of embryo transfer and the efficiency of human conception *in vitro*. *Fertil Steril* 1991; 55: 114–8.
79. Prapas Y, Prapas N, Jones EE et al. The window for embryo transfer in oocyte donation cycles depends on the duration of progesterone therapy. *Hum Reprod* 1998; 13: 720–3.
80. Lewin A, Benshushan A, Mezker E et al. The role of estrogen support during the luteal phase of *in vitro* fertilization–embryo transplant cycles: A comparative study between progesterone alone and estrogen and progesterone support. *Fertil Steril* 1994; 62: 121–5.

81. Devroey P, Camus M, Palermo G et al. Placental production of estradiol and progesterone after oocyte donation in patients with primary ovarian failure. *Am J Obstet Gynecol* 1990; 162: 66–70.
82. Scott R, Navot D, Liu HC, Rosenwaks Z. A human *in vivo* model for the luteoplacental shift. *Fertil Steril* 1991; 56: 481–4.
83. Reis Soares S, Rubio C, Rodrigo L et al. Does preimplantation genetic diagnosis improve the outcome of egg donation cycles? *19th Annual Meeting of ESHRE*, 2003, Madrid, Spain, P-568, xviii, 189.
84. Sauer MV, Kavic SM. Oocyte and embryo donation 2006: Reviewing two decades of innovation and controversy. *Reprod Biomed Online* 2006; 12: 153–62.
85. Reis Soares S, Troncoso C, Bosch E et al. Age and uterine receptiveness: Predicting the outcome of oocyte donation cycles. *J Clin Endocrinol Metab* 2005; 90: 4399–404.
86. Pados G, Camus M, Van Steirteghem A et al. The evolution and outcome of pregnancies from oocyte donation. *Hum Reprod* 1994; 9: 538–42.
87. Wolff KM, McMahon MJ, Kuller JA et al. Advanced maternal age and perinatal outcome: Oocyte recipiency versus natural conception. *Obstet Gynecol* 1997; 89: 519–23.
88. Soderstrom-Anttila V, Tiitinen A, Foudila T, Hovatta O. Obstetric and perinatal outcome after oocyte donation: Comparison with *in-vitro* fertilization pregnancies. *Hum Reprod* 1998; 13: 483–90.
89. Abdalla HI, Billett A, Kan AK et al. Obstetric outcome in 232 ovum donation pregnancies. *Br J Obstet Gynaecol* 1998; 105: 332–7.
90. Salha O, Sharma V, Dada T et al. The influence of donated gametes on the incidence of hypertensive disorders of pregnancy. *Hum Reprod* 1999; 14: 2268–73.
91. Keegan DA, Krey LC, Chang HC, Noyes N. Increased risk of pregnancy-induced hypertension in young recipients of donated oocytes. *Fertil Steril* 2007; 87: 776–81.
92. Soderstrom-Anttila V, Sajaniemi N, Tiitinen A, Hovatta O. Health and development of children born after oocyte donation compared with that of those born after *in-vitro* fertilization, and parents' attitudes regarding secrecy. *Hum Reprod* 1998; 13: 2009–15.
93. Paulson RJ, Boostanfar R, Saadat P et al. Pregnancy in the sixth decade of life. Obstetric outcomes in women of advanced reproductive age. *JAMA* 2002; 288: 2320–3.
94. Antinori S, Gholami GH, Versaci C et al. Obstetric and prenatal outcome in menopausal women: A 12-year clinical study. *Reprod Biomed Online* 2002; 6: 257–61.
95. Kort DH, Gosselin J, Choi JM et al. Pregnancy after age 50: Defining risks for mother and child. *Am J Perinatol* 2012; 29: 245–50.
96. Frattarelli J, Miller K, Miller B et al. Male age negatively impacts embryo development and reproductive outcome in donor oocyte assisted reproductive technology cycles. *Fertil Steril* 2008; 90: 97–103.
97. Bodri D, Guillen JJ, Lopez M. et al. Racial disparity in oocyte donation outcome: A multiethnic, matched cohort study. *Hum Reprod* 2010; 2: 436–42.
98. Yen JS, Steward RG, Dude AM, Shah AA, Goldfarb JM, Muasher SJ. Pregnancy outcomes decline in recipients over age 44: An analysis of 27,959 fresh donor oocyte *in vitro* fertilization cycles from the Society for Assisted Reproductive Technology. *Fertil Steril* 2014; 101: 133–6.
99. Sauer MV, Paulson RJ, Francis MM et al. Preimplantation adoption: Establishing pregnancy using donated oocytes and spermatozoa. *Hum Reprod* 1995; 10: 1419–22.
100. Lindheim SR, Sauer MV. Embryo donation: A programmed approach. *Fertil Steril* 1999; 72: 940–1.
101. Sehnert B, Chetkowski RJ. Secondary donation of frozen embryos is more common after pregnancy initiation with donated eggs than after *in vitro* fertilization–embryo transfer and gamete intrafallopian transfer. *Fertil Steril* 1998; 69: 350–2.
102. The American Society for Reproductive Medicine. Guidelines for gamete and embryo donation. Guidelines for embryo donation. *Fertil Steril* 1998; 70(Suppl 3): 7S–8S.
103. St.John JC, Barratt CL. Use of anucleate donor oocyte cytoplasm in recipient eggs. *Lancet* 1997; 350: 961–2.
104. Weissman A, Leong M, Sauer MV, Shoham Z. Characterizing the practice of oocyte donation: A web-based international survey. *Reprod Biomed Online* 2014; 28: 443–50.

Gestational surrogacy

69

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OVERVIEW

Third-party reproduction includes gamete (sperm and/or oocyte) or embryo donation, surrogacy, and adoption.

Surrogacy has been practiced as a means of helping women who are unable to bear children for centuries. The earliest mention is in the Old Testament of the Bible (1). Before the advent of modern assisted conception techniques, "natural surrogacy" was the only means of helping certain barren women to have babies. Before the introduction of artificial insemination, babies were conceived the "natural way," as practiced by Abraham (1). Later, with the introduction of artificial insemination techniques, it became more socially acceptable to use these than "natural means." Later still, when assisted conception techniques such as *in vitro* fertilization (IVF) were introduced, embryos created entirely from the gametes of the "genetic" or "commissioning couple" could be transferred to the "surrogate host," who therefore provided no genetic contribution to any child that resulted from the arrangement. She bore the child and handed it over to the full "genetic parents." "Gestational surrogacy," otherwise known as "IVF surrogacy" or "full surrogacy," is now generally accepted in many countries as a treatment option for infertile women with certain clearly defined medical problems. The first report of a baby being born by gestational surrogacy was from the U.S.A. in 1985 (2).

Gestational surrogacy is now accepted in the U.K. as a treatment option for infertile women, provided there are clearly defined medical indications. A report commissioned by the British Medical Association (BMA) in 1990 (3) provided the first evidence that surrogacy was formally accepted as a legitimate treatment option. In the same year, the Human Fertilization and Embryology Act (1990) (4) was passed through the U.K. Parliament and did not ban surrogacy. The most recent report of the BMA (5) states that "surrogacy is an acceptable option of last resort in cases where it is impossible or highly undesirable for medical reasons for the intended mother to carry a child herself." During the years of this protracted debate in the U.K., most other European countries had decided to ban the practice of surrogacy of any kind. In Israel, the surrogacy law was accepted in 1996 and is in a process of updating. The largest experience of both natural and gestational surrogacy is in the U.S.A., where commercial surrogacy arrangements are allowed. Relatively few publications of experience with gestational surrogacy appeared in the literature in the early years, and there were few long-term follow-up studies of the babies or of the couples involved in surrogacy arrangements (6–9), in spite of strong recommendations to do so (10,11). However, more recently, a number of studies have been published on couples' long-term experiences and those of their children, which are reassuring and are further discussed in the "Results" section of this chapter.

METHODS

Definitions of terms

There has always been confusion among patients, practitioners, and between different countries on the definition of the different forms of surrogacy. It is common practice to use the term "surrogate mother" or "surrogate" for the woman who carries and delivers a baby.

Since the woman who gives birth is initially the legal mother of that child, further confusion is added. "Gestational surrogacy," "full surrogacy," or "IVF surrogacy" are defined as treatment by which the gametes of the "genetic couple," "commissioning couple," or "intended parents" in a surrogacy arrangement are used to produce embryos, and these embryos are subsequently transferred to a woman who agrees to act as a host for these embryos. The "surrogate host" is therefore genetically unrelated to any offspring that may be born as a result of this arrangement. With "natural surrogacy" or "partial surrogacy," the intended host is inseminated with the semen of the husband of the "genetic couple." Any resulting child is therefore genetically related to the host. In this chapter, only treatment by "gestational surrogacy" is considered and the couple who initiates the surrogacy arrangement and whose gametes are used will be known as the "genetic couple" and the woman who subsequently carries the child will be known as the "surrogate host."

Indications for "gestational surrogacy"

The genetic couples are divided into two categories: those without a uterus and those with a uterus. In those without a uterus, the need for surrogacy can be congenital or acquired. The congenital absence of the uterus is known as Mayer–Rokitansky–Küster–Hauser syndrome (MRKHs). The acquired form is post-hysterectomy due to various uterine pathologies. The second category—patients with their uterus but unable to conceive—includes repeated implantation failure in IVF, repeated fetal loss, and various underlying maternal conditions where pregnancy is medically contraindicated.

The principal indications for treatment by "gestational surrogacy" in Assaf Harofeh Medical Center are shown in Table 69.1. The main indication for surrogacy (40%) at Assaf Harofeh Medical Center is MRKHs. Acquired reasons for absence of the uterus (8%) were following hysterectomy for endometrial carcinoma, placental site tumor, complicated cornual pregnancy, complicated cervical

Table 69.1 Indications for "gestational surrogacy" among 100 patients during the years 1997–2015: The Assaf Harofeh Medical Centre Israel experience

Genetic mothers without a uterus	
Rokitansky syndrome	43
Post-hysterectomy	8
Testicular feminization	1
Total	52
Genetic mothers with a uterus	
Implantation failure in *in vitro* fertilization	12
Habitual abortions	10
Maternal diseases	14
Renal failure	6
Intrauterine adhesions, thin endometrium	6
Total	48

pregnancy, huge fibroid uterus, and cesarean hysterectomy. In those without a uterus, pregnancy is impossible under any condition.

Those patients with a uterus include women who have suffered repeated miscarriages and are deemed to have little or no chance of carrying a child to term (10%). Repeated failure of treatment by IVF is also a relative and rare indication for surrogacy, but it has only been used for women who have never shown any signs of implanting normal embryos in an apparently normal uterus after at least six to eight IVF–embryo transfer (IVF–ET) cycles (12). A "non-functional" scarred uterus with massive intrauterine adhesions is known as Asherman's syndrome. To these we add the patients with constant ultrasound evidence of thin ecogenic endometrium (4 mm or less).

A large subgroup in our center consists of those with medical conditions that would threaten the life of a woman were she to become pregnant, such as renal failure (6%), antiphospholipid antibody syndrome with systemic involvement, and severe heart disease, such as maternal cardiomyopathy. Other non-frequent underlying diseases are severe recurrent pre-eclamptic toxemia, uveitis potentially causing blindness in pregnancy, complicated systemic lupus erythematosus, hormone-dependent congenital hemangioma of the neck, Budd–Chiari syndrome, post-removal of adenoma causing Cushing's syndrome, and post-lung transplantation.

Discussion is always held with the specialist looking after the medical problems of these women, and the Ethics Committee require evidence that the female partner of the "genetic couple" will be able to look after the child adequately and that her life expectancy is reasonable.

Women who request gestational surrogacy purely for career or social reasons are not considered for treatment. Because the indications for treatment are relatively limited, the actual need for treatment by gestational surrogacy is also limited. In Bourn Hall, U.K., treatment by surrogacy accounted for 1% of the total annual throughput of cases out of a total of about 2500 IVF and frozen embryo replacement cycles. It is practiced in only a limited number of IVF centers in the U.K. (12), Israel (13), and the U.S.A. In a worldwide survey on behalf of the International Federation of Fertility Societies (IFFS), Jones et al. (14) reported that of the 57 countries surveyed, 20 allowed and/or practiced surrogacy.

Mayer–Rokitansky–Küster–Hauser syndrome

Since MRKHs is a very frequent indication for gestational surrogacy, it is described in more detail: MRKHs is a congenital anomaly of the genital tract with an incidence of 1:4000 female births (15). Primary amenorrhea leads to its diagnosis in adolescence. The syndrome includes vaginal aplasia with an absent or rudimentary uterus consisting of two small bilateral fibromuscular remnants, normal fallopian tubes, and normally functioning ovaries (16). The ovaries are sometimes found in a sub-costal location, and in these exceptional patients, a transabdominal rather than transvaginal puncture for oocyte retrieval in IVF is needed (17).

The karyotype as a rule is 46XX, and the secondary sex characteristics are usually feminine. As an exception, a rare combination of MRKHs with 46XXX manifesting prominent low ovarian response during ovarian stimulation in repeated IVF cycles has been reported (18).

MRKHs is frequently associated with urinary (19), skeletal, and cardiac defects. Urinary tract anomalies include renal agenesis/aplasia, pelvic kidney, horseshoe kidney, renal sclerosis, and double ureter (20). Skeletal anomalies related to MRKHs (vertebral, rib, digits, and palate) have been linked to the gene on the long arm of chromosome 12 (12q24.1) that encodes a T-Box containing the transcription factor TBX5 (21).

Oppelt et al. (22), in a series of 53 MRKHs patients, created a new clinical diagnostic classification of the syndrome: the typical form in which the fallopian tubes, ovaries, and renal system are generated and well developed; and the atypical form in which additional malformations of the ovaries and/or the renal system are present. Duncan et al. (23) proposed the term MURCS (Malformations Urinary Cardiac and Skeletal) for cases where systemic involvement was present. This term was "incorporated" as a third form in Oppelt et al.'s clinical classification.

In the past, the main medical interest in this syndrome was to enable normal sexual intercourse by the use of different operative techniques for the reconstruction of the vagina (24). With the advent of various assisted reproduction techniques, the focus of medical interest in patients with MRKHs has reportedly shifted to their options for motherhood by means of IVF surrogacy (25), and, most recently, by the potential of uterine transplantation (see Chapter 63).

The IVF performance of MRKHs patients was different in the two subtypes mentioned above. Based on follow-up data on a total of 102 cycles of surrogate IVF in 27 MRKHs patients, women with the typical form of MRKHs require less gonadotropins and a shorter duration of ovarian stimulation. The mean number of follicles, oocytes, and metaphase II oocytes, the fertilization rate and cleaving

embryos were higher among women with the typical form. Pregnancy rates were similar since the available number and quality of transferred embryos to the surrogate mothers was not affected (26).

Petrozza et al. (27) sent questionnaires to all treatment centers performing surrogacy procedures and asked them to follow-up on the frequency of congenital abnormalities among the progeny born to MRKHs women. Results of 162 IVF cycles produced 34 live-born children, half of whom were female. No congenital anomalies were found amongst these females. These results appear to suggest that congenital absence of the uterus and vagina, if it is genetically transmitted, is not inherited commonly in a dominant fashion.

Selection of patients for treatment

In the Assaf Harofeh Medical Center IVF program in Israel, all "genetic couples" are referred by their gynecologist and are therefore already selected as probably being suitable for treatment. The "genetic couple" is seen alone in the first instance and in-depth consultation on all medical aspects of the treatment is carried out. Surrogacy is allowed in Israel only in the set-up of a couple. Israeli law does not allow access to surrogacy programs to single women and same-sex male partners. The genetic couple should be Israeli citizens, with similar religion, and with an age limit of 48 years for the female and 58 years for the male. Once the couple is considered to be medically and legally suitable for treatment, they must find a host surrogate either by a surrogacy agency or by themselves.

According to the Israeli guidelines, the age of the surrogate should be between 22 and 38 years. She is expected to be unmarried (single or divorced) with at least one child, but with a history of no more than four past deliveries and no more than one cesarean section. It must be at least one year since any delivery of her own child. Other groups have also reported using sisters (28), mothers (29), and support groups or other agencies (7,30). The Ethics Committee of the American Society for Reproductive Medicine (ASRM) concluded that many intrafamilial arrangements (such as sister to sister) and some intergenerational arrangements (such as daughter to mother) are ethically acceptable, but others should be rejected. The most problematic are child to parent surrogacy due to possible undue pressure on the host or undue unhealthy family dynamics (31).

All groups practicing gestational surrogacy are adamant about the need for in-depth medical and psychological screening of all "genetic couples," the surrogate hosts, and other members of the family, especially any existing children of the surrogate host, and possibly also the parents of the hosts and genetic couples (30,32).

In our own practice, when a suitable host has been found, she and her partner are interviewed at length and a full explanation of the implications of acting as a surrogate host are explained to them. If the host is thought to be suitable, then both the genetic couple and the host are counseled in depth. If this process is satisfactory and there are no obvious reasons why the arrangement should not be allowed to proceed from a medical and counseling standpoint, a report is prepared and submitted to a Ministry of Health-nominated independent Ethics Committee. This Committee includes two gynecologists, two internal medicine physicians, a lawyer, a senior social worker, a religion representative (a priest or a rabbi), and a senior psychologist. The goal of this respectable Ethical Committee is to ensure that, at the end of the process, the surrogate and her child/children will not be negatively affected and the genetic couple will have accomplished their wish by having a successful delivery of their own genetic child. It must be stressed that in all surrogacy arrangements the welfare of any child born as a result of treatment and of any existing children of a family is given the utmost importance. The Committee then approves the arrangement, holds it over for further information and discussion, or rejects it. The medical process of surrogacy can be started only after written official approval is granted by the Ethics Committee.

Counseling

In-depth counseling of all parties engaged in surrogacy arrangements is of paramount importance and aims to prepare all parties contemplating this treatment of last resort to consider all the facts that will have an influence on the future lives of each of them. They must be confident and comfortable with their decisions and have trust in each other, so that no one party is felt to be taking advantage of the other. The BMA in its 1990 report (3) produced a most useful statement: "The aggregate of foreseeable hazards should not be so great as to place unacceptable burdens on any of the parties—including the future child."

Many issues must be discussed with both the genetic couple and the proposed host surrogate. These include, for the genetic couples (8):

- A review of all alternative treatment options
- The need for in-depth counseling
- The need to find their own host (U.K. and Israel)
- The practical difficulty and cost of treatment by gestational surrogacy
- The medical and psychological risks of surrogacy
- Potential psychological risk to the child
- The chances of having a multiple pregnancy
- The degree of involvement that the host may wish to have with the child
- The possibility that a child may be born with a handicap
- The possibility that the host may wish to retain the child after birth
- The importance of obtaining legal advice
- The genetic couples are advised to take out insurance cover for the surrogate host

Issues for discussion with the host include (8):

- The full implications of undergoing treatment by IVF surrogacy
- The possibility of multiple pregnancy including fetal reduction
- The possibility of family and friends being against such treatment

- The need to abstain from unprotected sexual intercourse during and just before the treatment
- The normal medical risks associated with pregnancy including an abortion, ectopic pregnancy, premature delivery, and the possibility of cesarean section
- Implications and feelings of guilt on both sides if the host should spontaneously abort a pregnancy
- The possibility that the host will feel a sense of bereavement when she gives the baby to the genetic couple
- The possibility that the child may be born with a handicap

Other issues that must be discussed with both parties to a surrogacy arrangement include whether and what both parties will tell the children born as a result of treatment in the future about their origins and also what the host mother will tell any children she has. There is an increasing willingness of all couples involved with treatment by assisted reproductive techniques to be more open about their treatment, whether by IVF, the use of donor gametes, or surrogacy.

Patient management

Management of the genetic mother

The majority of "genetic mothers" treated at Bourn Hall are fully assessed by their gynecologist before referral. The work-up usually includes a laparoscopy, if there are congenital anomalies. Evidence of ovarian function and reserve can often be obtained from a history of cyclical premenstrual symptoms or symptoms of ovulation. This can be confirmed by one or more estimations of serum follicle-stimulating hormone and luteinizing hormone, and possibly timed progesterone levels in the estimated luteal phase. Anti-Mullerian hormone and antral follicle count may be very useful for assessment of ovarian reserve and subsequent assignment of an individualized stimulation protocol.

The blood groups of the genetic parents are requested in case the host is rhesus negative and both the genetic parents are tested for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) status. The latest recommendations of the ASRM for screening and testing and for complex medical and psychological issues of genetic parents and gestational hosts were recently published (32). Ultrasound scanning of the ovaries is carried out on some patients to confirm the presence of one or both ovaries, their position, and possible evidence of their activity. Other investigations are carried out as necessary on an individual basis.

On completion of the full medical assessment and the counseling process, and when the approval of the Ethics Committee has been obtained, treatment of the genetic couple is started, provided the host has already been identified, fully counseled, and approved. Since most women requesting treatment by gestational surrogacy are perfectly normal with regard to their ovarian function, the management of their IVF treatment cycles is straightforward. Ovarian follicular stimulation, monitoring, and oocyte recovery methods are as in any IVF treatment. If appropriate, the gonadotropin-releasing hormone (GnRH) antagonist protocol with GnRH agonist trigger may be preferred in order to eliminate the risk of ovarian hyperstimulation syndrome. Oocytes are usually collected by the standard transvaginal ultrasound-guided technique. In all treatment cycles in Israel, the genetic woman undergoes a "fresh" IVF cycle and the developing embryo/embryos are transferred immediately to the surrogate host, whereas in the U.K., the embryos obtained from the genetic couple must be frozen for a six-month "quarantine" period pending a repeat negative HIV report prior to their transfer to the uterus of the surrogate host (33).

Management of the surrogate host

In Israel, the surrogate host's age must be 38 years or less and they must have given birth at least once. Other groups will consider any woman less than 45 years of age who is willing to act as a host (34). The Ethics Committee in Israel recommend an unmarried/divorced woman as a host. The Ethics Committee in the U.K. have recommended that hosts should be married and that the husband or partner should be made fully aware during the counseling process of the implications of his partner acting as a surrogate host.

Fertility investigations of the proposed host have not been necessary. All hosts and their partners are tested for HBV, HCV, and HIV status before the ET is carried out, and the HIV status of the "genetic couple" is retested. In Israel, if the surrogate host is not taking the oral contraceptive pill, it is recommended to resume administration to ease synchronization between the genetic mother and host and to prevent undesired pregnancy. In other places, the host is placed on a GnRH agonist regimen, combined with hormone replacement therapy for synchronization between genetic mother and host. The management of the hormone-controlled cycles for the transfer of frozen–thawed embryos has been described previously (35).

RESULTS

Treatment by gestational surrogacy generally achieves satisfactory pregnancy and live birth rates per genetic couple and per surrogate host. To date, there have been only a few published series reported in the literature. In Born Hall, U.K., live birth rates of between 37% and 43% per genetic or commissioning couple and 34% and 39% per host surrogate have been achieved, with a mean of two embryos transferred (33,36). Another U.K. series in which all the female partners in the "genetic couple" had had a hysterectomy achieved a pregnancy rate of 37.5% per surrogate host and 27.3% (6/22) per cycle of treatment begun (37).

In the original series reported by Utian and colleagues (6), a clinical pregnancy rate of 18% (7/59) per cycle initiated and a 23% clinical pregnancy rate per ET was achieved. A later series of 180 cycles of IVF gestational surrogacy, reported by the same group, gave an overall pregnancy rate per cycle of 24% (38/138) and a live birth rate of 15.8% (25/158) (37). Another larger series from

the U.S.A. showed ongoing or delivered pregnancy rates of 36% (172 of 484 surrogate hosts) (38), with a mean of 5 ± 1.3 embryos transferred. Corson and colleagues reported a clinical pregnancy rate of 58% per commissioning couple and 33.2% per ET in women where the genetic women were less than 40 years of age (39).

What has recently become apparent is that very little investigation of the immediate and long-term outcomes of the babies born as a result of gestational surrogacy has been carried out. However, Parkinson et al. (40) have reviewed the prenatal outcomes of pregnancies from IVF surrogacy and compared them to the outcomes of pregnancies resulting from standard IVF. As would be expected, the surrogate hosts who carried twin and triplet gestations delivered substantially earlier than those who gestated singleton pregnancies and the twin newborns were significantly lighter than singleton infants born through IVF surrogacy. Interestingly, the occurrence of pregnancy-induced hypertension and bleeding in the third trimester of pregnancy was up to five-times lower in the surrogate hosts than in the standard IVF patient controls. Apart from birth weights and prematurity, little other information is given about the outcomes of the babies.

There have been very few long-term follow-up studies of women who have acted as surrogate hosts, but there is little to suggest any long-term harm or regret among them (9,10,41). Jadva et al. investigated the psychological well-being of 20 hosts from 1 to 10 years following the birth of their child. The hosts did not show signs of depression as measured by Beck Depression Inventory. The relations with the genetic couples were more than expected and, for the future, most of them were ready to be available to the child if requested. At age 10, 90% of children who had been informed of the nature of their conception had a good understanding of this (42). Soderstorm-Anttila et al. recently summarized the literature that addressed the outcomes for surrogate mothers, their children, and the resulting families (43). The psychological well-being of children whose mother had been a host between 5 and 15 years earlier was good. Most surrogate mothers scored within the normal range in personality tests. No major difference in psychological state was found between intended mothers, other IVF patients, and regular conception.

There are very limited data on same-sex male couples seeking surrogacy to achieve parenthood (44). In a survey of 104 men in New Jersey, their mean age was 36–45 years and 90% were in a relationship. During the first IVF surrogacy cycle, 68% transferred two embryos, 19% transferred one embryo, and 13% transferred three or more embryos. The rate of twins was 50%. The reasons for the high multiple pregnancy rate were: for a higher chance to achieve successful IVF (42%); a specific desire for twins (26%); and a desire to have an embryo derived from the genes of each partner (11%). In the set-up of gay men and IVF surrogacy, better counseling for the risks of multifetal pregnancies is recommended (45).

The overall experience by participants in IVF surrogacy arrangements is that it has been considered to be a good experience. Studies of hosts and commissioning couples show reassuring data and positive outcomes, particularly for the hosts (46–49).

COMPLICATIONS

Problems encountered with gestational surrogacy

The major problems that have been reported with surrogacy arrangements have almost entirely arisen from "natural surrogacy" arrangements. The major problems have been legal and mostly revolve around the "ownership" and rights of both the "genetic couple" and the birth mothers. These are not further considered in this chapter, but they are well documented in a number of papers published on the subject (3,10,11,50–52). The main reason these problems have arisen is that the majority of the arrangements were largely unsupervised and did not involve careful clinical and psychological assessment, counseling, and discussion with lawyers.

With gestational surrogacy, professionals in all of these areas are invariably involved and, as a consequence, the number of complications arising out of these treatments is very low. In the past 19 years of the Israeli experience with surrogacy, no serious clinical, ethical, or legal problems have been encountered. The same experience was reported by the U.K. groups.

The major ethical and practical problems that might be encountered with IVF surrogacy include:

- The host may wish to keep the child.
- An abnormal child may be rejected by both the genetic and host parents.
- The question of whether it is ethical to pay hosts and, if so, how much, has always caused concern. In the U.S.A. and Israel, payment is "up front" and revealed, whereas in the U.K. and most of Europe, altruistic surrogacy is what everyone aspires to, but it is in effect impractical and payment is often hidden as "reasonable expenses." Many also consider it unethical not to pay hosts for the sacrifices that they make to help other couples. The European Society of Human Reproduction and Embryology (ESHRE) Task Force on Ethics and the Law (2005) (33) state that payment for (surrogacy) services is unacceptable, while the IFFS Surveillance Report 2010 (14) states only that "The payment to the surrogate raises special concerns."
- The long-term effects on the children born as a result of gestational surrogacy are not known.
- The long-term psychological effects on both the "genetic couple" and "host surrogates" are not known, although surveys are being conducted in a few centers.
- The impact on the hosts' existing children, namely the mother–child relationship. Golombok et al. (47) found no difference in maternal negativity, maternal positivity, mother–child interaction, and child adjustment between surrogacy and egg donation compared with natural conception. However, surrogacy and egg donation families showed less positive mother–child interaction compared with natural conception.

- Poor ovarian response of "genetic mothers" to stimulation. In the post-hysterectomy cases, this reduced follicular response may be due to reduced vascular supply to the ovaries (53).

This has been reported particularly after Wertheim's hysterectomy.

We (AR and RR-E) encountered a case of a handicapped woman who was closely treated daily by a nurse. She decided to create a child by herself using egg donation, sperm donation, and transfer of the embryo to a surrogate who was a family member. The surrogacy was done in East Asia and the delivery in Israel. After delivery, the newborn came under the care of the handicapped woman treated by the nurse. The case was reported to the Ministries of Welfare and Health and, in order to ensure the best interests of the child, he has now been placed under the care of a foster family.

Simultaneous pregnancies coming from embryos generated by the genetic mother, transferred to the host, and, at the same time, to the biologic mother in the same IVF cycle have been reported. It is our policy to transfer embryos of the genetic mother to the host surrogate mother only, even in genetic mothers with their own intact uterus.

CROSS-BORDER SURROGACY

Because there are a number of leading countries, particularly in Europe, such as Italy, Germany, and France, where surrogacy is not permitted, and as the ease of worldwide travel increases, couples now travel for treatment that is unavailable in their own countries. Cross-border reproductive care, which means crossing borders to have children, is a rapidly growing phenomenon (54). The concern is that these practices may lead to disputes and exploitation of desperate couples seeking this particular treatment (8), such as in Eastern Europe.

Cross-border surrogacy in Israel occurs when:

- Patients are outside of the criteria of Israeli surrogacy law, such as same-sex couples (homosexual relationships), single women who want a child through surrogacy, unmarried heterosexual couples, and couples in which the female and male partners are of different religions
- Couples with two or more children desiring an additional child
- Patients with only a relatively minor medical indication or a transient indication for surrogacy
- Couples who wish to travel to countries where the costs are lower
- Patients who prefer a long-distance relationship with the gestational host rather than close contact

In order that the child will be permitted to be a legal citizen of Israel, homosexual or heterosexual couples have to undergo an histocompatibility leucocyte antibody (HLA) typing test to show that the child is genetically related to at least one of his or her parents. In one still unsolved legal situation, the child delivered to Israel had HLA similarity with his surrogate host and not with his parents. Though undesired by the host but desired by the commissioning couple, the status of the baby remains unclear (55).

The Ethics Committee Report of the ASRM in 2006 carefully considered "the changing nature of reproduction and the family." They concluded that "…there is no sound basis for denying to single persons and gays and lesbians the same rights to reproduce that other individuals have," and they finally state: "As a matter of ethics, we believe that the ethical duty to treat persons with equal respect requires that fertility programs treat single persons and gay and lesbian couples equally with married couples in determining which services to provide" (56).

FUTURE DIRECTIONS AND CONTROVERSIES

In the U.K. and the U.S.A., the public generally accepts that treatment by surrogacy, particularly gestational surrogacy, is a reasonable treatment option if there are good clinical indications.

If the best interests of the child are considered at all times as the priority, then the other issues will invariably fall into place. For instance, the fitness and welfare of the proposed host to go through with the treatment, the age and physical and psychological "fitness" of the female partner in the commissioning couple, and the seeking of proper legal advice will nearly always become apparent if due consideration is given to the welfare of the child.

Legal issues

The majority of the legal problems that have arisen as a result of surrogacy have been associated with cases of "natural surrogacy." There have been two cases that have received particular publicity: the "baby M case" (57) and also the case of Smith versus Jones (58,59). In the "baby M case," the final decision was that the genetic couple would have precedence for custody of the child over the birth mother. In the case of Smith versus Jones, which involved "gestational surrogacy," the District Court recognized the genetic parents to be the legal parents and gave them the right to put their names on the birth certificate of the baby (58,59). In the U.S.A., a number of states have specific regulations regarding surrogate motherhood, but some are more specific than others about the rights of the "genetic mother" over those of the "birth mother." The complex differences between states have been well summarized by Schuster (51,52). Similarly, in the case of Johnson versus Calvert in the California Superior Court, where Johnson was the "gestational surrogate," the Calverts—the "genetic parents" of the child—were ruled to be the natural parents of the child (60).

As in the U.S.A., Australian states have different regulations. Surrogacy is freely available in New South Wales, Western Australia, and the Australia Capital Territory. In Tasmania, South Australia, and Victoria, it is not illegal, but very strict controls on payment and the lack of binding legal arrangements make it almost impossible to carry out (61). There is a tendency, therefore, for couples seeking surrogacy arrangements to move from state to state (62).

Religious issues

Religious attitudes toward surrogacy differ widely.

The Catholic Church is strongly against all forms of assisted conception, particularly those that involve gamete donation and surrogacy (63). Therefore, surrogacy is banned in the Catholic countries of Europe: Italy, France, and others. The Anglican Church is less rigid in its view on surrogacy and has not condemned it.

The Jewish religion, which is very much family oriented and puts a duty on Jewish couples to have children, does not forbid the practice of gestational surrogacy (64). From the religious point of view, a child born through gestational surrogacy to a Jewish couple will belong to the father who gave the sperm (therefore sperm donation is not allowed in gestational surrogacy in Israel) and to the woman who gave birth (65–67).

The Islamic view appears absolute and, in the same way that the use of donor gametes is strictly forbidden, so surrogacy is not allowed. It is suggested that it may be permissible between wives in the same marriage, but the debate continues (68).

CONCLUSION

It is now 31 years since the birth of the first child following a gestational surrogacy arrangement in the U.S.A. (2). It has been shown that the treatment of young women with very specific indications is successful and relatively free of complications. The practice of gestational surrogacy can only be carried out in clinics licensed by the Human Embryology and Fertilization Act (HEFA) in the U.K., in a very few countries in Europe, in Israel, and in the U.S.A.

The indications for treatment by gestational surrogacy are limited to a small group of women who have no uterus (congenital or acquired), suffer recurrent abortions, or suffer from certain medical conditions that would threaten the life of a woman were she to become pregnant. Times are changing, however, and gestational surrogacy has recently been used to help gay couples who wish to have families.

The treatment process in itself is straightforward. The woman from the "genetic couple" undergoes a normal stimulated IVF cycle and the embryos are transferred to the surrogate host after a short medical preparation of the endometrium. The difficult aspects of the treatment concern the extreme care with which the surrogate host must be selected by the genetic couple to ensure complete compatibility, and also the in-depth counseling that is required, both in the short and the long term, on all aspects of the treatment. The support and advice of an independent counselor and lawyer are absolutely essential, and we believe the advice of an independent Ethics Committee is also essential in assessing the suitability of individual cases. As clinicians and counselors, we are inclined to become so deeply involved in the problems of individual couples that some of the more obvious pitfalls in the social, religious, or ethical aspects of treating a particular couple may easily be overlooked.

During our long-term experience both in Israel and in the U.K., no serious clinical, ethical, or legal problems have been encountered. A minor problem that has been encountered is that both parties very often have unreasonably high expectations of the success of treatment, in spite of very frank explanations and counseling being provided to them. Because the host is fit, young, and known to be fertile, she and the genetic parents invariably expect success, and they feel badly let down if this is not achieved. Full support counseling for both couples is essential when this occurs. Gestational surrogacy services should be part of a comprehensive infertility treatment program that larger centers should offer now that it is an ethically accepted form of treatment in several countries worldwide.

REFERENCES

1. Holy Bible, The Book of Genesis; 16: 1–15; 17: 15–19; 21: 1–4.
2. Utian WH, Sheean LA, Goldfarb JM, Kiwi R. Successful pregnancy after *in vitro* fertilization and embryo transfer from an infertile woman to a surrogate. *N Engl J Med* 1985; 313: 1351–2.
3. British Medical Association. *Surrogacy: Ethical Considerations. Report of the Working Party on Human Infertility Services*. London, U.K.: BMA Publications, 1990.
4. Human Fertilisation and Embryology Act. London, U.K.: Her Majesty's Stationery Office, 1990.
5. British Medical Association. *Changing Conceptions of Motherhood. The Practice of Surrogacy in Britain*. London, U.K.: BMA Publications, 1996.
6. Utian WF, Goldfarb JM, Kiwi R et al. Preliminary experience with *in vitro* fertilization-surrogate gestational pregnancy. *Fertil Steril* 1989; 52: 633–8.
7. Marrs RP, Ringler GE, Stein AL, Vargyas JM, Stone BA. The use of surrogate gestational carriers for assisted reproductive technologies. *Am J Obstet Gynecol* 1993; 168: 1858–63.
8. Fisher S, Gillman I. Surrogate motherhood: Attachment, attitudes and social support. *Psychiatry* 1991; 54: 13–20.
9. Blyth E. Interviews with surrogate mothers in Britain. *J Reprod Infert Psychol* 1994; 12: 189–98.
10. Ethics Committee of the American Fertility Society. Ethical considerations in the new reproductive technologies. *Fertil Steril* 1986; 46(Suppl 1): 62–8.
11. American College of Obstetricians and Gynecologists. *Committee on Ethics: Ethical Issues in Surrogate Motherhood*. Washington, DC: American College of Obstetricians and Gynecologists, 1990.
12. Balen AH, Hadyn CA. British fertility society survey of all licensed clinics that perform surrogacy in the UK. *Hum Fertil* 1998; 1: 6–9.
13. Raziel A, Schachter M, Strassburger D, Komarovsky D, Ron-El R, Friedler S. Eight years' experience with an IVF surrogate gestational pregnancy programme. *Reprod Biomed Online* 2005; 11: 254–8.
14. Jones HJ, Cooke I, Kempers R, Brinsden PR, Saunders D. IFFS surveillance 2010. *Fertil Steril* 2011; 95: 491.

15. Capraro VJ, Gallego MB. Vaginal agenesis. *Am J Obstet Gynecol* 1976; 124: 98–107.
16. Egarter CH, Huber J. Successful stimulation and retrieval of oocytes in patient with Mayer–Rokitansky–Küster–Hauser syndrome. *Lancet* 1998; 6: 1283.
17. Raziel A, Vaknin Z, Schachter M, Strassburger D, Herman A, Ron-El R, Friedler S. Ultrasonographic-guided percutaneous transabdominal puncture for oocyte retrieval in a rare patient with Rokitansky syndrome in an *in vitro* fertilization surrogacy program. *Fertil Steril* 2006; 86: 1760–3.
18. Raziel A, Friedler S, Gidoni Y, Ben-ami I, Strassburger D, Ron-El R. IVF surrogacy in rare coexisting Mayer–Rokitansky–Kuster–Hauser syndrome and triple X karyotype. *Fertil Steril* 2011; 95: 1788–90.
19. Creatsas G, Malhotra N, Malhotra J, Malhotra P, Malhotra RM. Vaginal agenesis associated with renal ectopia. *Adolesc Pediatr Gynecol* 1990; 3: 103–5.
20. Wottgen M, Brucker S, Renner SP, Strissel PL, Strick R, Kellerman A, Wallwiener D, Beckmann MW, Oppelt P. Higher incidence of linked malformations in siblings of Mayer–Rokitansky–Kuster–Hauser syndrome patients. *Hum Reprod* 2008; 3: 1226–31.
21. McDonough PG. Letters: Hot clues to the etiology of Mayer–Rokitansky–Küster–Hauser syndrome? *Fertil Steril* 2005; 84: 545.
22. Oppelt P, Renner SP, Kellermann A, Brucker S, Hauser GA, Ludwig KS, Strissel PL, Strick R, Wallwiener D, Beckmann MW. Clinical aspects of Mayer–Rokitansky–Kuester–Hauser syndrome: Recommendations for clinical diagnosis and staging. *Hum Reprod* 2006; 21: 792–7.
23. Duncan PA, Shapiro LR, Stangel JJ, Klein RM, Addonizio JC. The MURCS association: Mullerian duct aplasia, renal aplasia, and cervicothoracic somite dysplasia. *J Pediatr* 1979; 95: 399–402.
24. Chapron C, Morice P, La Tour MD, Chavet X, Dubuisson JB. Laparoscopic management of asymmetric Mayer–Rokitansky–Kuster–Hauser syndrome. *Hum Reprod* 1995; 10: 369–71.
25. Beski S, Gorgy A, Venkat G, Craft IL, Edmonds K. Gestational surrogacy: A feasible option for patients with Rokitansky syndrome. *Hum Reprod* 2000; 15: 2326–8.
26. Raziel A, Friedler S, Gidoni Y, Ben Ami I, Strassburger D, Ron-El R. Surrogate *in vitro* fertilization outcome in typical and atypical forms of Mayer–Rokitansky–Kuster–Hauser syndrome. *Hum Reprod* 2011; 27: 126–30.
27. Petrozza JC, Gray MR, Davis AJ, Reindollar RH. Congenital absence of uterus and vagina is not commonly transmitted as dominant genetic trait: Outcome of surrogate pregnancies. *Fertil Steril* 1997; 67: 387–9.
28. Leeton J, King C, Harman J. Sister–sister *in vitro* fertilization surrogate pregnancy with donor sperm: The case for surrogate gestational pregnancy. *J In Vitro Fert Embryo Trans* 1988; 5: 245–98.
29. Michello MC, Bernstein K, Jacobsen MJ et al. Mother–daughter *in vitro* fertilisation triplet surrogate pregnancy. *J In Vitro Fert Embryo Trans* 1988; 5: 31–4.
30. Sheean LA, Goldfarb JM, Kiwi R, Utian WH. *In vitro* fertilisation (IVF)-surrogacy: Application of IVF to women without functional uteri. *J In Vitro Fert Embryo Trans* 1989; 6: 134–7.
31. Ethics Committee of the American Society for Reproductive Medicine. Using family members as gamete donors or surrogates. *Fertil Steril* 2012; 4: 797–803.
32. Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Recommendations for practices utilizing gestational carriers: A committee opinion. *Fertil Steril* 2015; 1: e1–8.
33. Brinsden PR. IVF surrogacy. In: *A Textbook of In Vitro Fertilization and Assisted Reproduction*. Brinsden PR (ed.). London, U.K. and New York, NY: Taylor and Francis, 2005, pp. 393–404.
34. Shenfield F, Pennings G, Cohen J et al. ESHRE Task Force on Ethics and Law 10: Surrogacy. *Hum Reprod* 2005; 20: 2705–7.
35. Sathanandan M, Macnamee M, Rainsbury P et al. Frozen–thawed embryo replacement in artificial and natural cycles; a prospective study. *Hum Reprod* 1991; 5: 1025–8.
36. Brinsden PR, Appleton TC, Murray E et al. Treatment by *in vitro* fertilisation with surrogacy—The experience of a single centre in the United Kingdom. *Br Med J* 2000; 320: 924–8.
37. Meniru GI, Craft IL. Experience with gestational surrogacy as a treatment for sterility resulting from hysterectomy. *Hum Reprod* 1997; 12: 51–4.
38. Goldfarb JM, Austin C, Peskin B et al. Fifteen years of experience with an *in-vitro* fertilization surrogate gestational pregnancy programme. *Hum Reprod* 2000; 15: 1075–8.
39. Corson SL, Kelly M, Braverman A, English ME. Gestational carrier pregnancy. *Fertil Steril* 1998; 69: 670–4.
40. Parkinson J, Tran C, Tan T et al. Peri-natal outcome after *in vitro* fertilization-surrogacy. *Hum Reprod* 1999; 14: 671–6.
41. Van den Akker OBA. Organisational selection and assessment of women entering a surrogacy agreement in the UK. *Hum Reprod* 1999; 14: 262–6.
42. Jadva V, Blake L, Casey P et al. Surrogacy families 10 years on: Relationship with the surrogate, decisions over disclosure and children's understanding of their surrogacy origins. *Hum Reprod* 2012; 10: 3008–14.
43. Soderstrom-Anttila V, Wennerholm UB, Loft A et al. Surrogacy: Outcomes for surrogate mothers, children and the resulting families—A systematic review. *Hum Reprod Update* 2016; 22: 260–76.

44. Notron W, Hudson N, Culley L. Gay man seeking surrogacy to achieve parenthood. *Reprod Biomed Online* 2013; 27: 271–9.
45. Syvestre-Margolis G, Vallejo V, Rauch E. Gestational surrogacy/egg donation IVF: Behavior of gay men intended parents with respect to numbers of embryo transferred. *O-145 ASRM Annual Meeting*, Baltimore, MD, October 17–21, 2015.
46. Kleinpeter CB. Surrogacy: The parent's story. *Psychol Rep* 2002; 91: 201–19.
47. Golombok S, Murray C, Jadva V et al. Families created through a surrogacy arrangement: Parent–child relationships in the first year of life. *Dev Psychol* 2004; 40: 400–11.
48. MacCallum F, Lycett E, Murray C et al. Surrogacy: The experience of commissioning couples. *Hum Reprod* 2003; 18: 1334–42.
49. Cohen B, Friend TL. Legal and ethical implications of surrogacy mother contracts. *Clin Perinatal* 1987; 14: 281–92.
50. Imrie S, Javada V. The long-term experiences of surrogates: Relationships and contact with surrogacy families in genetic and gestational surrogacy arrangements. *Reprod Biomed Online* 2014; 29: 424–35.
51. Shuster E. Non-genetic surrogacy: No cure but problems for infertility? *Hum Reprod* 1991; 6: 1176–80.
52. Shuster E. When genes determine motherhood: Problems in gestational surrogacy. *Hum Reprod* 1992; 7: 1029–33.
53. Siddle N, Sarrel P, Whitehead M. The effect of hysterectomy on the age at ovarian failure: Identification of a subgroup of women with premature loss of ovarian function and literature review. *Fertil Steril* 1987; 47: 94–100.
54. Crockin SL. Growing families in a shrinking world: Legal and ethical challenges in cross-border surrogacy. *Reprod Biomed Online* 2013; 27: 733–41.
55. Knoche JW. Health concerns and ethical considerations regarding international surrogacy. *Int J Gynaecol Obstet* 2014; 126: 183–6.
56. Ethics Committee of the American Society for Reproductive Medicine. Access to treatment by gays, lesbians, and unmarried persons. *Fertil Steril* 2006; 86: 1333–5.
57. Andrews LB. The stork market: The law of the new reproductive technologies. *Am Bar Assoc J* 1984; 78: 50–6.
58. Annas G. Using genes to define motherhood: The California solution. *N Engl J Med* 1992; 326: 417–20.
59. Smith VJ. Los Angeles Superior Court, Los Angeles County. 1987:No CF 025653.
60. Oxman RB. California's experiment in surrogacy. *Lancet* 1993; 341: 1468–9.
61. Leeton J. The current status of IVF surrogacy in Australia. *Aust N Z J Obstet Gynaecol* 1991; 31: 260–2.
62. Johnson I. Regulation of assisted reproductive technology: The Australian experience. In: *A Textbook of In Vitro Fertilization and Assisted Reproduction*. Brinsden PR (ed.). Carnforth, U.K. and New York, NY: Parthenon, 1999, pp. 424–7.
63. McCormick RA. Surrogacy: A Catholic perspective. *Creighton Law Rev* 1992; 25: 1617–25.
64. Schenker JG. Assisted reproduction practice in Europe: Legal and ethical aspects. *Hum Reprod Update* 1997; 3: 173–84.
65. Hirsh AV. Infertility in Jewish couples, biblical and rabbinic law. *Hum Fertil (Camb)* 1998; 1: 14–9.
66. Schenker JG. Infertility evaluation and treatment according to Jewish Law. *Em J Obstet Gynaecol Reprod Biol* 1997; 71: 113–21.
67. Benshushan A, Schenker JG. Legitimizing surrogacy in Israel. *Hum Reprod* 1997; 12: 1832–4.
68. Hussain FA. Reproductive issues from the Islamic perspective. *Hum Fertil* 2000; 3: 124–8.

The evolving role of the assisted reproduction technology nurse

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A contemporary review

JOANNE L. LIBRARO

INTRODUCTION

Advances in assisted reproduction technologies (ARTs) continue to offer millions of patients, couples, and families around the world the opportunity to parent. As the "art" of ARTs advances, multifaceted changes are seen in the roles, responsibilities, and commitments of each member of the ART treatment team. Clinical programs continue to modify their policies and procedures to meet the needs of the changing culture of medicine, science, and research that surrounds the field of reproductive medicine. The term "*in vitro* fertilization" (IVF) has become synonymous with *hope* for those patients seeking the advances in care options to fulfil the dream of parenthood. In the early days of ART, treatment was considered something available only for the privileged or wealthy. Today, with the advent of ART treatment coverage by healthcare insurance, even more patients are able to seek and successfully pursue treatment and realize their goal of becoming parents. Historically, ART was seen as a "laboratory science," research-oriented and even "experimental." As time passed, physicians, nurses, laboratory personnel, and researchers have developed the requisite skills necessary to remain current in the field of reproductive endocrinology and infertility (REI). Additionally, the available technologies have become accepted as standard treatment modalities.

Nearly 50 years ago at Bourne Hall in the U.K., under the guidance of Drs. Robert Edwards and Patrick Steptoe, the future role of ART nurses was conceived and established (1). Jean Purdy (Figure 70.1), the first recognized "nurse" of distinction in the field of REI, created and established the foundation for clinical growth, scientific development, holistic supportive therapies, and nursing research. The birth of Louise Brown—the first "test-tube" baby—on July 25, 1978 not only thrilled her parents, but also introduced the world to the future of assisted reproduction. This triangle of success included Drs. Edwards and Steptoe and Jean Purdy. On the basis of their early commitment to the advancement of research and science surrounding infertility options, many ground-breaking discoveries have evolved, further supporting today's scientists and professionals to continue to enhance the available options for those seeking assistance.

The current trends in reproductive health support the need for nursing professionals to assume an ongoing, visionary, scientific, and academic approach to advancement. Nurses remain a primary influence in the industry and define the nature and future of nursing standards in reproductive health. As Jean Purdy demonstrated through her determination to partner with scientific professionals and focus on the goals of success, many of today's eager professionals seek the same alignment with clinical and research teams.

Many nursing roles in REI-ART have evolved in recent years to assume a significantly more comprehensive responsibility that is in alignment with the rapidly changing trends in research, science, and treatment options. Over the last several decades, nursing roles have continuously transformed, further defining the need for specialized education, training, and competencies. Now, more than ever, those professionals seeking success as an REI professional must support their clinical and theoretical knowledge base and remain current with regulatory and ethical trends. In this chapter, the critical role of the REI-ART nurse is reviewed, highlighting the major changes and ongoing trends in the field.

ART: A RAPIDLY PROGRESSING FIELD

Many in the field of assisted reproduction phrase the comprehensive clinical, emotional, and scientific process of infertility treatment as a *journey*. The sometimes arduous journey to success challenges many patients and couples as they strive to become parents. Initially, patients must face the challenge of accepting the need for ART and the associated failures from which it stems. Many layers of decision making—emotional, cultural, ethical, financial, and religious—are involved before initiating treatment. In many cases, these challenges lead to the development of unique relationships established with patients seeking treatment; the ART nurse plays a critical role in their care. Since the 1980s, significant changes in the field of REI have stimulated the need for reflection as professionals adapt to the changing trends. Technological advances, research initiatives, and factors linked to regulatory influences drive the role of today's ART nursing professional.

The comprehensive staff contribution that includes REI physicians, nurses, psychologists, embryologists, andrologists, reproductive urologists, genetic counselors, financial counselors, reproductive endocrine laboratory staff, and administrative staff support the rapidly changing culture of REI and positively impact the role of nurses. Additional support in many ART programs with academic affiliations

Figure 70.1 Jean Marion Purdy, 1945–1985. (From Edwards R et al. *Implantation of the Human Embryo*. London, U.K.: Academic Press, 1985.)

includes the three-year fellowship for obstetricians and gynecologists interested in pursuing REI. Educational and scientific advances expand for all involved in patient care as the team grows to meet the national trends. As the team melds their efforts surrounding coordination of care, tremendous improvements are noted in patient care delivery. The collaborative nature of the professional REI team-driven focuses further supports the goal of providing supportive, clinically necessary care to patients and increases the likelihood of a successful outcome.

REI NURSING ROLE

The number of REI centers across the country continues to increase, with an ensuing growth in the number of nurses needed to support the clinical needs of these centers. The integral roles of REI nurses have concomitantly increased and professional nursing involvement in patient care is becoming ever more complex. Many advanced nursing professionals are now expanding into leadership, clinical, research, and administrative roles. This growth in partnership with physician teams has allowed nurses to extend their clinical reach far beyond that of the traditional nursing model. In many ART centers, significant increases in autonomy are noted, as supported by the framework of a highly specialized team of professionals. The resulting multidisciplinary responsibilities of today's REI nurse expand to include a variety of roles including, but not limited to, those listed here:

1. ART clinical nurse
2. Patient educator
3. IVF coordinator
4. Regulatory compliance officer
5. Counselor
6. Nurse researcher
7. Egg donor/oocyte coordinator
8. Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) coordinator
9. Fertility preservation coordinator
10. Male factor infertility coordinator

REI nurses nationally support both large and small ART centers with similar goals for success. The diverse roles of today's REI nurses drive the critical need for enhanced education and competency in the medical aspects of care, as well as psychological support needs, participation in quality assurance initiatives, and patient education. Experienced REI practitioners bring many of these core skills from other areas of nursing. Dedicated training at the center-specific level in the field of infertility further enhances these transferable skills. Furthermore, there are ethical and religious dimensions inherent in treatment of infertility (e.g., through the use of IVF and other related techniques) that may present considerable challenges for nurses and patients. For example, the use of donor sperm, directed donor sperm, donor oocytes or embryos, oocyte cryopreservation for cancer patients, PGD, PGS, fertility preservation, or issues related to multiple pregnancies can be particularly challenging when compared to standard IVF. It is this diversity in the role of the ART nurse, combined with the reliance of patients on the nurse for education and, importantly, support, which means that ART nursing might be better suited for a more seasoned nursing professional rather than a recent nursing school graduate. Those most likely to succeed as ART nurses are mature, articulate, experienced, and flexible individuals who will consistently seek to redefine their role within a rapidly evolving field.

To remain successful in this highly demanding and constantly changing environment, REI nurses must support their professional commitment to education, certifications, and the development of co-workers. Training others enhances the team's ability to grow, supports the vision of the center to offer current technology within the guidelines of care, and supports the clinical needs for the team and patient population. Included in this training is an emphasis on resilience, which is needed due to the emotional and physical pressures of the role. At a minimum, nurses working in this specialty must demonstrate the confidence to articulate accurate information and support the emotional and clinical needs of the patients, all while ensuring the application and governance of the regulatory aspects of care. Each of these diverse, dynamic pieces lays the foundation for success in REI. Nurses with personality traits that can be described as "shy," "apprehensive," or "reactive" are more likely to suffer burnout and may be less successful professionally. Indeed, there is evidence suggesting that ART nurses are at high risk of burnout, which is positively correlated to the length of time spent working in the field and to a low perceived sense of emotional support (2). As the nursing shortage in the U.S.A. continues

to escalate, ART nursing positions remain difficult to fill. Thus, the workload carried by many ART nurses can be compounded by this resource challenge. In addition, the complexity of contemporary ART adds to the obstacles that many programs face when hiring and trying to retain professional nursing staff.

ART CLINICAL NURSE

The traditional role of the nursing professional presents a marked contrast when compared to the role of today's REI nurse. The academic structure that creates the ART center and the long-term needs of the varied patient populations determine the specific roles, education, and training necessary for success. Nurses have traditionally performed—or at least participated in—many of the technical steps involved in initiating a patient's ART cycle. Following initial consultation with the physician, the ART nurse becomes intimately involved in each patient's treatment cycle and will participate in that patient's care through pregnancy evaluation and treatment failure follow-up. In addition to their role in patient education, counseling, treatment planning, and coordination, nurses have commonly performed certain technical aspects of IVF treatment cycles, including but certainly not limited to:

1. REI and significant medical history with record review
2. Pretreatment evaluation of required diagnostic testing and screening
3. Evaluation of daily laboratory results
4. Medication administration and instruction
5. Preoperative, intraoperative, and postoperative care
6. Pregnancy evaluation and follow-up
7. Ovulation induction monitoring and intrauterine insemination partner and donor programs (3)

PATIENT EDUCATOR

Reproductive endocrinology nurses are foundational resources for patients and couples seeking ART treatments. The comprehensive, individualized care needs dictate the specific educational approaches used to direct treatment, evaluation, and follow-up care. As a key source of information to patients, continuing education is a primary requirement for the professional REI nurse, with the understanding that advances in technology have continued to positively impact clinical and research successes. However, with advancing technology comes the need for extensive, global education across REI centers. Each center differs in the available techniques, outcomes, and successes, yet the need for nurses to remain current on clinical, research, and regulatory trends is extensive. Through such education, nurses are best set to provide information related to current techniques and practices specific to their program.

Comprehensive patient education and support should be considered mandatory at every ART clinic. The goal of IVF patient orientation and education is to ensure that patients understand the "big picture" of their treatment. Ultimately, successful patient education fosters confidence in the care team when undergoing treatment. As a result, patients are more likely to be confident about providing informed consent, to be satisfied with their treatment, and to be more inclined to accept its final outcome (4).

Key aspects of patient education include discussions that focus on:

1. The patient or couple's specific infertility challenge
2. An overview of program practices, including members of the care team, clinical workflow, and treatment
3. Available ARTs, including intracytoplasmic sperm injection (ICSI), genetic screening and testing (PGD/PGS), assisted hatching, testicular sperm extraction (TESE), third-party reproduction, and fertility preservation, among others
4. Timing of the patient's cycle, including drug injections, overview of the hospital experience from oocyte recovery to embryo transfer, and other important aspects of treatment
5. Preparation of the cycle, including prestimulation protocols, stimulation protocols, and possible adverse events
6. Post-transfer management, such as pregnancy testing and follow-up appointments
7. Medication education for injection and reconstitution
8. Available support services that are center-specific, center-supported, or outside advocacy groups
9. Possible IVF concerns, such as premature ovulation, poor ovarian response, ovarian hyperstimulation syndrome, etc.
10. Regulatory considerations specific to the individual treatment cycles

ART remains a complex specialty with ever-changing medical terminology, a vast number of acronyms and abbreviations, and complex drug regimens and protocols. For this reason, it is important for the ART nurse to fully prepare patients on what to expect during the ART process. These professionals focus on alleviating anticipated stress and fear associated with ART treatment, and foster a sense of confidence with the care team.

In many ART centers, training occurs one-on-one and includes the patient (and partner where applicable) in addition to, or at times as a substitute for, group instruction. The importance of involving the partner or spouse in the educational process cannot be overemphasized. Fortunately, the unique relationship that develops between the patient and the nursing team in conjunction with the support team enhances communication. During the educational sessions, the nurse is able to evaluate the patient's understanding of clinical needs and gains insight into each patient's specific treatment requirements. Nurses support educational needs specific to their treatment cycle including clinical applications relating to the patient's religious, cultural, psychological, and medical needs.

Increases in clinical successes and outcomes are linked to increased patient compliance with drug regimens. Educational programs and patient comprehension increase the understanding of protocols and procedures. The

literature continues to support the notion that success or failure of treatment depends on a clear understanding of the current treatment regimen, regardless of the patient's prior experience with the process. For example, although human chorionic gonadotropin (hCG) is the single most important injection of the patient's treatment cycle, poor cycle outcomes have been attributed to errors in hCG administration. Patients have made reconstitution errors, such as injection of a tenth of the dose or administration of diluent only, or timed the injection incorrectly (5). A recent report found that 10 patients (15.2%) undergoing IVF or ovulation induction treatment received hCG incorrectly, and in these cycles only one pregnancy occurred (6).

Historically, an REI nurse could expect to conservatively spend approximately two hours on a patient education session. Today, with the recent improvements to the instruments used for medication administration, nurses have noted a significant time saving associated with education and follow-up questions during stimulation. For example, urinary-based products required intramuscular administration and therefore necessitated comprehensive educational sessions. These products were exclusive to REI medication protocols and included preparations of human menopausal gonadotropins and hCG. In the mid-1990s, with improvements in laboratory procedures, the movement from sub-zonal injection to ICSI, the evolution from urinary to recombinant medications, improvements in PGD procedures, and the expansion of other available technologies, the time and complexity of patient education increased dramatically. In particular, the late 1990s saw the introduction of recombinant follicle-stimulating hormone, followed by other recombinant gonadotropins. These medications were suitable for subcutaneous self-injection by the patient (or with assistance from a partner or family member). In recent years, the advancement of endometrial co-culture techniques, the broadening application of PGD and PGS testing, the addition of gonadotropin-releasing hormone antagonists as an alternative to the agonists for the prevention of a premature luteinizing hormone surge, and the development of new and more complicated protocols involving techniques such as TESE/testicular sperm aspiration have brought additional patient education challenges to the ART nurse. All these advances are associated with the need for nurses to provide appropriate and accurate education to patients, which of course requires ART nurses to continually update their knowledge and remain current in trends in assisted reproduction.

Despite a continued willingness to extend therapeutic options for patients, there are ongoing efforts to simplify treatment regimens and, thus, minimize confusion. ART nurses frequently consult with colleagues on ways to streamline the educational process for patients, ultimately improving the quality and efficacy of patient care.

EDUCATIONAL RESOURCES

The provision of center-specific ART materials outlines the procedures, policies, and requirements of each center. Nurses respond to the changing trends in REI clinical, research, and treatment aspects as necessary for their respective centers. Updating educational materials is critical to supporting the needs of patients, as well as the clinical care team. Written materials are integral to supporting in-person educational sessions and enhancing the at-home reference for patients. Demonstration remains an educational format that serves this population well. The steps involved in reconstituting medication and proper injection techniques derive great benefit from demonstration. In more recent years, the introduction of liquid formulations and user-friendly medication injection devices has significantly improved and changed the education dynamics. The diversity of available preparations supports the need for comprehensive, individualized education that integrates both demonstration and written resources. The most obvious example of this is the education provided to patients and partners to self-inject medications. Nurses employ hands-on, tactile-type teaching with instructional materials, including an injection buttocks and videos, to facilitate patients' understanding of the process. Utilizing this approach also allows the nurse to assess comprehension and the ability to correctly perform medication administration and/or reconstitution, which is essential to supporting a favorable outcome. Today, nurses value the additional support of industry-driven resources to enhance the educational setting. The use of CDs, DVDs, and online media has updated the at-home references for patients with great success.

Additionally, web-based resources and industry-supported written instructions are available for distribution to patients. Moreover, several of the national patient organizations also provide written materials (books, newsletters, fact sheets, and online FAQs), CDs/DVDs, and reliable websites that are updated with current treatment options, support sessions, relevant insurance, and provider information.

IVF NURSE COORDINATOR

The ART nurse coordinator is an integral component of the care team in REI. The role of the ART nurse coordinator is extensive and includes the evaluation of each research, clinical, and regulatory component of care. The nurse as a coordinator is part of the traditional nursing role that continues to this day. In ART programs, the overall goal of the nurse coordinator is to serve as a clinical liaison between patients and the scientists, clinicians, and nurses, ensuring a smooth navigation through the treatment cycle. This requires a great deal of flexibility, as well as acute attention to detail and strong delegation skills. As clinical advances continue to emerge, ART programs need well-informed coordinators and support staff to meet the needs of their patients. Timing, scheduling, and pre-testing requirements are complex and necessitate dedicated detail-oriented staff for oversight and management. Ongoing education and staff reinforcement are essential to this success. Rather humorously, the role of nurse coordinator has been described as "organizing the world based on another woman's menstrual cycle … or organized chaos" (4).

Table 70.1 Coordinating the assisted reproduction technology team: Some aspects to consider

- Alerting the pharmacy regarding drug requirements
- Scheduling interventional procedures (e.g., ultrasound examinations and oocyte retrievals)
- Documenting treatment results and outcomes
- Updating patient records
- Communicating results to patients
- Amending existing protocols or developing new ones
- Updating the staff counselor on specific patient needs
- Obtaining legal consents for new or updated procedures

Coordinating the unit

The success of an ART program is dependent upon the coordination of the collective team of professional physicians, scientists, support staff, and nurses. The inclusive evaluation of clinical and academic responsibilities and prior commitments, such as lectures, vacations, and conferences, is essential for timing and supporting the team. Table 70.1 highlights some of the aspects of treatment that must be incorporated into the overall treatment plan for all patients.

Nationally, ART centers continue to expand, offering additional services and sometimes even conveniently located satellite-based centers. This enhanced availability affords patients greater access to care when they live significant distances from the main center, supporting their desire to remain at home and/or working for as long as possible during treatment. Additionally, the success with specific technologies achieved by some ART centers has attracted potential patients both locally and internationally. Coordinating care for patients who live abroad is much more complex than for those who live in closer proximity to the ART center. The requirements for long distance coordination vary according to the patient's situation and selected treatment options. All in all, well-organized management of care contributes to the smooth running of an efficient unit, ensuring patient satisfaction and overall success.

PATIENT COORDINATION: FOCUS ON COMMUNICATION

As ART programs continue to grow, both in numbers and in expertise, there are ongoing initiatives to ensure quality of care and patient satisfaction. Patients are increasingly focused on communication, privacy, and accuracy of information. Maintaining and supporting communication is essential to the process for both the care team and the patient. The development of dedicated nursing teams in many ART centers has supported effective communication and highlights the benefits resulting from such commitments. Limiting the number of staff that interface with patients increases confidence, limits errors, and enhances satisfaction for both the care team and the patient. Continuity of care and team collaboration are effective communication tools in an REI program. Building confidence through communication influences patient satisfaction and successes within the REI programs.

In ART centers that have expanded to include satellite offices, the establishment of a primary care nursing team structure (an IVF coordinator and assistant coordinator assigned to each physician) supports goals for improved communication and team building. Patients seek access to members of the care team to support their personal goals. With advances in communication practices by way of sophisticated but user-friendly IT systems, written and verbal communication trails, and team collaboration, ART centers support their practice mission to expand with the confidence that patient care is accented and that patients are treated safely within both the regulatory guidelines and the recommendations of the American Society for Reproductive Medicine (ASRM).

REI nurses in ART centers engage other members of the care team in the process of treatment and support the goal of access to the primary REI physician. The nursing team handles the medical aspects of the treatment cycle, while other coordinators, such as third-party liaisons, finance teams, and research nurses, manage other issues related to the non-medical, more administrative aspects of care (e.g., consents and contracts). Following comprehensive evaluations of such revisions to practice, it has been noted that accountability and communication improved, both within the teams and with the center, and there were fewer complaints from patients regarding communication and call-back issues (7).

Other centers have successfully adapted a more modified approach, designing nurse and physician teams that coordinate phases of the treatment cycles. Once the patient is fully engaged in treatment, a more coordinated approach is employed. Nurses tend to benefit from this practice as well through the development of a closer rapport with patients and increased job satisfaction.

More subtly, nurses tend to be able to empathize better with patients and to develop a greater insight into patients' physical and emotional needs (8).

PATIENT COUNSELOR

REI nursing professionals fully recognize the impact that infertility treatment has on the emotional state of patients and partners. Considerable emotional demands are noted in those seeking treatment. While infertility itself is recognized as a "life crisis," provoking a variety of emotional responses, the range of ART treatment options now available to couples also raises complex emotional issues. These options may present significant financial, medical, legal, and religious or ethical implications. As patients proceed along the treatment journey, the psychological impact may become increasingly important and, at times, more pronounced. Several studies have suggested that emotional and psychological factors may be leading causes of patient dropout (9). In fact, there are several reports suggesting that approximately 60%–65% of patients who begin REI

treatment may drop out before treatment completion (10–12). In addition, the proportion of patients dropping out of treatment appears to increase with each subsequent failed cycle (13). Although emotional/psychological factors are important, other factors may also contribute to patient dropout (13). In recognition of the importance of psychological factors in treatment success, many clinics offer supportive counseling on a routine basis. Since the ART nurse is often the first to recognize the patient's need for counseling, s/he serves as an important advocate.

NURSE VERSUS COUNSELOR

Although there may be significant overlap in the emotional support provided by the ART nurse and a professional counselor, in general, counseling involves the use of psychological interventions based on theoretical frameworks for which specialized training is required. The nurse's perspective includes a thorough understanding of the patient's clinical scenario. The ART nurse is in a unique position to provide emotional support to the patient and her partner because of the close relationship that develops, based on a high level of trust, sensitivity, and discretion. This is very different from other clinical settings, because infertile patients rarely discuss the "private" side of their infertility outside of the nurse/counselor relationship.

Specific areas that the nurse can focus on to help promote psychological well-being include:

1. Talking through individuals' emotional responses to their infertility
2. Identifying couples' sources of stress, such as the success of procedures like ICSI for severe male factor infertility, PGD/PGS for the diagnosis of chromosomal and genetic abnormalities or donor gametes, and the associated issues of disclosure and ethical and religious ramifications
3. Providing support regarding infertility patients' concerns and emotions
4. Discussion of therapeutic options, including:
   a. Providing guidance on realistic expectations
   b. Anticipating emotional responses
   c. Discussing ethical and religious concerns
5. Helping patients to maintain self-esteem and interpersonal relationships
6. Encouraging patients to continue with "life" outside of infertility

In many instances, there will be an overlap between the nurse as "educator" and the nurse as "counselor." In many settings, nurses discuss treatment options and confirm patient understanding through appropriate questions and monitoring feedback, enabling patients to provide informed consent and ultimately feel more in control, therefore reducing potential anxiety. In addition, nurses collaborate with the psychological support team to offer patients additional services. ART nurses can take on a supportive role, drawing on clinical experiences to guide patients in the decision-making process related to other treatment options or for those seeking closure after treatment failure.

SPECIALIST COUNSELING

Some ART programs require patients to undergo psychological counseling prior to pursuing treatment. Certainly, in cases where third-party parenting options are being considered, ART programs mandate patient counseling. This trend toward promoting emotional wellness is on the rise and offers tremendous advantages to those seeking treatment. With the increase in third-party reproduction and the use of donor sperm and gestational carries, the risks are greater and suggest that more counseling opportunities support favorable outcomes. Additionally, some patients will require support that extends beyond the type required by most undergoing "routine" ART procedures; it is imperative that this need is recognized and addressed.

Research indicates that three particular groups of patients are likely to benefit from specialist counseling.

They are patients:

1. Experiencing high levels of stress (e.g., after a failed treatment, during a multiple pregnancy, undergoing PGD/PGS, TESE, failed ICSI, etc.)
2. Requiring donated gametes, directed-donor settings, surrogacy, or adoption (third-party reproduction)
3. Seeking fertility services because of their special social or ethical circumstances (14).

NURSING RESEARCH

The driving force behind the acceptance of ART nursing as a separate specialty is nursing-directed research, ideally inspired, motivated, and supported through collaborations with physician colleagues. While the outcomes of nursing interventions are already used as sources for nursing-based research, generally the type of research undertaken by nurses has a more subjective approach, including investigation of the psychological, nurturing, and educational aspects of ART. This contrasts with the more objective research likely to be conducted by physicians. For example, a nurse-based study considering administration of progesterone for luteal-phase support would focus more on the tolerability of the drugs administered than a physician-driven study that would be more likely to concentrate on aspects such as in-phase endometrial development. Regardless of topic, nurses are already participating in clinical care research in partnership with other healthcare professionals, and are benefiting from the support given to them by the National Institute of Nursing Research and Nursing Research Mentors.

Increased acceptance of nursing-driven research will come through translation of their research into practice. Thus, it is clear that forums are needed to allow nurses to disseminate the knowledge they are acquiring. Every opportunity should be afforded to nurses to present their studies, both locally and at an international level, including the annual meetings of major societies such as the ASRM. In addition, there is a need for a specialized journal

devoted to ART nursing. Such a journal could play an important role in stimulating ART nursing research and in setting high standards for the publication of research studies.

THE ROLE OF AN EFFECTIVE REI NURSE LEADER

The nurse leader role in REI is an interesting meld of mentoring, leadership, clinical expertise, nurturing, and vision. As part of the daily practice, REI leaders focus on coordinating and directing teams on clinical and administrative tasks to ensure that the center functions effectively and efficiently on all levels. Frequently, the nurse leader manages administrative roles and interacts on a personal level with each member of staff, as well as with individual patients. Nurse leaders are integral members of the center's quality assurance program, quality control, research, patient satisfaction team, and financial focus. As such, the responsibility of the ART nurse manager is not only to ensure that their staff receives the training needed, but also to continuously monitor and assess their activities so that training standards are maintained, ensuring that the ongoing concerns of the patients and the ART center are addressed.

ART nurse leaders have the opportunity to contribute to all aspects of patient care, from the moment the patient enters the clinic to the time they leave, whereas in other areas of medicine the patients often receive "segmented" and often disjointed and confusing information. The quality of care the nurse leader provides offers overall responsibility, and contributes directly to the success of the ART program both in clinical terms and in terms of patient satisfaction. Many times the role revolves around mentoring, nurturing, and reassuring on clinical, research, and personal levels. Burnout is a significant concern to the ART nurse leaders, due to the inherent differences between ART nursing and traditional nursing roles. Unlike other areas of medicine, where nurses can adopt a more detached attitude, ART nurses are more likely to become personally involved with the infertile couple and patients, regardless of the inconvenience to themselves in terms of additional time and energy. Teaching ART staff nurses how to allocate time to particular tasks, to more efficiently organize their day, and to seek personal education and supportive care from physicians and staff psychologists is an ongoing role of the ART nurse manager. With the appropriate support, nurses can remain emotionally and physically healthy and will be able to perform their role effectively. Nurse managers' interactions in center-specific quality assurance concerns assist with the evaluation of the ongoing needs of the department and its staff, and therefore ensure that a healthy clinical balance is maintained.

The ability to recognize staff educational needs and to offer the appropriate materials and opportunities to address these is another way to ensure the provision of quality care, as well as to address issues of nursing burnout. Since ART is such a specialized field, the ART nurse manager not only becomes the nurse educator within the ART center (e.g., to new nurses), but is also called upon to educate other specialties, such as the neonatal intensive care unit or high-risk antepartum staff, on issues pertinent to ART.

Table 70.2 Professional organizations supporting the assisted reproduction technology nurse

- American Infertility Association (AIA; patient support group)
- Nurses Professional Group (NPG) of the American Society for Reproductive Medicine (ASRM)
- Regional nursing associations
- RESOLVE (patient support group)
- Biosymposia
- Society for Assisted Reproductive Technology (SART)
- Fertile Hope (patient support group)

ART: NURSE TRAINING

As technology advances, the need for ongoing educational support within ART centers has increased. As previously mentioned, ART nurses benefit tremendously from supportive physicians and colleagues within their center. Furthermore, many ART nurses are fortunate to gain educational support or insight for their research interests through participation in or attendance at educational symposia, or being supported in their research endeavors through sponsored ART clinics. ART nurses are also supported in their evolving role by a number of organizations that offer professional development guidance, research mentors, conferences, lecture opportunities, information on policies, procedures and position statements, state-of-the-art medical information, and networking opportunities (Table 70.2). For example, the Nurses Professional Group (NPG) of the ASRM provides a forum for networking and information exchange among nurses. It also offers continuing medical education opportunities through roundtables, seminars, and workshops at its annual meeting. The NPG has developed *Protocols and Procedures for Nurses*, a publication that encompasses a variety of topics such as:

1. Nursing management of patients undergoing ART
2. Patient preparation for infertility-related treatments
3. Protocols on procedures performed in reproductive medicine practice

While such resources are valuable aids to the ART nurse, they are not substitutes for the one-on-one instruction that remains key to the successful training of a new ART nurse. Introductory training must include a review of basic gynecology, including the integration of the reproductive/endocrine cycle, the characteristics of normal and abnormal cycles, and physical anomalies that interfere with fertility, together with a less academic but equally important overview of the responsibilities of key ART team functions. It is crucial that each member of the team knows not only their own responsibilities, but also those of their fellow team members and, importantly, how these roles interact. Ultimately, it is the practical experience of patient management and the experience gained through

repeatedly performing assigned tasks that form the basic core of the ART nurse's education and training.

While expanding the skills of nurses will obviously increase the number of tasks they can perform competently within a treatment program, less obvious are the benefits such as increased job satisfaction and greater continuity of patient care. Well-defined responsibilities, standards, and protocols for clinical practice are required and should be continually refined. As an example, the recent inception of U.S. Food and Drug Administration regulations established national guidelines for ART centers, which required ongoing education for the experienced ART nurse and the development of new nurse training.

THE FUTURE OF ART

The role of the REI nurse continues to see significant expansion. Clinical responsibilities are expanding in part due to the continued introduction and revision of techniques and treatment modalities, clinical goals, and patient focuses. Much of the significant changes involve nursing allocations of time and education, further supporting the need for visionary learning and goals. Despite the rapid advances made in ART and the constantly evolving role of nurses working in this field, there is currently no specific certification that officially recognizes REI/IVF/ART nursing as a specialty. The NPG continues to support the development of education modules, and is currently supporting a certificate program for REI nurses. Unlike neonatal and high-risk antepartum nurses, who receive specialty training in an academic setting and who qualify for certification, ART nurses have more limited educational resources. There is inadequate exposure to the field of infertility at any level of academic training, and when it is offered, often as a one-day course, the level of its content is highly variable. As outlined earlier, ART nursing is emotionally demanding, and it is not recommended as an entry-level position due to the level of autonomy that is required in this field. A certain level of professional maturity is mandatory; infertility treatment is elective and patients are generally well-educated in terms of infertility treatment options, and are often able to make informed decisions regarding their treatment regimen. The nurse needs to be confident enough to face the challenges presented by very determined and oftentimes well-informed patients. As research and treatment options evolve, so does the role of the nurse in reproductive medicine. In turn, the prospects for a variety of professional opportunities will develop, providing broader career options and greater job satisfaction.

CONCLUSIONS

REI nurses are in a unique position professionally. The autonomous, interdisciplinary role that many nurses support allows for an extended, participative role with both the patient and the clinical team. Nurses provide a deep empathy for a patient's infertility struggle, and play a critical role in the patient's treatment success. With the indicated education and mentoring from others on the team, including physicians and scientists, nurses continue to frame the profession with an eager sense of pride and determination. Appropriate continuing education and opportunities for clinical certification are essential to support this determined group of nursing professionals. Professional growth and clinical expertise come to those who seek excellence, show a willingness to improve, and further expand the future of the REI-ART nursing practice. Continued participation in nursing research initiatives offers professionals a collaborative role with others in the field who are interested in the development of state-of-the-art standards. These goals are in alignment with the deep desire of professionals focused on ensuring patients benefit from the ongoing emotional, educational, and practical support offered by the contemporary ART nurse. Although ART nursing may be perceived as "stressful," it remains a profoundly rewarding career that offers tremendous value, satisfaction, and a future of unknown dimensions.

REFERENCES

1. Edwards R, Purdy JM, Steptoe P. *Implantation of the Human Embryo*. London, U.K.: Academic Press, 1985.
2. Rausch DT, Braverman AM. Burnout rates among reproductive endocrinology nurses: The role of personality and infertility attitudes. *56th Annual Meeting of the American Society for Reproductive Medicine*. San Diego, CA, October 21–26, 2000, abstract.
3. D'Andrea KG. The role of the nurse practitioner in artificial insemination. *J Obstet Gynecol Nurs* 1984; 13: 75–8.
4. James CA. The nursing role in assisted reproductive technologies. *NAACOGS Clin Issu Perinat Womens Health Nurs* 1992; 3: 328–34.
5. Ludwig M, Doody KJ, Doody KM. Use of recombinant human chorionic gonadotropin in ovulation induction. *Fertil Steril* 2003; 79: 1051–9.
6. Markle RL, King PI, Martin DB et al. Characteristics of successful human chorionic gonadotropin (hCG) administration in assisted reproduction. *58th Annual Meeting of the American Society for Reproductive Medicine*. Seattle, WA, October 13–16, 2002, abstract.
7. Norbryhn G, Fontanilla T, Rogoff R et al. Establishment of a primary care nursing team in a rapidly expanding multi-site reproductive endocrine center. *56th Annual Meeting of the American Society for Reproductive Medicine*. San Diego, CA, October 21–26, 2000, abstract.
8. Muirhead M, Lawton J. A team approach to assisted conception treatment. *Hum Fertil* 1998; 1: 40–3.
9. Domar AD. Impact of psychological factors on dropout rates in insured patients. *Fertil Steril* 2004; 81: 271–3.
10. Land JA, Courtar DA, Evers JLH. Patient drop out in an assisted reproductive technology program: Implication for pregnancy rates. *Fertil Steril* 1997; 68: 278–81.

11. Olivius C, Friden B, Borg G et al. Why do couples discontinue *in vitro* fertilization treatment? A cohort study. *Fertil Seril* 2004; 81: 258–61.
12. Malcom CE, Cumming DC. Follow-up of infertile couples who dropped out of a fertility specialist clinic. *Fertil Steril* 2004; 81: 269–70.
13. Rajkhowa M, McConnell A, Thomas GE. Reasons for discontinuation of IVF treatment: A questionnaire study. *Hum Reprod* 2006; 21: 358–63.
14. Boivin J, Appleton TC, Baetens P et al. Guidelines for counseling in infertility: Outline version. *Hum Reprod* 2001; 16: 1301–4.

Patient support in the assisted reproduction technology program

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SHARON N. COVINGTON

OVERVIEW

Reproduction is considered the most basic of human needs, propelled by powerful biological and psychological drives. When the ability to reproduce is thwarted, a crisis ensues—the life crisis of infertility. The psychological crisis of infertility has been well documented in the literature. It is considered an emotionally difficult experience that impacts on all aspects of a couple's or an individual's life: relationships with others, life goals, social roles, self-image, self-confidence, and sexuality, to name a few (1). The losses associated with infertility are multifaceted, including the loss of hopes, dreams, future plans, marital satisfaction, self-esteem, sense of control, belief in the fairness of life, health, and well-being, and, most important, the "dream child" (2). Further, these losses evoke feelings of grief—shock, disbelief, sadness, anger, guilt, blame, and depression—which occur in a repetitive and predictable process as patients move through medical diagnosis and treatment. It is through the experience and expression of emotions involved in the grieving process that the infertile couple moves toward an acceptance of their infertile state, engages in the exploration of alternative plans, and begins to move forward with their lives (3). During the past 50 years, we have seen a shift from the psychogenic infertility model, in which demonstrable psychopathology was thought to play an etiologic role in infertility, to a psychological sequelae model, in which numerous psychological factors were considered the result of infertility (4). In this concept, infertility is viewed as an emotionally difficult experience affecting all aspects of an individual's and a couple's life. Thus, emotional distress is a consequence and not a cause of infertility, as conceptualized previously. The application of a broader spectrum of theoretical approaches has led to a less individualistic perspective and a more holistic approach to infertility. In this sense, the interactions among individuals/couples and social/medical components are considered and must be factored into medical treatment. These perspectives have also increased understanding of individual and couple differences and resilience, the impact of reproductive medical treatments, and the efficacy of therapeutic psychological interventions.

Research examining the psychosocial context of infertility has burgeoned during this period. In a comprehensive review of the literature, Greil and associates (5) expanded on earlier work (6) by assessing research published in the last 10 years to determine how it has changed, where methodological progress has been made, and what generalizations can be drawn about the experience of infertility. They note the change from viewing infertility as a medical condition with psychological consequences to placing infertility within a larger sociocultural construct that shapes the experience.

There is increasing consensus among all reproductive medical organizations that patients seeking assistance need a "patient-centered" approach whereby an individual patient's needs and values are respected, responded to, and guide all clinical decisions. Optimal patient care will include ways to minimize patients' psychological distress, while providing effective clinical care in a positive environment (7). With a patient-centered approach, the provision of routine psychosocial care can reduce distress related to medical procedures and the experience of infertility, as well as improve patient well-being and compliance with treatment. Taking an evidence-based method, the European Society of Human Reproduction and Embryology (ESHRE) recently published extensive guidelines for routine psychosocial care in assisted reproduction technology (ART) programs (8), an approach supported in this chapter.

Stress and ART

ARTs, while opening up expanded opportunities for the treatment of infertility, have generated their own psychological challenges for patients. For most couples, ARTs are the last, best options for having a child, and are used after long months, and sometimes years, of treatment failure, often at tremendous emotional, physical, and financial cost. Patients entering ART programs usually do so with the burden of grief and disappointment from infertility, seeming depressed, angry, tired, dependent, and anxious. Although emotionally depleted, couples are attracted to a technology that offers hope where, a few years ago, none existed. They find themselves drawn into a new emotional turbulence of contrasting feelings of hope and despair, which seem to be generated in part by the experience of the technology itself. The intensity and high-tech nature of ART create a stressful atmosphere, where the stakes are high and the chance of success may be relatively low. ART is a gamble, and, like gamblers, patients may have unrealistically high expectations of success (9) or feel compelled to try "just one more time," finding it difficult to end treatment without success. Of all infertility treatments, *in vitro* fertilization (IVF) is considered the most stressful (10), with 80% of IVF patients ranking it as "extremely" to "moderately" stressful (11). Furthermore, after a failed cycle, almost all couples report acute depression (12), with elevated anxiety and anger levels persisting weeks later (13). Despite the stressful

consequences of infertility and ART, numerous studies report that the vast majority of patients are generally well adjusted (14–17). In one of the most extensive reviews of scientifically rigorous research on the psychological effects of infertility, Stanton and Danoff-Burg concluded that the majority of infertile men and women are psychologically resilient and maintain adequate psychosocial functioning (18). Boivin found little evidence that infertile patients, as a group, experience significant, long-term maladjustment on measures of anxiety, psychiatric disturbance, marital conflict, and sexual dysfunction, when compared with population norms (19). Overall, this group reports marital adjustment in the normal range, and that the crisis of infertility may actually improve marital communication and emotional intimacy (20–23).

Gender differences and ART stress

The majority of studies of stress during ART are in women, and, overall, women react more intensely to infertility and ART than do men (24). Prior to IVF, women report more anxiety and depression, less life satisfaction, lower self-esteem, and more anticipatory stress than their male partners (21). During IVF, the intensity of a demanding treatment protocol—daily ultrasound monitoring, blood draws for hormone levels, injections, invasive procedures for oocyte retrieval, and embryo transfer—is frequently given by women as a cause of psychological distress (10). If treatment fails, depression persists longer for women than for their partners, lasting up to six months (13). Years later, women will recall the stress of IVF as more stressful for them than for their partner, regardless of the success or failure of treatment.

In one of the few studies examining men's distress during IVF, Boivin et al. found that men who were undergoing intracytoplasmic sperm injection (ICSI) reported more distress on the days prior to retrieval than did other IVF men (25). However, in all other areas, ICSI and IVF men were similar in their adjustment to infertility and in their distress during the treatment cycle. These findings were in contrast to those of early studies of distress among men with a male factor diagnosis, as these infertile men reported more negative feelings and psychiatric distress (12,26). The discrepancy between these studies may have been due to the fact that ICSI could circumvent the infertility, whereas at the time of the earlier studies the only medical option available was donor insemination. While the intensity of emotional reactions to particular aspects of ART may differ between men and women, the types of reactions are the same, with both experiencing a significant increase in anxiety and depressive symptoms from pre- to post-treatment (21). In addition, both men and women rank the relative stresses of each stage of IVF equally, and tend to overestimate the chances of success of IVF in general, showing a high level of hopefulness in their own cases (13).

Men and women tend to cope differently with the stress of ART and infertility (20). As frequently noted, women are more expressive of feelings, and are more likely to seek emotional and social support during ART by informal activities such as talking to spouse, family, and friends. In terms of the effects of coping post-IVF treatment, Hynes et al. found that women who used problem-focused coping had a higher level of well-being than those who used avoidance coping or social support (27). Men, on the other hand, who are often action oriented and solution focused, frequently cope with infertility through greater involvement in work or sports-related activities. While men and women may have different coping strategies, the use and effectiveness of these techniques may be influenced by the point in the infertility process and the existence of a gender-specific infertility diagnosis (28).

Gender differences may also be impacted by the perception of psychosocial support during ART (29). Since the nature of ART treatment is focused on women, men can feel more isolated and less emotionally supported than their partner, especially by family and friends. Increased distress may arise when infertile people do not get the emotional support they need during infertility treatment (30). Psychosocial support and intervention are equally beneficial for both men and women (31) and thus are recommended as part of treatment.

Levels of stress during ART cycles

While general assumptions may be made about stress levels during ART cycles, the experience for infertility patients will be personal and unique: each patient will experience the stress differently, based upon his or her own personality and life experiences. Newton et al. noted that stress has been conceptualized both as a stimulus or event (distressing circumstances outside the person) and as a response (internal disturbance) (24). A contrasting approach describes stress as neither an event nor a response, but rather a combination of factors: the perceived meaning of the event and self-appraisal of the adequacy of coping resources (32). Thus, it is not the stress itself but the perception of the stress that determines how ART patients experience and handle it.

The aspects of ART perceived to be stressful to patients are multifaceted and affect all parts of their life: marital, social, physical, emotional, financial, cultural, and religious. Time is stressful, both in the time commitment to an intense treatment that leads to disruption in family, work, and social activities, and, for some, in long waiting periods for IVF or third-party reproduction. ART stress impacts on the marital relationship with an emotionally laden experience, and, by removing the conjugal act for procreation, sexual intimacy is lost. Also, couples are stretched financially, paying for the high cost of ART treatment with a relatively low probability of success. Dealing with the medical staff and with the side effects or potential complications of medical treatment has its own stress: hot flushes, headaches, mood fluctuations, shots, sonograms, future health concerns, and decision-making about embryos and multiple pregnancies. Religious, social, cultural, and moral issues may also make ART cycles stressful, especially for those dealing with third-party reproduction, when these values are in conflict with the choice of treatment.

The first treatment cycle has been found to be the most stressful for patients, with high levels of confusion, bewilderment, and anxiety (10,13). This may be due to inexperience with the process, or possibly inadequate preparation of the patient by staff in terms of information and discussion of care. Slade et al. found that for couples attempting three cycles of IVF, distress diminished during the middle cycle, but rose after they discovered that the intervention had not been successful, with the last cycle being as stressful as the first (13).

Within a treatment cycle, patients view IVF/ART as a series of stages that must be successfully completed before moving on to the next phase of treatment: monitoring, oocyte retrieval, fertilization, embryo transfer, waiting period, and pregnancy test stages. The level of stress, anxiety, and anticipation rises with each stage, peaking during the waiting period. A number of studies have confirmed what clinicians know anecdotally: in order of perceived stress for patients, waiting to hear the outcome of the embryo transfer is the most stressful, followed by waiting to hear whether fertilization has occurred, and then the egg retrieval stage (11,33). Patients are aware of the importance of these key phases in the IVF process, and the uncertainty of the outcome is highly distressing.

Understandably, patients who are experiencing emotional distress from infertility will have their quality of life impacted. To identify these patients, several years ago an international effort was undertaken by the American Society for Reproductive Medicine (ASRM), the ESHRE, and Merck-Serono to develop a psychometric tool that would be reliable, cross-cultural, and easy to access and interpreted. Published in 2011, the Fertility Quality of Life (FertiQoL) is able to measure treatment quality (interactions with staff and quality of information) and treatment tolerability (effects on mood and disruptions to daily life), and proves to be an invaluable tool for clinicians (34). It is free of charge and completed online by patients, with results sent to the clinician. FertiQoL is available in 31 languages, with more being developed, and takes about 10–15 minutes to complete (www.fertiqol.org).

METHODS

Who provides patient support services in ART?

Given the host of research on the emotional consequences of infertility and on the distressing nature of ART, it is clear that patients need psychological support as an integrated part of the medical treatment process. Technology has become more complex, and so have the psychological, social, and ethical issues related to treatment, which challenges the resources of staff and patients. As a result of technological advances in ART and the recognition of the psychosocial issues and demands facing infertile patients, mental health professionals have become increasingly important members of the reproductive medical team (35). The specialization of "infertility counseling" has emerged internationally, combining the fields of reproductive health psychology and reproductive medicine, for mental health professionals including social workers, psychologists, psychiatrists, marriage and family therapists, counselors, and psychiatric nurses (36).

Infertility counselors serve as a resource to patients and staff by providing specialized psychological services that support and enhance quality care. For example, the complex medical and psychological issues in third-party reproduction have social and legal implications that must be assessed carefully, and warrant involvement of a qualified mental health professional experienced in infertility counseling. In addition, the psychosocial impact on the offspring created by ART needs to be considered, and assistance given to families dealing with these issues pre- and post-treatment.

Nonetheless, the responsibility for patient support in the ART program is the duty of all staff members, not just the domain of nurses or infertility counselors. Interactions with each staff member, from administrative staff to physician, influence a patient's perception of care and, in turn, his or her stress level. Sensitivity, warmth, patience, and responsiveness create an environment of support. Also, the general clinic routine and ambience reflect support and respect of patients when it is provided in an efficient, organized, clean, uncrowded, and esthetically pleasing atmosphere. All staff need to be sensitive to and knowledgeable about the psychological needs and stresses of ART patients (7). While the primary focus of physicians, nurses, laboratory scientists, and other healthcare staff is the medical diagnosis and treatment of infertility, it must also entail "treating the patient, not the disease."

Types of ART support services

ART patient support services can be generalized into overall clinic administration and environment, to specialized services that need to be provided by a mental health professional who is experienced in infertility counseling. For the purpose of this chapter, while specialized services provided by an infertility counselor are described, a detailed explanation of methodology is not addressed (4). Moving from specific to general, the methods of providing patient support services can be categorized as:

1. Psychological assessment and evaluation
2. Therapeutic counseling
3. Supportive counseling
4. Information and education
5. Clinic administration

Psychological assessment and evaluation

Psychological preparation and assessment of participants using ART often vary from program to program, with the purpose often debated: should it be "mandatory" or "voluntary"? Is it "counseling" and/or "evaluation"? Currently, there are only a handful of jurisdictions that require counseling prior to ART treatment (36).

While the Human Fertilisation and Embryology Authority (HFEA), which regulates assisted reproduction in the U.K., has stipulated that psychosocial counseling must be offered to patients seeking IVF or donor gametes (37), one study found that fewer than 25% of patients took

up the suggestion (38). In the U.S.A., recommendations and guidelines for the provision of psychological services to ART participants are voluntary (39), and the decision concerning which patients should be screened or counseled, and for what procedures, is left to each individual fertility practice. Thus, available guidelines for assessment and evaluation are usually tailored to the specific requirements or preferences of a particular program. Whether a clinic adopts formal or recommended guidelines or chooses to develop its own, the program's policy regarding infertility counseling, screening, exclusion criteria, and so on should be clearly defined for the protection of the medical team, the infertility counselor, and patients (40).

Notwithstanding the voluntary nature of screening ART participants, it has become the standard of care to require psychological evaluation and psychoeducational preparation of oocyte donors, surrogates, and gestational carriers by experienced mental health professionals. The evaluation usually involves both psychological testing of the donor/carrier, with the Minnesota Multiphasic Personality Inventory-2 (MMPI-2) being used most often (41,42), and clinical interviews with the donor/carrier and, when available, her partner. Assessment and counseling of recipients of donor gametes are also strongly recommended or required by many programs, especially when the donor/carrier is known or related. Other situations where programs may require screening and assessment involve patients undergoing IVF who are considered psychologically or physically vulnerable, previous IVF patients donating frozen embryos, single recipients of gamete donation, and older infertility patients.

The established protocol for psychological evaluation and assessment within the author's program includes:

1. All recipients of anonymous donor eggs, sperm, and embryos, and genetic parents using a gestational carrier, are required to see a staff infertility counselor. The psychoeducational counseling and assessment usually take place in one or two counseling sessions. Reading materials and support resources are provided, and issues related to raising children conceived through third-party reproduction are discussed.
2. Psychological evaluation of all anonymous oocyte donors is mandatory. Psychological testing (MMPI-2) is administered, and then scored and interpreted. A minimum of two clinical interviews—one with the donor and one with her and her partner—are conducted with a staff infertility counselor to assess psychological functioning, and the process, motivations, and implications of gamete donation are discussed.
3. All known donors or gestational carriers and recipients are required to undergo evaluation and counseling, which includes administering the MMPI-2 to the donor and gestational carrier. Clinical interviews are held with the donor or carrier and patient separately, including their partners, and a joint "group" session is conducted to discuss how they will deal with issues in known donation. Legal consultation and contracts are also strongly recommended with gestational carriers.
4. Assessment and counseling of any infertility patient is required when the physician is concerned about psychological vulnerability or marital instability, or if a situation is presented to our internal ethics committee where additional psychosocial information is needed before a decision about treatment can be made.

Our mental health professional staff follow the criteria established for acceptance or rejection of participants in the recommended ASRM practice guidelines for gamete donors and gestational surrogates (39,43). When a recommendation to withhold or postpone treatment is made by the infertility counselor, a team meeting takes place so that a decision is made by team consensus, rather than one member (usually the physician or the infertility counselor) being seen by the patient as the "gatekeeper." It is useful to view and interpret these recommendations to the patient as protection of the parties involved rather than rejection, since it is the first responsibility of all healthcare providers to "do no harm."

Therapeutic counseling

Another aspect of patient support services involves intervention and treatment for the consequences of infertility, or for underlying mental disturbances that could affect medical treatment. Treatment modalities of individual, couple, and group counseling provide an opportunity to assist patients in understanding and handling the emotional sequelae of infertility, identifying and developing a coping mechanism to deal with treatment, managing the effects of infertility or psychosocial history on interpersonal functioning (anxiety, depression, etc.) and on marital, sexual, and social relationships, considering the implications of ART treatment, decision making on treatment options and alternative family building, pregnancy, and parenting following treatment, and ending treatment and building a life after infertility. Group counseling has been shown to be a highly effective, cost-efficient intervention for producing positive change when education and skills training (e.g., relaxation techniques) are emphasized (31).

ART programs may provide psychological assessment and therapeutic counseling services through an infertility counselor on the staff (an employee) or on site (an independent contractor), or may choose to refer to a qualified mental health professional who works independently of the clinic. Guidelines for when to refer patients for psychological support and assistance are displayed in Table 71.1 (44).

Supportive counseling

1. Supportive counseling involves reproductive healthcare providers giving both advice (counsel) and comfort (console) to their patients. Although nurses often assume primary responsibility for patient support, it is the job of every member of the team to be empathic and sensitive to patients' needs. Services combine supportive and psychoeducational counseling, and may include a pre-IVF preparation session with an infertility counselor, which is offered as part of the treatment package.

Table 71.1 Situations in which to refer to an infertility counselor

The following situations serve as guidelines for referral for psychological assessment, counseling, and/or intervention (44):

- The use or consideration of third-party reproduction
- Psychiatric illness (past or present)
- History of pregnancy complications or loss
- Significant physical illness (past or present)
- Sexual or physical abuse (past or present)
- Conflicted gender identity, homosexuality, or bisexuality
- Chemical abuse or dependency
- Marital instability or chaotic social functioning
- Single patients
- Older patients

Symptoms

Referral to a mental health professional should also be considered when there is a change in current mental status and/or exacerbation of symptoms that are affecting normal functioning and relationships, including:

- Depression or persistent sadness and tearfulness
- High levels of anxiety or agitation
- Increased mood swings
- Obsessive–compulsive behaviors
- Strained interpersonal relationships
- Social isolation
- Loss of interest in usual activities
- Diminished ability to accomplish tasks
- Difficulty concentrating or remembering
- Difficulty making decisions
- Change in appetite, weight, or sleep patterns
- Increased use of drugs or alcohol
- Persistent feelings of pessimism, guilt, or worthlessness
- Persistent feelings of bitterness or anger
- Thoughts of or reference to death or suicide

2. Monthly support groups for IVF participants, patients considering or using donor gametes, and those with general infertility (non-ART), secondary infertility, miscarriage, and pregnancy after infertility. These groups are open-ended, of no cost to patients, and are run by a staff infertility counselor and, if needed, a nurse.
3. A monthly discussion series on infertility topics identified through a patient survey, such as adoption, donor issues, staff–patient communication, drug side effects, dealing with family and friends, decision making, marriage enhancement, and when to end treatment. These informal groups are facilitated by an infertility counselor, physician, nurse, and/or an invited guest from the community who is knowledgeable on the subject.
4. Stress management and relaxation classes taught by an infertility counselor and/or a nurse. Relaxation tapes and guided imagery tapes are also available to lend to patients for use before, during, and after retrieval and transfer.
5. Referral resources within the community for patients who request alternative approaches to help with quality of life during infertility, such as mind–body programs, yoga classes, acupuncture, and homeopathy.
6. Providing a network for patient-to-patient contact about aspects of treatment. Well-adjusted patients who have been through a procedure or have a specific diagnosis volunteer, or are asked by a staff member if they would be willing to speak one-on-one with other patients who request this contact. Common requests for contact are situations where patients have had a child via donor gametes, or who have undergone selective reduction or carried multiple pregnancies.
7. Giving each patient current information about local and national infertility support groups (e.g., RESOLVE, Inc.), such as monthly updates on meetings, support groups, living room sessions, telephone counseling, newsletters, and articles.

Information and education

Probably the most far-reaching opportunity for ART support is through patients' easy access to written information and education about the medical and psychological aspects of infertility. Patients rely heavily on the educational materials that document the processes and procedures of ART, and search out information at the clinic, through the media (TV, magazines, books, etc.), and on the internet. One study found that patients identified informational materials as their primary source of support, after talking with spouse, family, or friends (45).

Computer-based technology has become a powerful source of information, education, and support for patients. There is increasing evidence that the internet can provide an effective intervention in helping patients manage the distress of infertility (46), and receiving direction from the medical staff on reliable internet sites for information is needed (47). Social media, such as Facebook and Twitter, may become resources for support, interaction, and information for infertile patients when managed by the clinic, as well as providing a marketing tool for the practice. Multimedia methods, such as a CD with audio, video, interactive tasks, and personalized feedback, can serve as an effective psychosocial intervention (48) and should be considered by clinics as resources for patients. Finally, providing internet access to personal health records and medical data is increasingly being considered within reproductive clinics to improve patient empowerment and satisfaction with care (49,50).

Any information and treatment packets sent out to new patients should include material on the emotional aspects of infertility and on support resources available through the clinic, in the community, and via the internet. A clinic's website is also an important source of support information, and could connect to other internet resources, such as RESOLVE, for easy patient access. Examples of information and education support services from the author's program include:

1. Online, interactive webcasts (webinars) on medical and psychosocial topics of infertility (i.e., preparing for IVF, deciding on ovum donation, miscarriage, etc.).

These webinars are live and allow patients to ask questions, which are then archived on the clinic website for patients to access and review at a later time.

2. Monthly IVF and donor egg recipient preparation classes for new patients beginning a cycle. Presentations are made by a member of each treatment team—physician, embryology/laboratory, nurse, or infertility counselor—and the administrative/finance office, who discuss protocols and processes, describe treatment services, and answer questions. These classes are held in the evening, a light dinner is provided, written materials on the medical and emotional aspects of IVF or donor eggs are distributed, and the informal atmosphere allows for easy exchange with patients.
3. Ready access to pamphlets, articles, and written materials on the medical and emotional aspects of infertility, which are displayed in patient waiting areas. Ample supplies of these materials are available in the nursing, physician, and infertility counseling offices, as well as with administrative staff. For example, billing staff found that as patients were checking out from office visits they often talked about their stresses, and being able to give patients flyers on clinic support services or educational pamphlets was greatly appreciated.
4. A "fact sheet" of resources for patients with names, telephone numbers, and internet websites about clinic and community support services relating to infertility, endometriosis, primary ovarian insufficiency, polycystic ovary syndrome, adoption, pregnancy, pregnancy loss or termination, multiple gestation and parenting, and single parenting.
5. One-page "tip sheets" on topics that offer suggestions about coping with the emotional aspects of infertility (IVF, marital relationships, etc.) and "summary sheets" on medical treatments/procedures. Patient information "fact sheets" are also available through the ASRM's website (http://www.reproductivefacts.org/news-and-publications/patient-fact-sheets-and-booklets/), and can easily be downloaded and given to patients. These summary sheets are especially helpful, as the volume of information given to patients may be overwhelming, and research has shown that patients retain only a small portion of information given to them verbally.
6. A patient lending library of infertility-related books, videos, and audiotapes of instruction and information ranging from topics on sexual dysfunction and adoption to medical diagnosis and treatment of infertility.
7. Resources that can be accessed or downloaded from the clinic's website. These may include blogs and articles written by staff members on psychological and medical aspects of treatment, an "ask the expert" column for patients to write in questions, and online webcasts to present information on treatment programs and psychosocial issues of infertility.

Clinic administration

The manner in which an ART program is administered, along with the physical environment of the clinic, affects both patient stress levels and their perception of support. An esthetically pleasing, clean, well-maintained office staffed by friendly, professionally dressed, well-trained people goes a long way in communicating an impression of professional competence, caring, and confidence. One study found that patient satisfaction can be improved with organizational shifts, when the patient was assigned a primary physician as well as being seen by a fertility-trained nurse (50). Ways in which the author's program provides support through clinic administration include:

1. Patient waiting areas, with access to reading materials, water, telephones, and restrooms. During weekend monitoring, a continental breakfast is available for patients in this area while patients wait to see the physician. (If a clinic shares space with an obstetrics and gynecology department, sensitivity needs to be considered and reasonable efforts made to separate pregnant patients and small children from infertility patients by adjusting appointments/schedules and/or seating arrangements.)
2. Private rooms where nurses or other clinical staff can instruct or consult with patients.
3. Private sections where billing and scheduling issues can be discussed by administrative staff with patients in a confidential manner.
4. A quiet, secure "donor room" for men to give semen samples, with erotic magazines/materials, a video player, and a comfortable chair or bed.
5. Private recovery areas after egg retrieval and embryo transfer with safe places to store belongings, a television/video player or music, and a comfortable chair for husbands.
6. Soothing, calming background music piped throughout the office.
7. An annual or biannual "baby party" for patients to come back with their children and celebrate with staff.
8. Miscarriage/pregnancy loss cards sent by the clinical staff when it is learned that, after a patient has been discharged from care, a pregnancy has been lost.
9. Primary care nursing, where a patient is assigned to one nurse, facilitating better continuity and coordination of treatment.
10. A staff member "patient advocate/ombudsman," who patients may talk to when they perceive a problem with their care, or other conflicts with the clinic that cannot be resolved.
11. Patient surveys, suggestion boxes, and written feedback, which encourage open communication regarding satisfaction, thoughts on improving care or services, and constructive criticism.
12. In-service training of all staff on the emotional needs of infertility patients, communication skills, stress management techniques, and on strategies to deal with difficult, demanding patients.
13. Staff support offering confidential assistance, direction, and referral for personal problems and professional burnout by the staff mental health professional or through an employee assistance program. Ultimately,

happy staff members are productive workers who give the best support and service to patients.

RESULTS

Although most patients undergoing ART cycles are well adjusted and will cope adequately with the process, all will benefit from, and indeed need, emotional support during treatment. Numerous studies show that most patients believe psychosocial counseling is beneficial and that they would avail themselves of it were it offered during treatment (19,51,52). While a minority of patients experience significant emotional distress and use formal counseling services, the vast majority of those who use formal counseling report having found it helpful (19).

The efficiency of psychosocial interventions impacting mental health issues (i.e., depression, anxiety, and distress) and pregnancy rates during infertility is still being debated. A number of meta-analyses and systematic reviews have been reported over the years with mixed results (31,53). However, a recent study by Frederiksen and associates on research published between 1978 and 2014 suggests that psychosocial interventions for couples during infertility treatment, in particular cognitive–behavioral therapy, are effective at both reducing psychological distress and at improving pregnancy rates (54).

There is a growing body of research examining the burden treatment places on patients both physically and psychologically (7,55). While patient-centeredness is increasingly consider to be fundamental to quality care, medical professionals often misjudge themselves in how their patients experience their care and interactions (56). The result may be that patients discontinue treatment because the emotional burden is too great. Thus, factors within the patient, the clinic, and the medical treatment contribute to this decision, and interventions must be addressed on all levels (57).

This information, coupled with the high dropout rates in ART programs, most likely due to psychological reasons (58–60), suggests that IVF programs need to provide better and more comprehensive psychosocial support services. Studies have indicated that even when cost is not a factor in pursuing treatment, over half of patients drop out of treatment before depleting their entitled insurance benefits (61–63). Cross-culturally, the most common reason given for treatment termination is psychological burden and distress (55,61–65). Providing integrated psychological support services may be an important step in diminishing a patient's depression and anxiety, lowering dropout rates, and possibly even increasing pregnancy rates—the goal of all fertility programs (59,66,67). It may also increase patients' overall sense of satisfaction with care, even when pregnancy is not achieved (68).

Simple strategies for managing patients can help a great deal (7,57). Olivius and colleagues (60) found that ease in contacting the clinic or clinician by telephone, seeing the same doctor during treatment, and receiving sufficient oral and written information about treatment and complications helped with patient distress. At the very least,

Table 71.2 Strategies for assisted reproduction technology patient support

Before

- Educational classes presented by each member of the treatment team on IVF
- Pre-treatment counseling session with a mental health professional/infertility counselor
- Psychosocial preparation and assessment of gamete donors, recipients, and surrogates with a mental health professional/infertility counselor
- Extensive written materials available and distributed on the medical, emotional, and financial aspects of ART
- Educational videos and web-based support on the medical and emotional aspects of infertility and ART
- Support groups
- Stress management, relaxation, and guided imagery classes and CD/DVDs
- Resource lists of community support services, including RESOLVE, Inc.

During

- Access to the mental health professionals/infertility counselors and other team members
- Telephone support with a primary care nurse
- If a patient has met with an infertility counselor before starting the cycle, a brief visit in the OR on retrieval and/or transfer day
- Stress management, relaxation, and guided imagery classes and audio tapes
- Computer-based technology, including a clinic website with resource materials and interactive social media (e.g., Facebook and Twitter); online educational webinars; written materials identifying reliable internet sites for information and support; and educational CD-ROMs
- Support groups

After

- Psychosocial follow-up after a failed cycle or pregnancy loss
- Decision-making counseling regarding alternative therapies or ending treatment
- Counseling on alternative family building through adoption or third-party reproduction
- Counseling and support for the decision to remain child-free after infertility
- Counseling and preparation for multiple pregnancy, including selective reduction
- Counseling and follow-up for pregnancy after infertility, including support groups
- Counseling and follow-up for issues in parenting after infertility, including families created through donor gametes
- Support groups
- Patient feedback survey

Abbreviations: ART, assisted reproduction technology; IVF, *in vitro* fertilization; OR, operating room.

written materials and educational resources on the medical and psychosocial aspects of infertility need to be readily available and given to patients by their programs. Further, the more holistically a patient is handled—supported medically and emotionally—the more likely she/he is to be treatment compliant and satisfied with care, regardless of the outcome of treatment. In fact, the true mark of success of a program may be in the ability of the team to help patients feel that they, the patients, have done their best when treatment has failed (see Table 71.2 for a summary of strategies for ART patient support).

FUTURE DIRECTION

Reproductive medicine will continue to change as advancing technology presents increasingly complex options and choices for patients. As reproductive technology continues to advance and push the boundaries of social, psychological, religious, and ethical acceptance, the need for comprehensive support services for ART patients will continue to grow. Patients will request a more holistic approach to medical treatment, where their bodies and their emotions are treated with equal importance. As "educated consumers," ART patients will search for the most effective and comprehensive care program, often choosing a practice on the basis of whether psychological support services are integrated into treatment. There will continue to be a growing need for the specialized clinical skills and services of mental health professionals trained in infertility counseling to provide this assistance to patients and staff. ART programs that have the foresight to integrate comprehensive support services with specialized mental health professionals as part of the treatment team will succeed.

CONCLUSION

Infertility is an emotionally exhausting, psychologically demanding experience for patients and, at times, their caregivers. Since ART cycles are considered the most stressful of all infertility treatments, patients who undergo them need as much support psychologically as they do medically from their clinical team. Specialized support services are needed for the psychosocial preparation, assessment, and treatment of patients who are faced with the unique issues associated with and/or the consequences of assisted reproduction. Experienced mental health professionals trained in infertility counseling must provide these specialized psychological services as part of, or in close collaboration with, the treatment team (69). Finally, patient support is the responsibility of all employees of an ART program, and staff must be knowledgeable about and sensitive to the emotional needs of their patients.

REFERENCES

1. Menning BE. The emotional needs of infertile couples. *Fertil Steril* 1980; 34: 313–9.
2. Mahlstedt PP. The psychological component of infertility. *Fertil Steril* 1985; 43: 335–46.
3. Stanton AL, Dunkel-Schetter C. Psychological adjustment to infertility. In: *Infertility: Perspectives from Stress and Coping Research*. Stanton AL, Dunkel Schetter C (eds). New York, NY: Plenum Press, 1991, pp. 3–16.
4. Burns LH, Covington SN. Psychology of infertility. In: *Infertility Counseling: A Comprehensive Handbook for Clinicians*, 2nd edn. Covington SN, Burns LH (eds). New York, NY: Cambridge University Press, 2006, pp. 1–19.
5. Greil AL, Slauson-Blevins K, McQuillan J. The experience of infertility: A review of recent literature. *Soc Health Illn* 2010; 32: 140–62.
6. Greil A. Infertility and psychological distress: A critical review of the literature. *Soc Sci Med* 1997; 45: 1679–704.
7. Gameiro S, Boivin J, Domar A. Optimal *in vitro* fertilization in 2020 should reduce treatment burden and enhance care delivery for patients and staff. *Fertil Steril* 2013; 100: 302–9.
8. Gameiro S, Boivin J, Dancet E, et al. ESHRE guideline: Routine psychosocial care in infertility and medical assisted reproduction—A guide for fertility staff. *Hum Reprod* 2015; 30: 2476–85.
9. Kopitzke EJ, Berg BJ, Wilson JF, Owen D. Physical and emotional stress associated with components of the infertility investigation: Professional and patient perspectives. *Fertil Steril* 1991; 55: 1137–43.
10. Boivin J, Takefman J. The impact of the *in vitro* fertilization–embryo transfer (IVF–ET) process on emotional, physical, and relational variables. *Hum Reprod* 1996; 11: 903–7.
11. Connolly KJ, Edelmann RJ, Bartlett H et al. An evaluation of counselling for couples undergoing treatment for *in vitro* fertilization. *Hum Reprod* 1993; 8: 1332–8.
12. Litt MD, Tennen H, Afflect G, Klock S. Coping and cognitive factors in adaptation in *in vitro* fertilization failure. *J Behav Med* 1992; 15: 171–87.
13. Slade P, Emery J, Lieberman BA. A prospective, longitudinal study of emotions and relationships in *in vitro* fertilization treatment. *Hum Reprod* 1997; 12: 183–90.
14. Connolly KJ, Edelmann RJ, Cooke ID, Robson J. The impact of infertility on psychological functioning. *J Psychosom Res* 1992; 36: 459–68.
15. Paulson JD, Haarmann BS, Salerno RL, Asmar P. An investigation of the relationship between emotional maladjustment and infertility. *Fertil Steril* 1988; 49: 258–62.
16. Downey J, Husami N, Yingling S et al. Mood disorders, psychiatric symptoms and distress in women presenting for infertility evaluation. *Fertil Steril* 1989; 52: 425–32.
17. Edelmann RJ, Connolly KJ, Cooke ID, Robson J. Psychogenetic infertility: Some findings. *J Psychosom Obstet Gynecol* 1991; 12: 163–8.

18. Stanton AL, Danoff-Burg S. Selected issues in women's reproductive health: Psychological perspectives. In: *The Psychology of Women's Health: Progress and Challenges in Research and Application*. Stanton AL, Gallant SJ (eds). Washington, DC: American Psychological Association, 1996, pp. 261–305.
19. Boivin J. Is there too much emphasis on psychological counselling for infertile patients? *J Assist Reprod Genet* 1997; 14: 184–6.
20. Freeman EW, Rickels K, Tausig J et al. Emotional and psychosocial factors in follow-up of women after IVF–ET treatment. *Acta Obstet Gynecol Scand* 1987; 66: 517–21.
21. Newton CR, Hearn MT, Yuzpe AA. Psychological assessment and follow-up after *in vitro* fertilization: Assessing the impact of failure. *Fertil Steril* 1990; 54: 879–86.
22. Berg BJ, Wilson JF. Psychological functioning across stages of treatment for infertility. *J Behav Med* 1991; 14: 11–26.
23. Lalos A, Lalos O, von Schoultz B. The psychosocial impact of infertility two years after completed surgical treatment. *Acta Obstet Gynecol Scand* 1985; 65: 599–604.
24. Newton CR, Sherrard W, Glavac I. The Fertility Problem Inventory: Measuring perceived infertility-related stress. *Fertil Steril* 1999; 72: 54–62.
25. Boivin J, Shoog-Svanberg A, Andersson L et al. Distress level in men undergoing intracytoplasmic sperm injection versus *in vitro* fertilization. *Hum Reprod* 1998; 13: 1403–6.
26. Nachtigall RD, Becker G, Wozny M. The effects of gender-specific diagnosis on men's and women's response to infertility. *Fertil Steril* 1992; 57: 113–21.
27. Hynes GJ, Callan VJ, Terry DJ et al. The psychological well-being of infertile women after a failed IVF attempt: The effects of coping. *Br J Med Psychol* 1992; 65: 269–78.
28. Petok WD. The psychology of gender-specific infertility diagnoses. In: *Infertility Counseling: A Comprehensive Handbook for Clinicians*, 2nd edn. Covington SN, Burns LH (eds). New York, NY: Cambridge University Press, 2006, pp. 37–60.
29. Agostini F, Monti F, De Pascalis L et al. Psychosocial support for infertile couples during assisted reproductive technology treatment. *Fertil Steril* 2011; 95: 707–10.
30. Mindes EJ, Ingram KM, Kliewer W et al. Longitudinal analysis of the relationship between unsupportive social interactions and psychological adjustment among women with fertility problems. *Soc Sci Med* 2003; 56: 165–80.
31. Boivin J. A review of psychosocial interventions in infertility. *Soc Sci Med* 2003; 57: 2325–41.
32. Cohen SJ, Kessler RC, Underwood GL. Strategies for measuring stress in studies of psychiatric and physical disorders. In: *Measuring Stress: A Guide for Health and Social Scientist*. Cohen SJ, Kessler RC, Underwood GL (eds). New York, NY: Oxford University Press, 1995, pp. 3–25.
33. Boivin J, Takefman J. Stress level across stages of *in vitro* fertilization in subsequently pregnant and non-pregnant women. *Fertil Steril* 1995; 64: 802–10.
34. Boivin J, Takefman J, Braverman A. The Fertility Quality of Life (FertiQoL) tool: Development and general psychometric properties. *Fertil Steril* 2011; 96: 409–15.
35. Covington SN. The role of the mental health professional in reproductive medicine. *Fertil Steril* 1995; 64: 895–7.
36. Blyth E. Guidelines for infertility counselling in different countries: Is there an emerging trend? *Hum Reprod* 2012; 27: 2046–57.
37. Human Fertilisation and Embryology Authority. *Code of Practice*, 2nd edn. London, U.K.: HFEA, 1995.
38. Hernon M, Harris CP, Elstein M et al. Review of organized support network for infertility patients in licensed units in the UK. *Hum Reprod* 1995; 10: 960–4.
39. The Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Recommendations for gamete and embryo donation: A committee opinion. *Fertil Steril* 2013; 99: 47–62.
40. Klock SC, Maier D. Guidelines for the provision of psychological evaluations for infertile patients at the University of Connecticut Health Center. *Fertil Steril* 1991; 56: 680–5.
41. Klock SC, Covington SN. Minnesota Multiphasic Personality Inventory (MMPI-2) profiles in the assessment of ovum donors. *Fertil Steril* 2010; 94: 1684–8.
42. Klock SC. Results of the Minesota Multiphasic Personality Inventory-2 among gestational surrogacy candidates. *Int J Gynecol Obstet* 2015; 130: 257–60.
43. The Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Recommendations for practices utilizing gestational carriers: A committee opinion. *Fertil Steril* 2015; 103: e1–8.
44. Burns LH. An overview of the psychology of infertility. *Infertil Reprod Med Clin North Am* 1993; 4: 433–54.
45. Boivin J, Scanlan LC, Walker SM. Why are infertile patients not using psychosocial counselling? *Hum Reprod* 1999; 14: 1384–91.
46. Cousineau TM, Green TC, Corsini E et al. Online psychoeducational support for infertile women: A randomized controlled trail. *Hum Reprod* 2008; 23: 554–66.
47. Kahlor L, Mackert M. Perceptions of infertility information and support sources among female patients who access the Internet. *Fertil Steril* 2009; 91: 83–90.

48. Cousineau TM, Lord SE, Seibring AR et al. A multimedia psychosocial support program for couples receiving infertility treatment: A feasibility study. *Fertil Steril* 2004; 81: 532–8.
49. Tuil WS, Verhaak CM, Braat D, de Vries Robbe P, Kremer J. Empowering patients undergoing *in vitro* fertilization by providing Internet access to medical data. *Fertil Steril* 2007; 88: 361–8.
50. Van Emple I, Hermens R, Akkermans R et al. Organization determinants of patient-centered fertility care: A multilevel analysis. *Fertil Steril* 2011; 95: 513–19.
51. Baram D, Tourtelot E, Muechler E et al. Psychosocial adjustment following unsuccessful *in vitro* fertilization. *J Psychosom Obstet Gynecol* 1988; 9: 181–90.
52. Mazure CM, Greenfeld DA. Psychological studies of *in vitro* fertilization/embryo transfer participants. *J In Vitro Fert Embryo Transf* 1989; 6: 242–56.
53. Hammerli K, Hansjorg Z, Barth J. The efficacy of psychological interventions for infertile patients: A meta-analysis examining mental health and pregnancy rate. *Hum Reprod Update* 2009; 15: 279–95.
54. Frederiksen Y, Farver-Vestergaard I, Skovgard NG, Ingerslev HJ, Zachariae R. Efficacy of psychosocial interventions for psychological and pregnancy outcomes in infertility women and men: A systematic review and meta-analysis. *BMJ Open* 2015; 5: e006592.
55. Gamerio S, Boivin J, Peronace LA, Verhaak CM. Why do patients discontinue treatment? A systemic review of reason and predictors of discontinuation in fertility treatment. *Hum Reprod Update* 2012; 18: 652–69.
56. Aarts WM, Faber MJ, van Empel IWH, Scheenjes E, Nelen WLDM, Kremer JAM. Professionals' perceptions of the patients' experiences with fertility care. *Hum Reprod* 2011; 26: 1119–27.
57. Boivin J, Domar AD, Shapiro DB, Wischmann T, Fauser BC, Verhaak CM. Tackling burden in ART: An integrated approach for medical staff. *Hum Reprod* 2012; 27: 941–50.
58. Domar AD. Impact of psychological factors on dropout rates in insured infertility patients. *Fertil Steril* 2004; 81: 271–3.
59. Penzias AS. When and why does the dream die? Or does it? *Fertil Steril* 2004; 81: 274–5.
60. Olivius C, Friden B, Borg G, Bergh C. Why do couples discontinue *in vitro* fertilization treatment? A cohort study. *Fertil Steril* 2004; 81: 258–61.
61. Smeenk JMJ, Verhaak CM, Stolwijk AM, Kremer JA, Braat DD. Reasons for dropout in an *in vitro* fertilization/intracytoplasmic sperm injection program. *Fertil Steril* 2004; 81: 262–8.
62. Malcolm CE, Cumming DC. Follow-up of infertile couples who dropped out of a specialist fertility clinic. *Fertil Steril* 2004; 81: 269–70.
63. Brandes M, van der Steen J, Bokdam S, et al. When and why do subfertile couples discontinue their fertility care? *Hum Reprod* 2009; 24: 3127–35.
64. Domar AD, Smith Conboy L, Iannone M, Alper M. A prospective investigation into the reasons why insured United States patients drop out of *in vitro* fertilization treatment. *Fertil Steril* 2010; 94: 1457–9.
65. Terzioglu F. Investigation into the effectiveness of counseling on assisted reproductive techniques in Turkey. *J Psychosom Obstet Gynaecol* 2001; 22: 133–41.
66. Smeenk JMJ, Verhaak CM, Stolwijk AM, Kremer JAM, Braat DDM. Psychological interference in *in vitro* fertilization treatment. *Fertil Steril* 2004; 81: 277.
67. Mourad SM, Nelen W, Akkermans R et al. Determinants of patients' experiences and satisfaction with fertility care. *Fertil Steril* 2010; 94: 1254–60.
68. Olivius C, Friden B, Borg G, Bergh C. Psychological aspects of discontinuation of *in vitro* fertilization treatment. *Fertil Steril* 2004; 81: 276.
69. Covington SN, Adamson GD. Collaborative reproductive healthcare model. In: *Fertility Counseling: Clinical Guide and Case Studies*. Covington SN (ed.). Cambridge, U.K.: Cambridge University Press, 2015, pp. 1–32.

The relationship between stress and *in vitro* fertilization outcome

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OVERVIEW

The impact of patient stress on the process of *in vitro* fertilization (IVF) is a complex and multifaceted interplay between the mind and the body. The old model of psychogenic causation (e.g., the Freudian approach that speaks of the woman's fear of impregnation or motherhood) has long been supplanted by the careful exploration of the interplay of stress with the endocrine system. Considerations of dispositional characterological factors such as optimism (1) or happiness (2) have also led to the hypothesis that such factors may play a role in treatment outcome.

Many studies have considered the relationship between stress (or other psychosocial variables) and its effect on pregnancy outcome per treatment cycle (1–5). The results have been mixed, and have often been confounding factors when the concepts of stress reduction and support as agents of cause or intervention in infertility and pregnancy outcomes are considered (6–9). In a comprehensive review of psychosocial interventions in infertility, Boivin noted that "analysis of these studies showed that psychosocial interventions were more effective in reducing negative affect than in changing interpersonal functioning," and that pregnancy rates were not likely to be affected by these interventions (10). Boivin also noted that counseling interventions that focused on affective expression regarding the emotional aspects of infertility were significantly less effective at producing a positive change than were education and skills training. Psychosocial intervention has looked at pregnancy and implantation rates (11,12), but not at treatment persistence and retention.

Some of the major confounds that occur while considering psychological distress and pregnancy outcomes include: the relationship between distress and anxiety/depression; the influences of diagnosis or the influence of information or attitudes of the medical team; the habituation effects of chronic stress; other life stressors; coping styles; and baseline psychological issues. In Domar et al.'s (12) review of the association of psychological distress and pregnancy outcomes, the authors conclude: "Women undergoing [assisted reproduction technology (ART)] procedures report significant levels of negative psychological symptoms, both prior to beginning and especially after experiencing an unsuccessful cycle. Most of the research conducted with women undergoing ART treatment supports the theory that emotional distress is associated with treatment success." However, the authors note the limitations of many of these studies, including the lack of control groups or small sample sizes.

More recently, studies have turned their attention away from the tremendously complex relationship between stress or depression and pregnancy outcome, focusing instead on the causes behind the discontinuation of treatment and treatment perseverance. These studies clearly demonstrate that treatment dropout is associated with psychological factors (1–5,13,14). Even with the high pregnancy rates associated with IVF and cumulatively over cycles, the ability for a patient to remain in treatment (i.e., treatment persistence) gives a patient her best opportunity for achieving a pregnancy. Treatment persistence will allow a patient to optimize her biological potential.

A broader question remains as to why patients do not use psychological counseling and support to manage their stress (15). Increasingly, attention is turning to integrating support for the emotional aspects of treatment and stress management through the medical staff providing theses resources (16) and this concept of integrated care in the cycle for reducing the burden of treatment is considered best practice (17).

RECENT RESEARCH

In their 2010 review, Boivin et al. (18) performed a meta-analysis on prospective psychosocial studies that examined the association between pretreatment emotional distress, defined as anxiety or depression, and pregnancy. The subjects received one cycle of ART; the effect size was determined by comparing those who achieved a pregnancy and those who did not. The authors found that there was no association between pretreatment emotional distress and pregnancy outcome. Limitations of the meta-analysis were due to the heterogeneity of the study designs.

Other research identifies infertility as a stressful event and has looked at its impact on the dyadic relationship. Some studies (19–21) have found that the infertility experience can strengthen a couple's relationship, and a 2011 study (22) explored whether there can be relational benefit from the infertility journey. In this prospective study, couples were followed for a five-year period of unsuccessful treatments. Women experienced a greater percentage of high levels of marital benefit. Different coping strategies were employed, but the cause of infertility did not significantly contribute. Overall, a third of the couples experienced a longitudinal positive effect on the marital relationship over a five-year period.

Interventional approaches have ranged from targeting specific stressful times in the treatment cycle, such as the waiting period (23–26), in order to considering the effect of positive reappraisal as a coping strategy (27,28). Researchers are now considering quality of life, and

measures specific to infertility, such as the Fertility Quality of Life (29), are being incorporated into many studies.

Researchers have also begun to look at stress via biomarkers and how such stress affects fertility (30). In a longitudinal prospective study, researchers assessed salivary cortisol and amylase levels and their relationship to female fecundity. The study found a statistically significant relationship for a lowered probability of conception during the fertile follicular window as the woman's salivary amylase concentrations rise, but offered no explanation of what the mechanism of impact is. This new research direction for understanding how stress impacts the body and fecundity may offer new directions for interventions for the stress affecting infertile individuals.

SOURCES OF STRESS

The experience of infertility transcends borders and socioeconomic status. Nearly every society throughout history has produced art and literature that has spoken of the desire for offspring and the pain (or shame) that ensues when pregnancy does not occur. For many individuals, infertility is among the first life crises that they may face, having previously dealt with pregnancy's counterpoint (the prevention of pregnancy) through vigilant contraception. Messages from friends, family, and society reinforce the notion that fertility is within an individual's control. Public figures or movie stars who pursue infertility treatment with success at ages well into their 40s and 50s only contribute to some individual's incredulity that there may be a fertility issue with either or both partners. The inevitable fact that age may play a substantive role in fertility may be lost within these competing social messages.

The stress of infertility is felt in many ways by men and women and may change over time and as individuals experience failures in the cycles. In a groundbreaking 1985 study, Freeman and colleagues (18) evaluated 200 couples experiencing infertility. Half the women and 15% of the men endorsed the notion that infertility was the worst event of their lives. In a more recent study, Verhaak et al. (32) surveyed 148 IVF patients along with their 71 partners and measured numerous psychological factors (anxiety, depression, personality characteristics, the meaning of fertility problems, coping, marital relationship, and social support) at pre-treatment, post-treatment, and final treatment stages. Six months later, participants were assessed for anxiety and depression. Among women, anxiety and depression increased after unsuccessful treatment and decreased after successful treatment. There was no change in anxiety and depression levels for men after either successful or unsuccessful treatment.

Some of the major categories of stress that have been traditionally recognized are addressed below, but none of these factors unequivocally demonstrate a clear role in pregnancy outcome. Without question, entering into medical treatment for fertility places the individual and couple into a "patient" mode. The stress of being in medical care alone can be a psychological burden for some patients. Contributing to this stress may be the language of infertility to which patients are regularly exposed, such as the use of pejorative terms like "failed cycle," "incompetent cervix," "shooting blanks," and "advanced maternal age," which may impact patients' self-esteem and body image, and which may serve to reinforce the potentially stress-inducing notion that the individual is a patient with medical problems.

Self-esteem and body image

For many individuals, being in "patient" mode means that their bodies are not working correctly; this circumstance can take a toll on their self-esteem as well as on their body image. The disappointment in their own bodies felt by the patient may be exacerbated by their unconscious belief that fertility should "come naturally," that it should be in their exclusive control. The stated message by friends, family, and others to "just relax and you'll get pregnant" serves to reinforce the notion that individuals are failing if they cannot make their bodies work correctly. Doubts may arise in a patient's mind regarding his or her sense of masculinity or femininity. For example, a man who has low motility may emotionally confuse the diagnosis with personal feelings of the loss of virility. The evaluation and scrutiny of the intimate and private areas of a relationship contribute to the pressure of being evaluated for adequacy (33).

Sexuality and intimacy

Sexuality can also be affected by infertility. Sexual enjoyment has been found to be lessened during certain required tests, such as a post-coital test (34). Sexuality may be linked with procreation during fertility treatment and is often divorced from recreation or intimacy. Timed intercourse can add to the burden of feeling measured, pressured, and stressed. Some women report feeling distanced from their bodies because of procedures or timed intercourse; men report feeling performance pressure with timed intercourse. Adding to these stressors are the feelings associated with unhappiness, anger, or disappointment in one's own body. None of these feelings or stressors enhances the sense of being a sexual person with sexual desires. This further adds to the burden on the relationship.

Relationship with partner

Research has demonstrated that men and women experience, and are affected by, infertility in different ways, and that their coping strategies may differ as well (35). Repeated treatment failure may take its toll on the relationship. Partners may disagree about the timing of when to seek evaluation or pursuit of treatment; couples may also need to navigate differing levels of optimism about treatment outcome. Congruence can depend upon the degree to which the partners perceive the severity of the stress of the infertility; lack of congruence may in itself add to stress within the relationship.

Partners may adopt different coping strategies that may be intra-personally effective, but may add to the relationship burden. In a study of German men, researchers found that men tended to suppress their emotions and had more difficulty communicating and identifying their emotions

(36). Take, for example, the situation in which one partner feels great relief by being able to process the thoughts and feelings that arise and where the other finds discussion stressful and anxiety producing. The very coping strategies that bring personal relief only add to the relationship stress where one partner may feel "shut down" and the other partner feels unfairly burdened depending on which coping strategy is utilized. Couples may also experience lack of congruence with regard to disclosure or nondisclosure of their fertility issues. If one partner has been diagnosed, s/he may feel stigmatized by the disclosure; the other partner may feel burdened by the demands of not sharing the information with important support persons.

Overall, men with infertility tend to somaticize their reactions; this has been observed across cultures. Some cultures are protective of the stress infertility places on men because only female factor infertility is recognized in those cultures. In Western cultures, men may repress their feelings related to the infertility; this repression may correlate with sexual dysfunction during treatment (37).

The burden of infertility and its treatment

Many different emotions have been identified as arising from infertility: anger, denial, grief, guilt, anxiety, and depression. The literature remains equivocal about the impact that the length of diagnosis has on psychological burden and adaptation. Issues from the past may weigh emotionally on the individual, such as previous pregnancy termination(s) or sexually transmitted diseases that may have contributed to the fertility problem. The constant cycle of hope and disappointment has led to the description of infertility as an "emotional roller coaster."

Treatment for infertility places very concrete demands on the individual and couple, which adds to the stress and burden. For some, the time demands of physician consultation, monitoring, inseminations, or IVF may present real problems on a patient's demanding job or upon an individual who is juggling childcare. Women must choose between being open or private about their treatment, not just with those in their personal lives, but also within their careers; too many unexplained absences from work may result in a poor performance review. Many individuals and couples may also feel that they are in limbo, foregoing new jobs or promotions due to concerns about access to treatment or even financial coverage (depending on the country).

For all, the decision about being open or private about their fertility situation can frequently arise from simple questions (e.g., "Do you have children?"), often asked innocently in social situations. Social situations are sometimes avoided to escape painful stimuli such as encountering pregnant women or having contact with babies. This social isolation can also add to feelings of being different or of being cut off from the usual support structures that the individual or couple typically depends upon. Families may fail to understand why the individual does not participate in expected family events such as visits to the maternity ward after a delivery, attending baby showers, or even attending holiday events.

OVERVIEW OF STRESS AND PREGNANCY OUTCOME DATA

A primary consideration of the stress and pregnancy outcome inquiry must focus on whether stress is causative of factors that would prohibit pregnancy or whether it is the diagnosis and/or treatment of infertility that causes stress. This consideration is further complicated by the issue of how stress is defined by researchers and mediated by patients. Studies that have addressed stress as it is activated by the hypothalamic–pituitary–adrenal axis have been unable to clearly delineate the exact pathways or mechanisms (38–41).

Patients are bombarded with the adage to "just relax and you'll get pregnant," which leads to the integration of the assumption that stress has an immediate and direct impact upon their fertility. Despite the conflicting literature, it is generally concluded that stress does have an impact on the body (10,11). Stress also has an impact on energy level, optimism, patience, and perseverance. Stress is also mediated by personal coping styles. For example, one person's reaction to a very stressful event, such as public speaking, may elicit a large stress response. Yet, the intrapersonal experience of that stress may be energizing and allow the individual to feel that s/he is performing better. In a different study (42), the authors concluded that infertile women have a different personality profile and that their stress levels (as measured by their prolactin and cortisol levels) were elevated compared to the controls.

Coping with stress may ultimately provide assistance to conception through stress reduction. Developing more effective coping strategies helps people reduce treatment termination, thereby allowing patients to truly maximize their biological potential. Improved coping strategies may also reduce relationship stress and improve communication, leading to more congruence between partners in pursuing treatment. Stress reduction should also lead to an improved sense of well-being (10). The relationship between stress and outcome is multifactorial—both complicated and curvilinear.

Take, for example, the individual who experiences anxiety during the waiting period between insemination and the pregnancy test. If the anxiety increases over time, the patient's desire to avoid the distress may grow greater than her desire to achieve pregnancy. Alternatively, the negative experience of anxiety may lead to avoidance behaviors with treatment tasks and reduce the efficacy of treatment, such as missed monitoring appointments or poor timing with coitus or medication.

Another conflict that may prevent good coping strategies is when an individual's belief conflicts with his or her needs. Dysfunctional beliefs lead to poor choices in coping strategies. For example, consider the patient who believes that medical intervention is "unnatural." This belief may lead that person to pursue treatment slowly and, for many medical reasons, this slowness may be costly. A 39-year-old woman who operates on the belief that medical intervention is unnatural may delay more aggressive treatment for a critical year or longer, thereby missing more optimal fertility opportunities. In another example, if a male partner believes

that conception can take place without a doctor's intervention and his partner is ready to pursue treatment, conflict can quickly arise and tax the couple's communication skills.

Cognitive therapy

Cognitive therapy is an effective tool for understanding how stress can arise when a person's beliefs are dysfunctional. The formulation of the patient's dysfunction is based on his or her internal experiences and how those experiences are distorted through negative beliefs, assumptions, inferences, and conclusions. Change is mediated by the task of examining the accuracy of these beliefs (43). In cognitive therapy, the therapeutic relationship takes a "back seat" as compared to a psychodynamic approach. It is the empirical investigation of these internal experiences and the opportunity to test the automatic thoughts, assumptions, and negative beliefs that yield the opportunity to correct the faulty dysfunctional constructs (44). It is the ability to change the person's underlying cognitive structures that will in turn change his or her affective state and pattern of behavior. Behavioral techniques and homework help to elicit cognitions that contribute to or cause problematic behavior and also help the patients test their maladaptive cognitions and assumptions, thus mobilizing the patients into constructive activities and enabling them to develop better coping strategies (18).

In contrast to the psychoanalytic approach (which works by making the patient conscious of his or her unconscious past), cognitive therapy identifies current thinking and behavior (45). For example, the combination of helping the patient understand the narcissistic injury of his or her infertility, as well as its impact on his or her interpersonal relationships and life planning, is critical. An individual may feel stress or inadequacy when her belief that "I cannot be a real woman unless I get pregnant" leads her to avoid interpersonal relationships in order to escape the painful feelings that arise from feeling isolated from the fertile world.

In addition, the recognition, as well as differentiation, of infertility as a crisis leads to stress. For some clients, infertility awakens or aggravates long-term issues in their lives, such as anxiety about intimacy, poor communication skills, etc. The cyclic nature of infertility treatment creates the feeling in the patient of an immediate infertility crisis, rather than the patient identifying it as a chronic condition. In many situations, a woman may present as if she has persistent depressive symptoms, but in reality these symptoms remit during the two weeks of the follicular stage of her cycle. The stress may be chronic in that it exists all the time, but the woman experiences it intensely during the waiting period post-ovulation.

Understanding that feelings of worthlessness, purposelessness, poor self-esteem, poor body image, isolation, and withdrawal (because of painful stimuli relating to fertility), among others, are inherent in the infertility experience is important for a thorough understanding of how change in the individual's reactions can be made by identifying these dysfunctional cognitions and exchanging them for functional ones. For example, a woman who has always struggled with body image issues may find that infertility exacerbates these feelings; educating and disentangling the issues with the patient by identifying the dysfunctional thoughts gives her the opportunity to diminish the emotional impact and substitute other thoughts and behaviors. The stresses of infertility arise from many sources. Below are some examples of how dysfunctional thoughts are identified and responded to within a cognitive therapy model.

- *All-or-nothing thinking:*
  - I can never feel like a real woman if I cannot be pregnant; or
  - My ability to gestate a pregnancy is a small part of my femininity.
- *Overgeneralization:*
  - Everyone will think differently of my child because of the gestational carrier; or
  - Some people may be curious about the gestational carrier pregnancy; or
  - My child will still have the same genetics as it would, had I carried the pregnancy.
- *Selective negative focus:*
  - I cannot care for my own child *in utero*; or
  - I can care for my child by selecting the best gestational carrier and building a positive working relationship.
- *Disqualifying the positive:*
  - I will never carry my own baby; or
  - I will miss the pregnancy experience but I have the rest of my child's life to experience; or
  - I will not carry a pregnancy but I can still have my own genetic child.
- *Arbitrary inference:*
  - People think I'm selfish because I do not have any children; or
  - People simply think we have not chosen to start our family yet.
- *Emotional reasoning:*
  - My child may have very different feelings and thoughts from mine and is growing up in a world where families are created in many different ways.
- *"Should" statements:*
  - I should not feel sad, therefore I am not handling this well; or
  - It is alright to feel sad, and handling this event means working through the sadness.

Behavioral medicine

Behavioral medicine offers many strategies for coping with and managing stress. Employing a variety of strategies, the intervention will focus on introducing techniques such as cognitive–behavioral strategies, relaxation techniques, and guided imagery. Patients also consider how nutrition, acupuncture, massage therapy, and yoga may effectively manage their stress. Another potential aspect of infertility stress management involves communication training in that patients communicate more effectively with the medical professionals involved in their care. Better communication increases the ability to receive and understand

information as well as allows the patients to feel that they can effectively negotiate meeting their needs.

COMPLEMENTARY ALTERNATIVE MEDICINE: ACUPUNCTURE AND OTHER ALTERNATIVES

Infertility treatment has come a long way since the days when psychogenic reasoning placed the blame for infertility on infertile women themselves. Modern medicine has begun to consider the mind–body connection in the treatment of infertility. Hotly and passionately debated within the mental health and medical professional communities, the mind–body connection is sometimes considered an entity or process with a commonly understood definition when, in fact, the mind–body connection is still more conjecture than fact. Yet, in a recent study, when asked to what extent religion and spirituality influenced patients' health, 56% responded "much or very much," yet only 6% believed that it changed "hard" medical outcomes. With regard to ways of coping with health issues, 75% felt that religion and spirituality helped patients cope, and 75% felt it gave patients a positive state of mind (46).

It is estimated that, in the U.S.A. alone, complementary and alternative medicine (CAM) is utilized by at least a third of all adults (47). That number increases to 62% of adults when prayer, used specifically for health reasons, is included. Other research clearly shows that, globally, CAM is either a standard part of patient care or an emerging option (48). Treatment for infertility has evolved to include an understanding that the most effective treatment involves treating both the mind and the body.

Although evidence-based studies are still emerging for treatments such as acupuncture and relaxation approaches, more programs are opening their doors and their referrals to CAM. Accompanying the concept of treating the whole person is the paradigm of collaborative care in the treatment of the patient. A team approach composed of a physician, nurse, mental health professional, acupuncturist, yoga instructor, or other professionals represents the new model for providing patient care. Patient associations are emerging as leaders in providing these models for collaborative care. For example, in the U.S.A., the American Fertility Association and Resolve regularly bring these diverse providers together for the benefit of patients through the use of forums, in-person seminars, or internet seminars.

CAM has different meanings in different cultures and different countries. What is complementary in one country may be an accepted staple of regular treatment in another. Research internationally is expected to begin to integrate all these approaches and lead us to a more global understanding of how harmony between the mind and body can facilitate good health, and conversely, how disharmony or dysfunction between the mind and body can contribute to poorer results for the patient.

Acupuncture

The literature about the efficacy of acupuncture has been equivocal. The basic tenets of acupuncture posit that its efficacy is achieved by balancing the flow of qi (energy) through the patient's body (49). Fertility mechanisms for women undergoing acupuncture involve the stimulation of β-endorphin secretion that has its impact on the gonadotropin-releasing hormone pulse generator and then upon gonadotropin and steroid secretion. This process creates a favorable environment within the uterus for implantation of the embryos by virtue of the increased blood flow (50). Although some studies have demonstrated a higher clinical pregnancy rate for acupuncture (50–52), others studies have shown no positive effects (49,53). Acupuncture's efficacy has been explored by comparing traditional acupuncture (needle insertions along the meridians and points), to electroacupuncture, to laser acupuncture, and to sham acupuncture. Criticism of the comparability of all these approaches has been made in the Western medical community and in traditional Chinese medical communities alike (54).

The first published study of a prospective, randomized trial of acupuncture was published in 2002 (52), which demonstrated a significantly higher pregnancy rate in the acupuncture group ($n = 80$) versus the controls ($n = 80$), with pregnancy rates being 42.5% and 26.3%, respectively. In a later study that was not published but presented at a European Society for Human Reproduction and Embryology meeting (55), Paulus et al. used sham acupuncture in one group, and compared the results to a group where traditional acupuncture was used, and no significant difference was observed. The authors noted that the pressure from the sham needles could indeed have created an effect. As Domar (54) observed, concerns were raised because the study ultimately was not published. Domar went on to suggest that the effect that the belief by the patient that acupuncture is effective must be controlled for; he argued that it may be the belief that is the agent of change that leads to increased pregnancy rates, rather than the acupuncture itself.

In a recent study (50), patients were randomized into three groups: a control group; patients who received acupuncture on the day of embryo transfer; and patients who received acupuncture on the transfer day and then two days later. Clinical ongoing pregnancy rates were higher in both treatment groups than in the control group, but did not reach statistical significance. The authors concluded that acupuncture significantly improves the outcome for IVF, but that adding treatment two days after offered no other improvement. In a different study, 225 patients were randomized to receive either luteal-phase acupuncture or placebo acupuncture (56). Both clinical and ongoing pregnancy rates were higher in the acupuncture group. The authors concluded that acupuncture was safe and effective for women undergoing IVF.

In another recent study, 228 patients were randomized to receive acupuncture treatment or noninvasive sham acupuncture (49). The acupuncture group received three treatments. Traditional Chinese medicine was used to diagnose the infertility and treatment was rendered accordingly. In the sham acupuncture group, points near but not on the actual acupuncture points were used. No significant differences were found between the groups, but the authors suggested that a small treatment effect could not be excluded because the odds were 1.5-times higher

for the treatment group. The authors concluded that acupuncture was safe for women undergoing embryo transfer.

Finally, in a review article by Stener-Victorin and Hamaidan (57), the authors reviewed four studies and noted that three of these found a higher efficacy rate for the acupuncture groups. They cautioned that the different study protocols created challenges in drawing conclusions, but could state that acupuncture has a positive effect and no adverse effects on pregnancy outcomes.

CONCLUSION

Many factors contribute to the stresses placed on, and experienced by, women and men who have fertility problems. Research has yet to disentangle and adequately address the relationship between stress and infertility. Counseling with cognitive or behavioral approaches offers tools for individuals and couples in coping with their infertility. There is emerging literature suggesting that patients are utilizing CAM approaches and that acupuncture may be a safe and effective tool for assisting with infertility treatment.

REFERENCES

1. Smeenk JMJ, Verhaak CM, Stolwijk AM, Kremer JAM, Brat DDM. Reasons for dropout in an *in vitro* fertilization/intracytoplasmic sperm injection program. *Fertil Steril* 2004; 81: 262–8.
2. Olivius C, Friden B, Borg G, Bergh C. Why do couples discontinue *in vitro* fertilization treatment? A cohort study. *Fertil Steril* 2004; 81: 258–61.
3. VanderLaan B, Karande V, Krohm C et al. Cost considerations with infertility therapy: Outcome and cost comparison and preferred provider organization care based on physician and facility cost. *Hum Reprod* 1998; 13: 1200–5.
4. Sharma V, Allgar V, Rajikhowa M. Factors influencing the cumulative conception rate and discontinuation of an *in vitro* fertilization treatment for infertility. *Fertil Steril* 2002; 78: 40–6.
5. Roest J, van Heusden AM, Zeilmaker GH, Verhoef A. Cumulative pregnancy rates and selective dropout patients in *in-vitro* fertilization treatment. *Hum Reprod* 1998; 13: 339–41.
6. Campagne DM. Should fertilization treatment start with reducing stress? *Hum Reprod* 2006; 21: 1651.
7. Chen TH, Chang SP, Tsai CF, Juang KD. Prevalence of depressive and anxiety disorders in an assisted reproductive technique clinic. *Hum Reprod*. 2004; 19: 2313–8.
8. Domar AD, Gross J, Rooney K, Boivin J. Exploratory randomized trial on the effect of a brief psychological intervention on emotions, quality of life, discontinuation, and pregnancy rates in *in vitro* fertilization patients. *Fertil Steril* 2015; 104: 440–51.
9. Frederiksen Y, Farver-Vestergaard I, Skovgard NG, Ingersley HJ, Zachariae R. Efficacy of psychosocial interventions for psychological and pregnancy outcomes in infertile women and men: A systematic review and meta-analysis. *BMJ Open* 2015; 5: e006592.
10. Boivin J. A review of psychosocial interventions in infertility. *Soc Sci Med* 2003; 57: 2325–41.
11. Domar AD, Clapp D, Slawsby EA et al. The impact of group psychological interventions on distress in infertile women. *Health Psychol* 2000; 19: 568–75.
12. Domar AD, Clapp D, Slawsby EA et al. Impact of group psychological interventions on pregnancy rates in infertile women. *Fertil Steril* 2000; 73: 805–11.
13. Domar AD, Smith K, Conboy L, Iannone M, Alper M. A prospective investigation into the reasons why insured United States patients drop out of *in vitro* fertilization treatment. *Fertil Steril* 2010; 94: 1457–9.
14. Gameiro S, Boivin J, Peronace L, Verhaak CM. Why do patients discontinue fertility treatment? A systematic review of reasons and predictors of discontinuation in fertility treatment. *Hum Reprod Update* 2012; 18: 652–69.
15. Boivin J, Scanlan LC, Walker SM. Why are infertile patients not using psychosocial counselling? *Hum Reprod* 1999; 14: 1384–91.
16. Boivin J, Domar A, Shapiro D, Wischmann T, Fauser B, Verhaak C. Tackling burden in ART: An integrated approach for medical staff. *Hum Reprod* 2012; 27: 941–50.
17. Gameiro S, Boivin J, Domar A. Optimal IVF in 2020 should reduce treatment burden and enhance care delivery for patients and staff. *Fertil Steril* 2013; 100: 302–94.
18. Freeman EW, Boxer AS, Rickels K, Tureck R, Mastrionni L, Jr. Psychological evaluation and support in a program of *in vitro* fertilization and embryo transfer. *Fertil Steril* 1985; 43: 48–53.
19. Boivin J, Griffiths E, Venetis CA. Emotional distress in infertile women and failure of assisted reproductive technologies: Meta-analysis of prospective psychosocial studies. *BMJ* 2011; 342: d223.
20. Greil AL. Infertility and psychological distress: A critical review of the literature. *Soc Sci Med* 1997; 45: 1679–704.
21. Drosdzol A, Skrzypulec V. Evaluation of marital and sexual interactions of Polish infertile couples. *J Sex Med* 2009; 6: 3335–46.
22. Greil AL, Slauson-Blevins K, McQuiollan J. The experience of infertility: A review of recent literature. *Sociol Health Illn* 2009; 32: 140–62.
23. Boivin J, Lancastle D. Medical waiting periods: Imminence, emotions and coping. *Womens Health* 2010; 6: 59–69.
24. Lancastle D, Boivin J. A feasibility study of a brief coping intervention (PRCI) for the waiting period before a pregnancy test during fertility treatment. *Hum Reprod* 2008; 23(10): 2299–307.
25. Mandle CL, Jacobs SC, Arcari PM, Domar AD. The efficacy of relaxation response interventions with adult patients: A review of the literature. *J Cardiovasc Nurs* 1996; 10: 4–26.
26. Skiadas CC, Terry K, De Pari M et al. Does emotional support during the luteal phase decrease the stress of *in vitro* fertilization? *Fertil Steril* 2011; 96: 1467–72.

27. Lancastle D. Reasons to be cheerful: Part I, II, III Dispositional optimism, positive reappraisal coping and positive (re)appraisals of the situation: Effects on emotional well-being and physical outcomes, *Doctor of Philosophy*, Cardiff University.
28. Ockhuijsen HDL, van den Hoogen A, Eijkemans MJC, Macklon N, Boivin J. Clarifying the benefits of the positive reappraisal coping intervention for women waiting for the outcome of IVF. *Hum Reprod* 2014; 29: 2712–8.
29. Boivin J, Takefman J, Braverman A. The Fertility Quality of Life (FertilQol) tool: Development and general psychometric properties. *Hum Reprod* 2011; 26: 2084–91.
30. Buck GM, Lum KJ, Sundaram R et al. Stress reduces conception probabilities across the fertile window: Evidence in support of relaxation. *Fertil Steril* 2011; 95: 2184–9.
31. Peterson BD, Pirritano M, Block JM, Schmidt L. Marital benefit and coping strategies in men and women under- going unsuccessful fertility treatments over a 5-year period. *Fertil Steril* 2011; 95: 1759–63.e1.
32. Verhaak CM, Smeenk JM, van Minnen A, Kremer JA, Kraaimaat FW. A longitudinal, prospective study on emotional adjustment before, during and after consecutive fertility treatment cycles. *Hum Reprod* 2005; 20: 2253–60.
33. Keye WR. The impact of infertility on psychosexual function. *Fertil Steril* 1980; 34: 308–9.
34. Boivin J, Takefman JE, Brender W et al. The effects of female sexual response in coitus on early reproductive process. *J Behav Med* 1992; 15: 509–18.
35. Newton CR. Counseling the infertile couple. In: *Infertility Counseling a Comprehensive Handbook for Clinicians*. 2nd edn. Covington SN, Burns LH (eds). New York, NY: Cambridge University Press, pp. 143–55, 2006.
36. Conrad R, Schilling G, Langenbuch M et al. Alexithymia in male infertility. *Hum Reprod* 2001; 16: 587–92.
37. Petok WD. The psychology of gender- specific infertility diagnoses. In: *Infertility Counseling a Comprehensive Handbook for Clinicians*, 2nd edn. Covington SN, Burns LH (eds). New York, NY: Cambridge University Press, pp. 37–60, 2006.
38. Berga SL. Functional hypothalamic chronic anovulation. In: *Reproductive Endocrinology, Surgery, and Technology*, Vol. 1. Adashi EY, Rock JA, Rosenwaks Z (eds). Philadelphia, PA: Lippincott-Raven, pp. 1061–75, 1996.
39. Chrousos GP, Torpy DJ, Gold PW. Interactions between the hypothalamic–pituitary–adrenal axis and the female reproductive system: Clinical implications. *Ann Intern Med* 1998; 129: 229–40.
40. Ferrin M. Clinical review 105: Stress and the reproductive cycle. *Clin Endocrinol Metab* 1999; 2: 309–14.
41. Haimovice F, Hill JA. The role of psycho-neuro-endocrine-immunology in reproduction. In: *Cytokines in Reproduction*. Hill JA (ed.). Austin, TX: Landes Bioscience, 1998.
42. Cxemickzy G, Landgren BM, Collins A. The influence of stress and state anxiety on the outcome of IVF-treatment: Psychological and endocrinological assessment of Swedish women entering IVF-treatment. *Acta Obstet Gynecol Scand* 2000; 79: 113–8.
43. Beck AT, Rush AJ, Shaw BF, Emery G. *Cognitive Therapy of Depression*. New York, NY: Guildford, 1979.
44. Clark DM, Fairburn CG (eds). *Science and Practice of Cognitive Behavior Therapy*. New York, NY: Oxford University Press, 1997.
45. Bergin AE, Garfield SL (eds). *Handbook of Psychotherapy and Behavior Change*. 4th edn. New York, NY: Wiley & Sons, 1994.
46. Farr AC, Sellergren SA, Lantos JD, Chin MH. Physician's observations and interpretations of the influence of religion and spirituality on health. *Arch Intern Med* 2007; 167: 649–54.
47. Barnes PM, Powell-Griner E, McFann K, Nahin RL. Complementary and alternative medicine use among adults: United States, 2002. *Adv Data* 2004; 27: 1–19.
48. Astin JA. Why patients use alternative medicine: Results of a national study. *JAMA* 1998; 279: 1548–53.
49. Smith C, Meaghan C, Norman RJ. Influence of acupuncture stimulation on pregnancy rates for women under-going embryo transfer. *Fertil Steril* 2006; 85: 1352–8.
50. Westergaard LG, Maol QM, Krogslund M et al. Acupuncture on the day of embryo transfer significantly improves the reproductive outcome in infertile women: A prospective, randomized trial. *Fertil Steril* 2006; 85: 1341–5.
51. Margarelli PC, Cridenda DK. Acupuncture and IVF poor responders: A cure? *Fertil Steril* 2004; 81: S20.
52. Paulus WE, Zhang M, Strehler E, El-Danasouri I, Sterzik K. Influence of acupuncture on the pregnancy rate in patients who undergo assisted reproduction therapy. *Fertil Steril* 2002; 77: 721–4.
53. Quintero R. A randomized, controlled, double-blind cross-over study evaluating acupuncture as an adjunct to IVF. *Fertil Steril* 2002; 81: S11–2.
54. Domar AD. Acupuncture and infertility: We need to stick to good science. *Fertil Steril* 2006; 85: 1359–61.
55. Paulus WE, Zhang M, Strehler E, Seybold B, Sterzik K. Placebo controlled trial of acupuncture effects in assisted reproductive therapy. *Hum Reprod* 2003; 18(Suppl 1): 18.
56. Dieterle S, Ying G, Hartzmann W, Neuer A. Effect of acupuncture on the outcome of *in vitro* fertilization and intracytoplasmic sperm injection: A randomized, prospective, controlled clinical study. *Fertil Steril* 2006; 85: 1347–51.
57. Stener-Victorin E, Hamaidan P. Use of acupuncture in female infertility and a summary of recent acupuncture studies related to embryo transfer. *Acupunct Med* 2006; 24: 157–63.

The impact of legislation and socioeconomic factors in the access to and global practice of assisted reproductive techniques

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INTRODUCTION

Throughout the world, the availability of infertility services is the result of public health policies associated with a variety of socioeconomic, political, and, on many occasions, religious influences. Wide disparities exist in the access, quality, and delivery of infertility services within developed countries, but most of all between developed and developing countries. Relatively few of the world's infertile population have complete equitable access to the full range of infertility treatment at affordable levels. Even in wealthy countries, such as Japan and the U.S.A., access to assisted reproduction technologies (ARTs) is, or has been, marked by high disparity and inequality in the access to treatment, partly due to high costs and legislative decisions, but most of all due to the lack of recognition of infertility as a disease.

Access for men and women to healthcare and specifically to the treatment of infertility not only requires awareness of being infertile and the knowledge that there are treatment alternatives; large amounts of funds are also required, irrespective of whether they are provided by national health authorities, by individuals themselves, or by a combination of both.

In countries where access to infertility treatment is granted by law, fertility is understood as a right to which all women and men have equal access. Centralized policies are then established in order to have access to these goods. An example of policies regulating to who and under what conditions this access is granted is reflected in the establishment of an age limit for women for whom treatment will be provided. Another example is a restriction in the number of embryos to be transferred in ART cycles. In this way, the balance of the right to autonomy that all individuals are entitled to is harmonized with the right to equal treatment and non-discrimination because of economic reasons, thus allowing equal access to treatments irrespective of economic capacity.

When access to infertility treatment is not part of a governmental policy, individuals must rely on their personal wealth and/or private insurances covering medical care. Under this scenario, what regulates access to diagnosis and treatment is left to a free market model, thereby excluding all those who cannot afford the costs involved. Furthermore, in most countries, companies providing private health insurances do not cover the costs involved in the treatment of infertility.

Coverage of infertility treatments offers some additional difficulties. While nobody would discuss the use of all available tools in order to save the lives of people with cancer, the use of modern reproductive technology is controversial, and many legislators wonder whether specific treatments should be available or funded in order to generate a new life. Interestingly, there is much more social acceptance and legislative agreement in saving lives than in generating new ones.

Irrespective of whether a country is over- or under-populated, there seems to be less public concern in prolonging the lives of the elderly than in generating new young lives.

It is a rule of life that those promoting laws and regulations have already passed by the burden of existing—all they need to worry about is the quality of their aging and death. On the other hand, for those who have not yet come into existence, there are no chances of influencing policy makers unless the latter themselves have experienced infertility or been moved by someone with this condition.

Countries around the world either do not regulate ART or regulate ART in many different ways. It is the purpose of this chapter to review how different legislation as well as socioeconomic, demographic, and religious factors influence access to and the way ART is practiced.

Most of the information concerning ART procedures will be related to treatment cycles initiated in 2010. Information on the number of ART procedures performed is now available thanks to worldwide data collected by the International Committee Monitoring Assisted Reproductive Technology (ICMART) (1). In 2006, 51 countries reported 848,585 cycles, but in 2010, 58 countries reported 1,170,358 cycles (2). The major contributor to ART cycles continues to be Europe at 48%, followed by Asia at 27% and North America at 13% of initiated cycles reported in 2010. Between 2005 and 2010, the number of initiated cycles increased 10%; however, neither the relative contribution nor the percentage increment in cycles follow homogeneous patterns, since by 2010, Latin America and the Middle East altogether represented only 4% of cycles performed worldwide (Figure 73.1). In many ways,

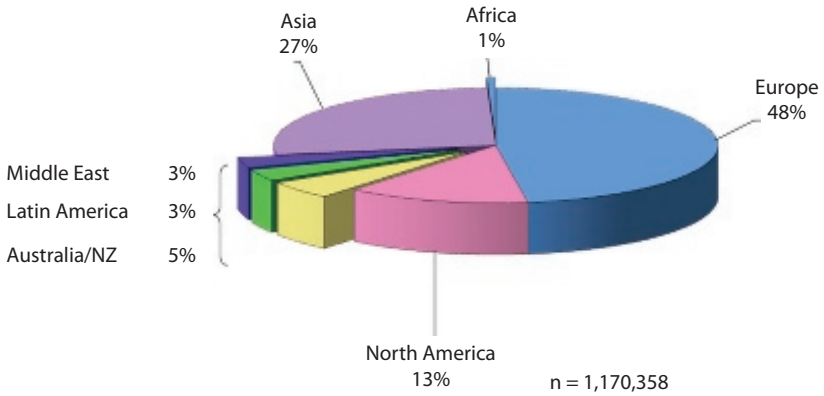

Figure 73.1 Regional contributions of assisted reproduction technology cycles to the world report (2010). *Abbreviations*: NZ, New Zealand. (From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.)

the proportion of ART cycles is a reflection of a balance between availability and access to this expensive form of infertility treatment.

Inequality in the access to ART

When access is expressed as the number of ART cycles per million women of reproductive age (arbitrarily set for the purpose of this publication to women between 25 and 40 years of age), the proportion of treatment cycles fluctuates between 18,600 and almost 29,000 cycles per million women of reproductive age in some Nordic countries such as Sweden and Denmark, as well as in Australia, and dropping to 8605 cycles in Germany. The disproportion is even greater between European countries and other regions of the world. Using the same calculations, access in a wealthy country such as the U.S.A. is only 4493 cycles per million, while Japan has had a steady increase from 8000 in 2006 to over 17,500 in 2010. Access to ART treatments is much lower in countries in Latin American such as Brazil, Chile, and Argentina, which perform between 695 and 1729 cycles per million. Similar numbers are found in other developing countries in the world such as Egypt, with approximately 800 cycles per million women of reproductive age.

Another way to look at the disparity in access to treatment in different populations is obtained by looking at the relative proportion of treatments performed in a certain population, and its theoretical need. This proportion (Figure 73.2) is calculated by dividing the number of initiated cycles per country by the number of women aged 25–40 years assuming 10% infertility, and 30% of those requiring ART (3% of all women aged 25–40 years). Using this calculation, the differences between Israel, several European countries, and Latin American countries are vast. Interestingly, the major source of difference in access to modern reproductive technology is not only based on the wealth of the country—it is also a reflection of the distribution of wealth. An example is the U.S.A., which is one of the wealthiest countries, with a disproportionately poor access to ART treatments in its population. Another reality is that of Israel where, due to a mixture of social and geopolitical reasons, access to ART is facilitated to an extent, leading to the provision of free access to all those in need.

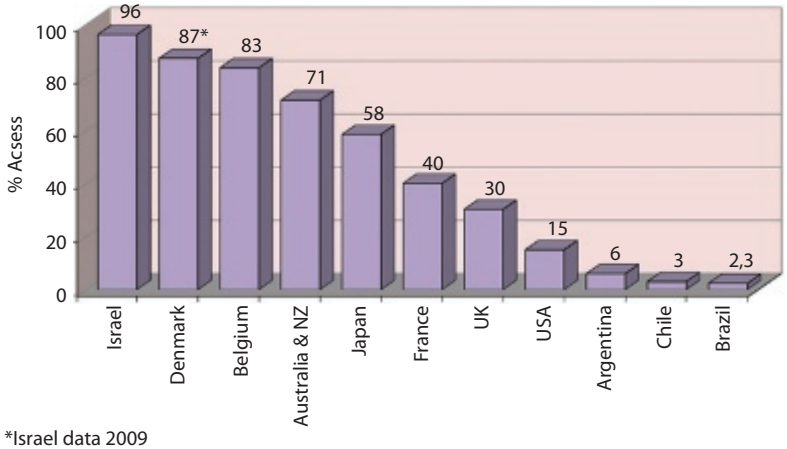

Figure 73.2 Access to assisted reproduction technology (women aged 25–40 years) (2010). *Abbreviation*: NZ, New Zealand. (From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.)

Table 73.1 Number of assisted reproduction technology cycles according to public/private health expenditure (2010)

GDP per capita (U.S. dollars)	Country (% general government expenditure)	Public (%)	Private (%)	ART cycles/million women in reproductive age (25–40 years)
33,000–41,000	Sweden (14.8)	81.0	19.0	18,587
	Denmark (16.4)	85.1	14.9	28,710
	U.K. (15.9)	83.2	16.8	9063
	Belgium (15.1)	75.6	24.4	25,040
	Australia (16.8)	68.5	31.5	21,397
	Japan (18.2)	80.3	19.7	17,527
48,000	U.S.A. (19.9)	48.2	51.8	4493
13,000–18,000	Brazil (10.7)	47.0	53.0	695
	Argentina (17.7)	64.4	35.6	1726
	Chile (15.8)	47.2	52.8	843

Source: From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.
Abbreviations: ART, assisted reproduction technology; GDP, gross domestic product.

Factors affecting access to ART

Access to ART can be the result of multiple variables. Table 73.1 describes the relationship between availability of ART and the ways funds allocated to health are distributed among the population. Countries from northern Europe allocate 14%–16% of their gross domestic product (GDP) to health expenditure, and more than 80% of it is allocated into public as opposed to private health expenditure. On the other hand, the U.S.A., with one of the highest GDPs per capita of US$44,000, also allocates the highest percentage of funds to health (19.9%), but due to a different economic policy, only 48.2% goes to public health expenditure. Countries in Latin American are not only much poorer; they also follow trends similar to the U.S.A., allocating the majority of their restricted funds into private rather than public health; an exception is Argentina, which allocates 64.45% of its health expenditure into public expenditure. The differences in access are immediately evident, as shown in Table 73.1. The consequence of favoring private as opposed to a public distribution of resources is that access to infertility treatments is restricted to those who can pay. While in the past, Japan and other Asian countries allocated a high proportion of their GDPs to health, and most of it as public health expenditure; their low coverage for ART treatments was the result of a political decision to refrain from funding infertility treatments altogether. However, Japan in 2004 and Korea in 2008 introduced policies in order to provide reimbursement for ART treatments, which varied according to the family income. Although ART remains privately funded in Japan, approximately half of the costs of *in vitro* fertilization (IVF) are reimbursed. This resulted in reimbursement of 65,468 out of 190,690 (34.3%) cycles performed in 2008 and in 96,458 of 241,189 cycles performed in 2010, representing 40% of treatment cycles. The consequences of this increased contribution to the costs of ART have immediately impacted the number of cycles performed in Japan and the way ART is practiced (Figure 73.3).

An opposite direction drove Germany, where severe restrictions in public coverage of ART treatments were imposed, resulting in a drop in the number of initiated cycles from 80,434 in 2003 to 54,695 in 2006. When treatment was again facilitated, the number of treatment cycles increased to 69,598 in 2010 (Table 73.1). These examples are crude reflections that above cultural and ethnic differences, it is economics that has the largest impact on a couple's decision or capacity to use modern reproductive technology in order to procreate.

The first conclusion would be that access to ART treatment is strongly influenced by socioeconomic policies. Countries where infertility treatment is considered a right to which all individuals are entitled as equals distribute their wealth through public facilities and have a much higher coverage of treatments. On the other hand, countries where access to infertility treatments is partly regulated by the market, requiring out-of-pocket funding, have a much lower coverage of fertility treatments, which in turn decreases the number of treatment cycles.

Access to ART treatment and insurance coverage

Indeed, access to healthcare and insurance coverage are intimately related.

Overall, Europe has made major changes in the last 10 years. Today, there is increasing homogeneity among different countries in mainly two aspects. The first is the policy towards coverage or reimbursement of treatments costs with public funding. This is now the case in 24 out of 29 countries responding to a survey published in *Focus on Reproduction* in May 2015, with the exceptions being Ireland, Switzerland, Lithuania, Romania, and Georgia. Consistent with the above, a second change has been the opening of many countries to allow a wider range of techniques. This is the case of Austria, which has introduced

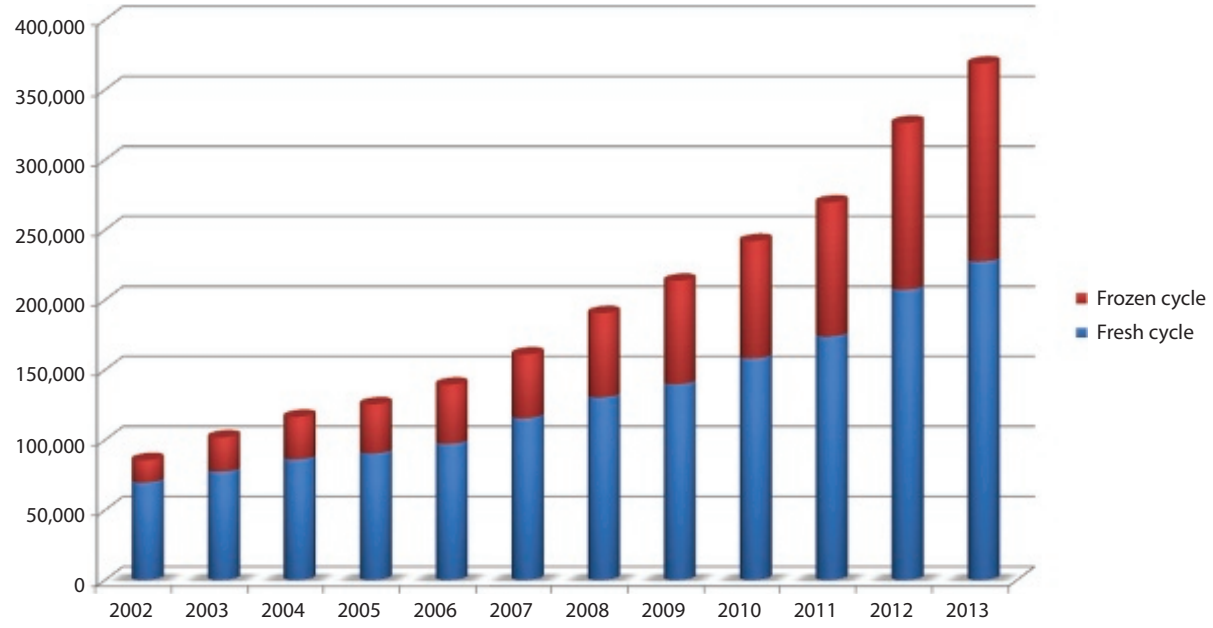

Figure 73.3 Numbers of fresh and frozen cycles performed in Japan.

legislation allowing preimplantation genetic diagnosis (PGD) and non-anonymous oocyte donation, and Poland, which introduced reimbursement in 2013.

In spite of these important changes in order to facilitate access to reproductive treatments, very few countries in the world have national health plans covering the full range of treatment. These countries consist of Australia, Belgium, France, Israel, Slovenia, and Sweden. In general, the differences between countries reside in the number of ART cycles that are covered by the national health plan and in the regulation imposed to have access to this facility (age limit of the female partner, maximum number of embryos to be transferred, etc.). An interesting observation results from the fact that countries with full health coverage have to simultaneously deal with the costs involved in pregnancy, delivery, and neonatal care. Today, all countries with reimbursed costs have regulations restricting the number of embryos to be transferred. Single-embryo transfer (SET) is the rule in Sweden and in young women in Belgium, Finland, and Australia. Therefore, the costs they have absorbed by covering ART have been compensated by decreasing the number of multiple births and the high costs involved in the care of preterm babies. Some other countries in Europe and the Middle East have only partial coverage from public sources, like the U.K., Denmark, Finland, and Tunisia, as well as Japan and Korea. What is more striking is that in Latin America, a region strongly influenced by Catholic tradition that opposes ART, access to infertility treatments is mostly left out of coverage both by public and private insurances.

When ART treatment is covered by out-of-pocket funding, it results in a source of inequality and disparity in the availability of health resources. The absence of insurance coverage determines that only wealthy couples can gain access to treatment, and as will be seen later in this chapter, this factor is strongly associated with high rates of multiple births.

Access to ART as a human right: The case of Latin America

Access to infertility treatment and especially to ART was discussed at the Inter American Court of Human Rights (IACHR) in 2012. In 2000, the Constitutional Chamber of Costa Rica declared ART as contrary to the right to life of embryos protected by their constitution, and therefore ART was forbidden. For 10 years, several infertile couples, medical organizations, and human rights organizations pledged against this ruling, and finally the case was judged by the IACHR, which declared that Costa Rica had violated the right to autonomy of persons with infertility, especially women, and had violated the right to found a family and the right to nondiscrimination, in this case due to economic reasons (3).

In its ruling, the IACHR obliged Costa Rica to restore IVF as part of its social security network in order to avoid any form of discrimination due to financial resources, and to compensate the infertile couples who were part of the accusation and had remained infertile because of the ruling of the Constitutional Court. Furthermore, the IACHR interpreted Article 4.1 of the Inter American Convention of Human Rights, which refers to the protection of the lives of persons and "*in general* from conception onwards." In its ruling, the IACHR stated that conception starts with embryo implantation and not fertilization, as was interpreted by the Constitutional Court. It continued by reflecting upon the acquisition of personhood, and defined it as a process rather than an instant acquired at fertilization. The Court then established that reproductive rights are in fact human rights, and it is the woman who is entitled to human rights and not the embryo.

This verdict had such impact in the region that Argentina in 2013 and later Uruguay in 2014 passed laws for the first time acknowledging infertility as a disease as stated by the World Health Organization, regulating the way ART ought to be practiced, and providing mechanisms for universal access to treatment.

This verdict has been extensively referred to in other fights for reproductive rights, such as the right to abortion. Only five countries in the world have absolute penalization of every form of abortion. Four of these countries are in Latin America (Chile, Dominican Republic, Nicaragua, and El Salvador). By establishing that the repository of human rights is always the woman and not the embryo, the court recognizes that any form of protection of the life of human embryos must be subordinated to the rights to which women are entitled.

Access to ART and demographic factors

It is interesting to observe that coverage of infertility treatments in many countries or regions is strongly associated with the mean age of women, the fertility rate (ratio between the number of births and the number of women exposed to the risk of pregnancy), and the population growth rate (the rate at which the number of individuals in a population increases). Table 73.2 describes the associations between access to ART and the median age of the female population, the fertility rate, and population growth rate in different countries.

Countries having the highest access to ART treatments are those with the lowest population growth rate and highest age of women. In contrast, younger populations with higher fertility rates, as in most countries in Latin American and the Middle East, have less coverage or no coverage at all. Again, as discussed before, Japan has reacted to the high median age of their female population (almost 45 years) and low population growth rate. As referred to before, Japan established a partial reimbursement policy, which immediately increased the use of ART, contributing to the renewal of its population through women who otherwise would have stayed childless or with fewer children than desired.

Perhaps the underlying factor responsible for these disparities is that in countries with an older female population and a negative growth rate, the nation as a whole needs to deal with population renewal. On the other hand, in countries with a young female population and a high growth rate, it is not the country but the individual who has to deal with his/her reproductive needs. This might perhaps explain why few Latin American countries consider infertility as a disease and therefore, in most countries, infertility treatments do not fall into the public health agenda.

The desire to have more children is not a national priority in regions with young female populations and high fertility rates. Furthermore, in the absence of a legislation regulating the practice of ART, access to fertility treatments is not on the agenda of national health policies, and companies responsible for health insurance do not cover expenses related to fertility treatments. Furthermore, for many legislators in Latin America, procedures such as IVF and embryo transfer are considered morally unacceptable and a luxury that should not be sustained with state funds. An extreme example of this moral dictatorship is Costa Rica where, as mentioned earlier, the high courts decided that IVF was morally unacceptable and illegal.

Influence of tradition and religion in the practice of ART

It is often difficult to determine the influences of religion, tradition, and other cultural factors on the application of laws regulating reproductive health. The difficulty is not necessarily the result of an imposition of certain religious morality; often, economic and political forces are strongly bound to religious organizations that, in the end, influence legislative processes.

Christian tradition

Although religion and public laws have been separated for centuries in countries in Western Europe and the Americas, Christianity, and most of all the Roman Catholic Church, is by far the most outspoken religious body when it comes to moral behavior concerning sex and reproduction. Catholic tradition has a strong influence in Latin America, less so

Table 73.2 Access to assisted reproduction technology (2010) according to age of female population and fertility rate

Country	Female median age (years)	Fertility rate (%)	Population growth (%)	Number of cycles/ million women aged (25–40 years)
Sweden	42.2 (in 2014)	2.0	0.9	18,587
Denmark	41.6 (in 2010)	1.9	0.4	28,710
U.K.	41.5 (in 2010)	1.9	0.8	9063
Japan	44.9 (in 2011)	1.4	0.0	17,527
U.S.A.	38.1 (in 2011)	1.9	0.8	4493
Brazil	31.4 (in 2010)	1.8	1.0	695
Argentina	31.3 (in 2010)	2.2	1.0	1726
Chile	32.8 (in 2010)	1.9	1.1	843

Source: From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.

in the U.S.A., and even though most European countries have a more rational, evidence-based approach to ethics in reproductive health issues, the Catholic tradition can still exert strong influence in that region, an example of which was the Italian law passed in 2004 that imposed severe restrictions to the practice of ART.

The fundamental basis for the Catholic opposition to any form of ART started in the late 1960s, when Pope Paul VI established in his Encyclical *Humane Vitae* that the uniting and procreative meanings of the conjugal act should not be voluntarily dissociated. Consequently, both contraception and assisted reproduction are considered immoral as they voluntarily dissociate these two meanings; one by allowing sexual intercourse devoid of its procreative meaning; and the other by allowing procreation not mediated by sexual intercourse. Later, in 1987, the Vatican published a document *Donum Vitae*, which contained an "instruction on respect for human life," issued by the Congregation for the doctrine of faith and signed by Cardinal Joseph Ratzinger, later Pope Benedict XVI. This document stated that a person, as we understand it, exists from conception onwards, and therefore condemned all forms of assisted reproduction, irrespective of its intention, the source of gametes, and marital status. This principle carried such power that later, the vast majority of countries in the Americas signed the "American convention on human rights, pact of Costa Rica," which states that "laws should protect the lives of those to be born—in general—from conception onwards." As mentioned before and based on this principle, the Supreme Court of Costa Rica stopped ART use in that country. In the rest of Latin America, ART cycles are performed and laws are only available in Argentina and Uruguay, while Brazil has some form of regulation that emanates from a Federal Council. In the majority of cases, this is mainly because no agreements are reached between legislators as to whether preimplantation embryos are entitled to rights of their own. Needless to say, in spite of the absence of regulatory bodies, genetic diagnosis is performed in several countries without the possibility of discarding abnormal embryos.

The influence of Catholicism concerning ART is less evident in the U.S.A., where more value is placed on the right to autonomy, from the perspectives of both couples and providers. It must be said, however, that the opposition of the U.S. government to therapeutic cloning and embryonic stem cell research, which lasted until president Obama was elected, was mainly the result of lobbying by the Catholic Church, under the argument that a preimplantation embryo is entitled to the same rights as an existing person.

For various reasons, the council of bishops in Europe has been more liberal in the application of directives arising from the Vatican. An example is the Catholic University of Leuven, Belgium, where ART, including embryo cryopreservation, PGD, and reproductive donation, are offered openly. A reverse example, however, was the law passed in Italy (40/2004), which forbade fertilization of more than three oocytes, embryo cryopreservation, use of donor gametes, genetic diagnosis, and so on. The reason behind this restrictive law was indeed the result of pressure from the Catholic Church on the basis of human rights attributable to embryos from conception onwards. Fortunately, the negative impact of this legislation gave way to a review, and this law was then reverted in 2009, allowing for cryopreservation and PGD (4).

In a different attitude toward reproduction, all protestant denominations (Baptist, Methodist, Lutheran, Mormon, Presbyterian, Episcopalian, and others) are very liberal concerning infertility treatments and the promotion of reproductive science. ART is accepted as long as gametes belong to spouses and there is no intention of destroying embryos.

Islamic tradition

Differently to religious laws regulating the Western world, Sharia law, which constitutes the basis for Islamic religion, also regulates political, public, and private lives. Its teachings and directions are open for interpretation as science and technology discovers new routes, and they serve humankind and society (5).

Concerning reproduction, almost all scholars agree that it is legitimate for infertile couples to pursue any form of therapy as long as both male and female gametes belong to the couple and pregnancy takes place in the woman's uterus (6). Consistent with this concept of genetic heritage, Islam does not approve of adoption. Thus, it is the duty of physicians to help infertile couples achieve conception with the freedom to use technology as long as this takes place inside the married couple (7). The embryo is entitled to due respect, and genetic diagnosis can be practiced as long as it does not harm the embryo (6). Although law allows for PGD in Islamic countries, couples cannot practice their autonomy to decide upon the fate of their embryos. In Islam, embryos cannot be discarded.

Jewish tradition

The application of the Jewish tradition is circumscribed to the teachings found in the Torah, subsequently followed by a compilation of traditions and interpretations, such as the Talmud and other ancient religious documents. Israeli laws are secular and rule public affairs, while private matters are the domain of Judaic law, enforced by special rabbinical courts. When it comes to procreation, both secular and religious laws are pragmatic and favor the stability and strength of the family, and in agreement with the first commandment "be fruitful and multiply," laws allow almost any form of assisted reproduction. Although marriage and/or a stable relationship are required to have access to ART, single mothers can also receive fertility treatments. Different religious branches of Judaism have marked differences in the interpretation of the law; nonetheless, in the end, the decision to use modern reproductive technology is dealt with freely by infertile couples, and is provided by the government. Israeli law allows gamete donation (with strict regulations on the source of male gamete). Most forms of ART including PGD and oocyte donation are allowed by legislation.

Israel holds the highest number of IVF clinics per capita and the National Health Insurance Fund provides IVF treatment for up to two live births for childless couples and for single mothers.

The coexistence of Jewish religion and law represents a remarkable example of equilibrium and tolerance between the strength found in tradition and the need to use science and technology to bear children and strengthen the family.

The purpose of reviewing religious morality is that, especially in the developing world, religion can have a strong influence in political decisions. In countries dominated by Catholic tradition, which today are concentrated mainly in Latin America, much of the discussion is not centered on the rights of infertile women and men. On the contrary, most of the discussion is centered on the moral rights of an embryo. As a consequence of the above, ART is accepted because it is there, but few countries have been able to reach a consensus on minimum standards to regulate the practice of ART. This lack of pragmatism in confronting biomedical and social realities is at least in part responsible for the low access to treatment, generating inequality and lack of autonomy, and therefore the absence of diagnostic and therapeutic procedures, such as PGD and other means of preventing the inheritance of genetic diseases. In countries where the influence of religion in public policies has been restricted, it is the right of persons that prevails, in as much as it does not affect society as a whole.

In general, the more separation there is as to how and who is entitled to impose religious and public laws, the more respect there is for the needs of women, men, and for children to be born.

How legislation and culture influence the practice of ART

Age of the female population receiving ART treatments

The age of the female partner has a great impact on the outcome of any fertility treatment and therefore on the mean number of embryos transferred. Therefore, when comparing ART policies and outcomes between countries or between different years, it is important to adjust the results by age. In 2010, the proportion of women aged ≥40 years receiving ART treatments ranged from 39.7% in Japan to 23% in Latin America and only 11.8% in Sweden (Figure 73.4). While in Latin America the high proportion of older women probably results from economic variables, in Japan there is a mix of economic, cultural, and demographic reasons. Conversely, in Nordic countries in Europe, women request infertility treatment much earlier in their life because access is easier. This impacts success rates as well as the way ART is practiced. It is indeed easier to implement programs such as mild controlled ovarian stimulation (COS) protocols and elective SET (eSET) in younger and therefore more fertile populations.

But the ease of access to treatment is not the only relevant factor. Both Sweden and Japan share similar policies toward embryo transfers; in fact, both countries have reached 70% SETs, in spite of major differences in population structures, reimbursement policies, and laws. The long-term responsibility expressed by the medical profession on the outcome of a specific treatment has a great impact on the way ART is practiced. Japan is an example where, in the absence of a law regulating the number of embryos to be transferred, the medical community has taken a decision to restrict the number of embryos based on the long-term negative effects of multiple births. Through this, 70% of transfers are SETs.

Number of embryos transferred and multiple births

The issue of multiple births is one of the most serious complications generated by ART. The risks related to multiple birth not only involve maternal and perinatal complications, but also generate financial and social problems, the majority of which have to be dealt with by the family alone. The rate of multiple births varies in different countries and regions. For 2010, the proportion of twins and triplets and more in Europe remained fairly constant compared with previous years at 20.6%, compared with 23.9% in Latin America, 30.5% in the U.S.A., and only 5% in Japan. In the past five years, every region has moved in a similar direction toward reducing the number of embryos transferred (Figure 73.5).

Perhaps the most remarkable difference is in the number of triplets and more, which increase from 0.1% in Japan and 1% in Europe to 1.5% in the U.S.A. and 1.7% in Latin

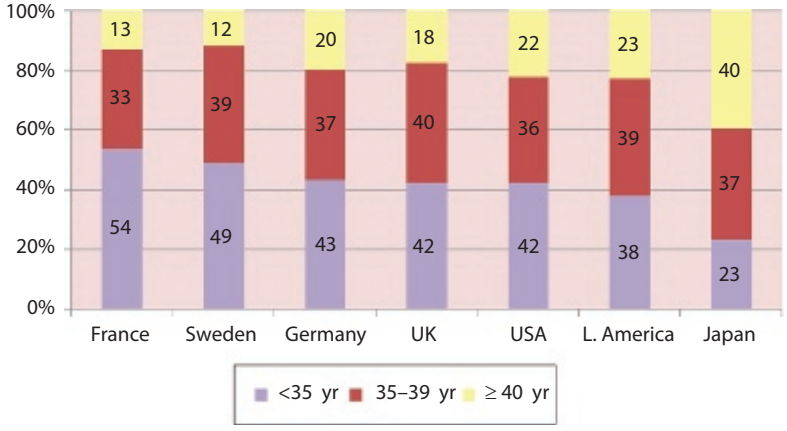

Figure 73.4 Age distributions of patients undergoing assisted reproduction technology cycles.

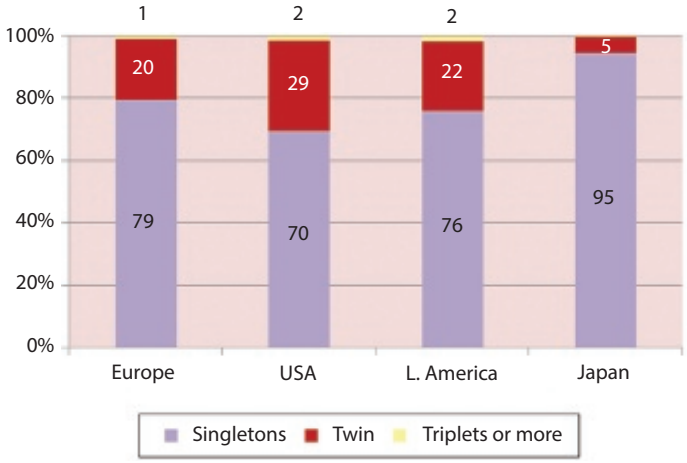

Figure 73.5 Regional variability in multiple births for *in vitro* fertilization and intracytoplasmic sperm injection (2010). (From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.)

America. The differences in high-order multiple births in Latin America and the U.S.A. are in part the result of embryo reduction in the latter. It is worth mentioning that since 2006 there has been a considerable reduction in the proportion of high-order births, which by 2010 dropped from 2% to 1.5% in the U.S.A. and from 4% to 1.7% in Latin America. Reports by the Latin American Registry of Assisted Reproduction show that between 1990 and 2009, a total of 92,791 babies were born, and 42,290 had cohabitated with at least one other fetus with a direct impact on perinatal mortality, which increased 2.8-fold in twin births, 6.4-fold with the birth of triplets, and 18.7-fold with the birth of quadruplets (8).

There is no doubt that the number of embryos transferred has a direct effect on the chances of becoming pregnant and is the single factor that by itself increases the risk of multiple gestation and birth. Indeed, this risk also increases as the age of the woman decreases. In 2010, 41.8% of transfer cycles in Latin America included three or four embryos, compared with 32% in the U.S.A., 10.5% in France, 5.1% in the U.K., and none in Sweden and Japan (Figure 73.6). It is worth mentioning that in the last five years, the number of cases of three or more embryos being transferred dropped by 15% in Latin America.

Many factors can be responsible for these regional differences, but the pressure for success placed on the couple and their family plays an important role. This pressure increases due to economic constraints. Thus, if for economic reasons the couple can afford only one treatment cycle, the risk/benefit evaluation of multiple births as opposed to no birth is considered differently than if couples can have six cycles for free. Table 73.3 compares the number of embryos transferred and the proportions of high-order multiple births in different countries during 2010. Data are presented according to whether the source of funding was public or out of pocket.

There is no doubt that the most efficient way to decrease the number of multiple births is by reducing the number of embryos transferred, and this is easier to do when the high costs are totally or partially covered by public or private sources other than the patients' own pockets.

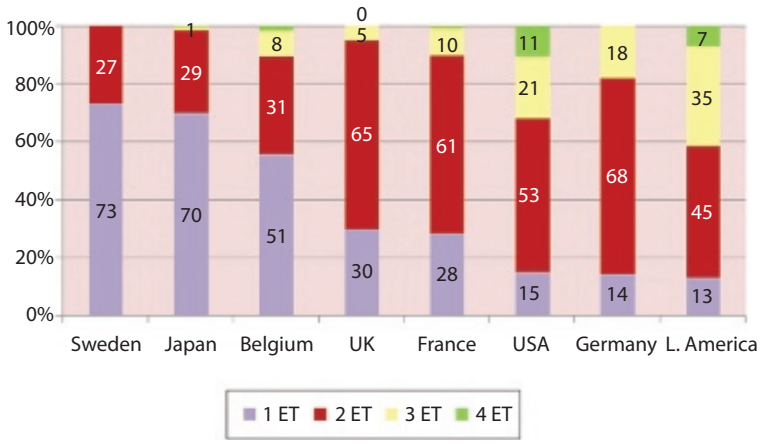

Figure 73.6 Percentages of transfer cycles according to the number of embryos transferred (2010). *Abbreviation*: ET, embryo transfer. (From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.)

Table 73.3 Source of funding influences the number of transferred embryos and high-order multiple births (2010)

Source	Country	Mean number of embryos transferred	High-order multiple births (%)
Public/private with partial/ total reimbursement	Denmark	1.6	0.4
	Sweden	1.3	0.1
	U.K.	1.8	0.3
	Belgium	1.6	0.2
	France	1.8	0.3
Out of pocket	Brazil	2.7	2.1
Out of pocket	Chile	2.1	0.8
Out of pocket + private insurance	U.S.A.	2.3	1.5
Out of pocket/partial reimbursement	Japan	1.2	0.1
Reimbursement	Australia and New Zealand	1.3	0.1

Source: From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.

The experience with eSET in the Nordic countries and Japan

These two different cultural and ethnic realities have dealt with the burden of multiple births using a similar strategy, which is prioritizing SET. In the case of countries like Sweden, infertile couples receive a substantial reimbursement (approximately 60% of costs) and 73.3% of transfers are SETs, while the rest are double-embryo transfers. The results of this policy, implemented in Sweden and Finland in the early 2000s, is that 94.1% of births are singletons, only 5.9% are twins, and only 0.1% are high-order multiple births. It took approximately five years for Japan to start ART treatments in similar directions, but in the absence of, or with little, reimbursement. The main motives for implementing the SET policy were its benefit in the prevention of multiple births without severely affecting success rates. Thus, in 2010, 70% of ART treatments performed in Japan are SETs, and consequently, 94.6% of births are singletons. Differently to the Nordic experience, in Japan, where there are no laws regulating this treatment, it was the Japanese professional society that decided to implement this therapeutic strategy, and it has been followed by the majority of today's 590 institutions providing ART treatments.

Mode of fertilization: Intracytoplasmic sperm injection versus IVF

Since its introduction, the use of intracytoplasmic sperm injection (ICSI) has increased yearly. Today, in certain regions of the world, ICSI is used in approximately 65% of ART cycles. Worldwide, the proportion of ICSI over IVF increased from 24% in 1995 to 63% in 2005 and has been quite stable to 2010. However, this proportion has regional variations that, similarly to what happens with the number of embryos transferred, are influenced by different legislations, especially by socioeconomic variables, such as who is responsible for the costs of treatment. In countries where ART is subsidized by public funds, the proportion of ICSI is relatively low: 60.7% in Australia and 67.9% in Europe. In regions where ART is paid directly by consumers, the proportion of ICSI rises to 86.1% in Latin America and over 90% in the Middle East (Figure 73.7). Irrespective of whether it is right or wrong, there is a tendency to avoid unexpected failed fertilization or low fertilization rates with regular IVF, and centers tend to use more ICSI to "ensure" fertilization.

Examples of the effects of laws on the outcomes of ART treatments

Perhaps the best examples of the impacts of legislation on the outcomes of ART can be found with the implementation of the new laws in Belgium and twice now in Italy.

The Belgian example

In July 2003, the Belgian health authorities introduced legislation to improve financial access to ART treatments and to reduce multiple births. The main decision included that laboratory costs would be refunded for six cycles in a lifetime for women under the age of 43 years. This benefit is conditioned by the number of embryos that can be transferred, which varies from one to three depending on the age of the woman and the cycle number. Furthermore, it obliges each center to report all its data to a centralized registry, which can evaluate trends. This policy not only eliminates inequality in access, but also decreases the risks of multiple births. In this way, the reduced neonatal costs should be enough to cover the costs of treatment.

This is perhaps one of the best examples of how a legislative body examines the available data and implements a solution that brings equality and benefit to all members of society. Belgium is a multi-religious community coexisting with a proportion of non-religious community members. The important fact is that this policy does not enter

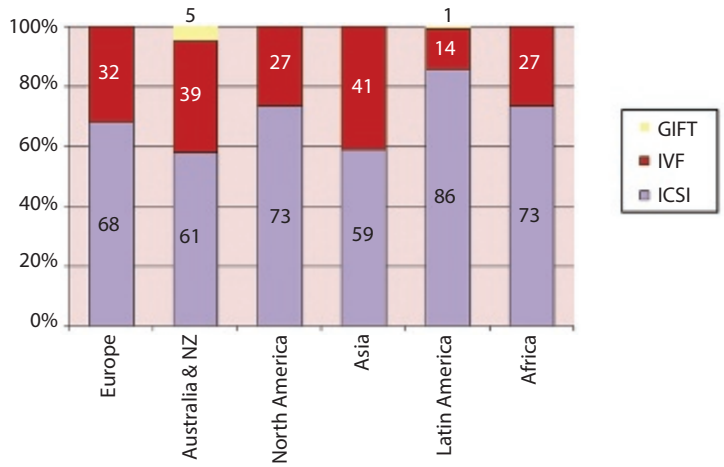

Figure 73.7 Types of assisted fertilization according to region (2010). *Abbreviations*: NZ, New Zealand; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; GIFT, gamete intrafallopian transfer. (From Dyer S et al. *Hum Reprod* 2016; 31(7): 1588–609, with permission.)

into the philosophical discussion of when personhood begins. On the contrary, it looks at the economic, social, and biological evidence with pragmatism and implements legislation that can deal with it in the best possible way, allowing individuals to decide by themselves. The consequences of this legislation are still under evaluation, but so far, at least two conclusions can be extracted. First, the policy does not jeopardize the chances of a couple having a baby. It can take more cycles to achieve the goal, but the cumulative birth rate is not affected. Second, there is a marked reduction in the rate of multiple births. A good review of these data can be found in a publication by Gerris (9).

The Italian example

In March 2004, a new Italian law imposed a number of limitations on the medical profession and on infertile couples. In fact, no more than three oocytes could be inseminated because no more than three embryos could be generated. Furthermore, all embryo transferals that needed to follow embryo cryopreservation, PGD, or any form of embryo manipulation were not allowed. In a different dimension, only married couples or heterosexual couples living in a stable relationship had access to ART treatment. No treatment was available for gay couples or single women.

This law established the right of an embryo over the rights of his or her progenitor. This is especially confusing in a country where the termination of a clinical pregnancy is legal. Furthermore, by restricting ART to only married and cohabiting heterosexual couples, it specifically discriminates against infertile women since if they were fertile, they could become pregnant without requiring a stable heterosexual relationship. This discrimination is only restricted to those infertile women requiring ART, since infertile women are not required to have a stable heterosexual partner to have access to ovarian stimulation, pelvic surgery, or any other form of infertility treatment. The ethical and clinical implications of this law have been discussed by Benagiano and Gianaroli (10).

Fortunately, after extensive lobbying and the use of scientific evidence, the rights of women and men prevailed, and in May 2009, the Italian Constitutional Court declared that the statement "the prevision of the creation of a number of embryos in any case not exceeding three and the mandatory transfer of all the embryos created for a maximum of three" was "unconstitutional." Today, the law says "ART techniques must not create a number of embryos exceeding the one strictly necessary," entrusting the decision of the right number of embryos to be created to the doctor, according to different patients' conditions.

With the Italian national registry created in 2005, it is now possible to evaluate the effects on multiple births and success rates of the restrictive law passed in 2004 and, at the same time, evaluate its reversal in 2009 (4).

These examples represent two different ways of legislating in areas related to sex and reproduction.

In the Italian experience in 2004, the main intention of legislators was to defend a moral principle—"the respect of a person from conception (fertilization) onwards"—irrespective of the effects this might have on actual persons (men and women) in the family and in society.

In the Belgian experience, the main intention of legislators was to defend the rights of actual persons to receive medical treatments. It also aimed to protect the quality of life of those to be born by facilitating the birth of singletons.

Other global examples

There is a global tendency to procure safety over efficacy, or at least satisfactorily balance safety with efficacy, particularly in countries where access to ART is guaranteed or at least facilitated by public resources. In the search for safety, a SET policy has been established in Sweden, Finland, and Belgium. There is more than one strategy for

reaching this goal, and these three countries have arrived at a SET policies by different roads.

In Sweden, which is currently in the lead in terms of the SET transition, 73.3% of all embryo transfers are now SETs, with the eradication of triplets, and a reduction of twinning from over 25% to now only 5.8%. This transition is a result of a combination of several factors: a professional decision, rapidly supported by the national patient organization, and later followed by governmental regulation. The process took four years, from 2002 to 2005.

The main factor behind this move was a cooperative effort between the professional societies of gynecologists and pediatricians and governmental authorities, in which the medical risks for IVF children were thoroughly investigated. A national IVF register of all women giving birth after IVF was formed and, using the personal identification number given to each Swedish citizen, cross-links were made to five different population-based health registers already in operation. Very convincing evidence emerged, showing that the much higher risk profile of IVF children was caused not by the IVF technique per se, but by an elevated multiple delivery rates. A large randomized clinical study followed using national data and demonstrated that pregnancy rates did not drop after a substantial increase in the proportion of SETs. This, finally made the case for SET as the norm. A very important driver for the transitions was the support of the lay press.

In the midst of this process, the law was changed to say that SET must be the norm. The change of law merely confirmed what was already happening, and was therefore welcomed in the country. Discussions regarding the economy of this approach were never instigated.

In Finland, the transition to SET as the norm was the result of a professional decision only, with no governmental interference, and again economic arguments were not raised.

In Belgium, on the other hand, economic arguments on the high national costs for the post-natal healthcare of prematurely born multiple-birth IVF children convinced the government to change reimbursement policies to strongly favor SET.

There is thus not a single road to procuring an equilibrium between safety and efficacy. For example, in Sweden, a preferential SET policy has been implemented through a state regulation, while in Finland the same policy was adopted by the medical profession themselves. On the other hand, a modification in the reimbursement policy caused Belgium to adopt a similar strategy. Thus, irrespective of how the policy is implemented, reducing multiple births will always carry a reduced risk for the children.

The most recent example of a national change toward a balance between safety and efficacy has been established in Japan. In contrast with the Nordic countries, reimbursement in Japan covers approximately 40% of ART cycles, and 39.8% of these women are $\geq$40 years of age compared to 11.8% in Sweden and 14.8% in Finland. A large proportion of women in Japan receive mild forms of COS, which include clomiphene citrate and alternative injections of human menopausal gonadotropin or recombinant follicle-stimulating hormone. Similarly to what took place in Sweden, in Japan, there are no laws regulating the practice of ART. The Japanese Society of Obstetrics and Gynecology made the relevant decision, and the vast majority of institutions followed its recommendation.

It is interesting to note that in countries where the influence of religious morality is well balanced by strong and independent lay organizations, laws tend to follow realistic and sensible evaluations of reality. Thus, public decisions are adopted after incorporating the lay public and society as equals in the discussion of public policies. By contrast, countries with strong religious influences tend to moralize in such a way that the value of embryos becomes the dominant issue, thereby overlooking the rights of actual persons, in this case infertile couples in the pursuit of effective and safe treatments in order to form families.

REFERENCES

1. Ishihara O, Adamson GD, Dyer S, de Mouzon J, Nygren KG, Sullivan EA, Zegers-Hochschild F, Mansour R. International committee for monitoring assisted reproductive technologies: World report on assisted reproductive technologies, 2007. *Fertil Steril* 2015; 103(2): 402–13.e11.
2. Dyer S, Chambers GM, de Mouzon J et al. International committee for monitoring assisted reproductive technologies world report: Assisted reproductive technology 2008, 2009 and 2010. *Hum Reprod* 2016; 31(7): 1588–609.
3. Zegers-Hochschild F, Dickens BM, Dughman-Manzur S. Human rights to *in vitro* fertilization. *Int J Gynecol Obstet* 2013; 123(1): 86–9.
4. Gianaroli L, Crivello AM, Stanghellini I, Ferraretti AP, Tabanelli C, Magli MC. Reiterative changes in the Italian regulation on IVF: The effect on PGD patients' reproductive decisions. *Reprod Biomed Online* 2013; 28(1): P125–32.
5. Serour GI. Traditional sexual practices in Islamic world. *Global Bioethics* 1995; 1: 35–47.
6. Yepren S. Current assisted reproduction treatment practices from an Islamic perspective. *Reprod Biomed Online* 2007; 14(Suppl 1): 44–7.
7. Serour GI. Medically assisted conception dilemma of practice and research. Islamic views. In: Serour GI (ed.). *Proceedings of the First International Conference on Bioethics in Human Reproduction Research in the Muslin World*, Cairo, Egypt, 1992. 11CPSR 2, pp. 235–42.
8. Zegers-Hochschild F, Schwarze JE, Crosby J, do Carmo Borges de Souza M. Twenty years of assisted reproductive technology (ART) in Latin America, 2011. *JBRA Assist Reprod* 2011; 15(2): 15–30.
9. Gerris J. Single-embryo transfer versus multiple-embryo transfer. *Reprod Biomed Online* 2009; 18(Suppl 2): 63–70.
10. Benagiano G, Gianaroli L. The new Italian IVF legislation. *Reprod Biomed Online* 2004; 9: 117–25.

Religious perspectives on human reproduction

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RAPHAEL RON-EL and BOTROS RIZK

Religion is an outcome of beliefs and heritage that are strongly related to history and folklore, which are influenced by environment, education, and interactions between people. Based on this definition, there is no wonder that views are different from one to another religion, which is part of human culture. This is also true when it comes to procreation, especially in what is connected to human reproduction in non-natural ways.

Since the extraordinary achievement of a baby by extracorporeal fertilization by Edwards and Steptoe in 1978 (1), the argument between the different views on the matter of human reproduction in non-natural ways has tremendously increased.

The core of the differences among the religions lies in the definition of what is a "human being." Is it an oocyte or sperm, or is a zygote (fertilized oocyte) a human being? Is a cleaved fertilized oocyte (i.e., a two- or four-cell stage embryo) a human being? Or can a blastocyst or a fetus with or without heartbeats be defined as a human being? Christianity claims that an embryo and fetus are considered as persons. Research on embryos, cryopreservation, and abortion is strongly disapproved (2,3). According to Judaism, the Jewish religion, the embryo is considered to be mere water until the fortieth day when the soul enters the body (Yevamot 69 b, the Babylonian Talmud). This means that here the human being is defined as such on the eighth week of pregnancy (4). In Islam, the fetus is first a "Nutfa" for 40 days, then an "Alaqa" for 40 days, and last a "Mudgha" for another 40 days. At the end of this period of 120 days, the soul enters the body (5). The old threshold of 40 days and upward from conception has been brought back to 14 days, because the new embryology has established this as the embryonic period of cellular activity before which individuation cannot begin.

In Hinduism, the soul is eternal. It has to live many earthly lives to purify itself in order to reach perfection and a higher state of existence called "Mokasa." In Buddhism, the long string of reincarnation and final purification of the soul that enters in a superior state into the body is called "Nirvana."

Is the zygote or embryo a human being? This question is the real basis for discussion among ethicists. Dr Iglesias, a Roman Catholic philosopher (6), claims that an embryo is a biological entity with the potentiality of morality and personality. Thus, given that the embryo has this potentiality, it should be considered as a human being from the very beginning of its creation. Dr Mill (7) completely opposes this concept by stressing that an embryo, being a biological entity, has a long process of developing to the stage of morality. Morality means the attitude of a person to another person and/or toward its environment process. As long as the embryo has no morality, it is a biological material, a result of fusion of two biological gametes, not a human being.

Since the definition of a "human being" is also not the same in every religion (8), there is a clear diversity of permissiveness for when manipulation can be performed on gametes, embryos, or fetuses. Moreover, the permissiveness varies from section to section within the same religion. In any case, all religions accept the principle that manipulation is prohibited on human beings.

CHRISTIANITY

The Christians in the world today are divided into more than 700 million Catholics, 325 million Protestants of different denominations, and 200 million Orthodox Christians.

The Roman Catholic Church

The main laws of the Catholic Church emerge from the holy book, the Bible, and the "Tradition," which are the Church's decisional boards, priests, and dogmatic teachings.

According to the latter, the three leading principles related to the family, the child, and reproduction are:

1. The protection of the human being from the moment of its conception.
2. God commands husband and wife to have children. The child is the fruit of marriage.
3. Integrity and dignity norms must be taken into consideration in all these matters.

In 1956, Pope Pius XII reemphasized these principles by stating that artificial fecundation is immoral and illegal, because it separates procreation and normal sexual function of the married couple.

Therefore, it was clear that the procedures of infertility treatment such as intrauterine insemination (IUI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection, embryo transfer (ET), and surrogate motherhood are not accepted. Moreover, the Catholic Church offers its respect and protection to the human being, starting with its first seconds of existence; it considers the embryo and fetus as persons and strongly disapproves research on embryos, cryopreservation, and abortion. Some of the mentors of the Catholic Church consider even the zygote as a human being, as was mentioned by Dr Iglesias (6).

The Eastern Orthodox Church

In a critical moment in Church history, the Church began to be pulled in two directions along the lines of East and West. At that time, "East" meant Greece, Asia, Alexandria, and the Middle East, and "West" referred to Europe. The causes of this rift were many, among which were language and culture, Latin versus Greek (9).

In 1054, Patriarch Michael Cerularius refused to recognize the Church of Rome's claim to be the head and mother of the churches. As Pope Leo IX had died, Cardinal Humbert excommunicated Cerularius, while Cerularius in return excommunicated Cardinal Humbert.

Thus, the "East–West Schism," or "The Great Schism," divided Christianity into the Eastern (Greek) Orthodox Church and the Roman (Latin) Catholic Church.

The primary locations of Orthodoxy in the world today are Greece, Russia, Eastern Europe, Egypt, and the Middle East, as in Syria, Lebanon, Palestine, and Israel. The Orthodox Church is larger in number than any of the Protestant denominations individually.

The Eastern (Greek) Orthodox Church is not as strict as the Roman (Latin) Catholic Church. It allows the medical and surgical treatment of infertility, including IUI, but is against IVF and other assisted reproduction technologies (ARTs), surrogate motherhood, and sperm and embryo donation.

The Protestant Church

Protestants vary in their beliefs on IVF. Unlike the Catholic Church, there is not one set of ethical guidelines for Protestant couples to follow regarding the use of ART.

Those who support IVF limit its use to married couples. All the embryos must be replaced into the uterus, meaning that no embryo wastage is permitted. Selective reduction is not allowed (10).

The Anglican Church

The Anglican Church allows ARTs, IVF, and ET and allows the use of sperm obtained after masturbation. However, it forbids gamete donation.

The Anglican Church does not see the embryo with a moral status. A moral status can only be given to an individual with a well-established personality. This attitude toward the status of the zygote and embryo is, of course, a major difference from the other churches, which permits the manipulation of the zygote, embryo, and blastocyst.

The Coptic Church

The word "copt" derives from the Greek Aigyptios ("Egyptian") via Coptic kyptaios and Arabic Qibti. Aigyptios derives from hikaptah, house of the Ka (spirit) of Ptah, one of the names for Memphis, the first capital of Ancient Egypt (11). The Arabs, upon arriving to Egypt in 640 CE, called Egypt dar al Qibt (home of the Egyptians), and since Christianity was the official religion of Egypt, the word Qibt came to refer to the practitioners of Christianity as well as to the inhabitants of the Nile Valley.

The first book on the opinion of the Coptic Orthodox Church on IVF and the transfer of embryos was published by His Grace, the late Bishop Gregorios, the Bishop of theological studies, Coptic culture, and scientific research (12). The introduction of his book starts by ascertaining that the success of IVF represents a great success for science by alleviating a great obstacle for married couples wishing to conceive a child. Although having children is not the only reason for marriage, it represents nature's first goal of marriage in all beings, including humans. He fully acknowledges that motherhood is the strongest instinct that a woman could have and that having children is the first wish for any mother, and certainly infertile women are among the unhappiest people, even if they are married to the richest, wealthiest, and most famous. He also acknowledges that the success of IVF has brought happiness to thousands of married couples and settled lives among many families.

The second chapter focuses on the pitfalls of IVF and assisted conception. He emphasizes that a key issue is the fertilization of a woman's oocyte by her husband's sperm, and extreme accuracy should be exercised in this important issue. He stresses the role of the treating physician in honesty so that there is no question that fertilization has occurred between the husband and wife and not any third party. He acknowledges that in certain situations fertilization might not occur, but does not accept that fertilization should be attempted between the wife's oocyte and any other man's spermatozoa, whether it is from a known or unknown donor. He does not accept the establishment of embryo banks and the buying and selling of gametes with money. This is unacceptable because it brings the relation of the value of marriage and conception and having children to a low level.

ISLAM

The teaching of Islam covers all the fields of human activity; spiritual and material, individual and social, educational and cultural, economic and political, and national and international. The instructions that regulate everyday activities of life to be adhered to by good Muslims are called Sharia. There are two sources of Sharia in Islam: primary and secondary. The primary sources of Sharia in chronological order are: the Holy Quran, the very word of God, and the Sunnah and Hadith, which are the authentic traditions and sayings of the Prophet Mohamed. The secondary sources of Sharia are Istihsan, the choice of one of several lawful options, the views of the Prophet's companions, current local customs if lawful, public welfare, and rulings of previous divine religions if they do not contradict the primary sources of Sharia. A good Muslim resorts to secondary sources of Sharia in matters not dealt with in the primary sources. Even if the action is forbidden, it may be undertaken if the alternative would cause harm. The Sharia is not rigid. It is flexible enough to adapt to emerging situations in different items and places. It can accommodate different honest opinions as long as they do not conflict with the spirit of its primary sources and

are directed to the benefit of humanity (11,13–15). Islam is a religion of Yusr (ease) not Usr (hardship), as indicated in the Holy Quran (16). The Broad Principles of Islamic Jurisprudence are permissibility unless prohibited by a text (Ibaha), no harm, and no harassment; necessity permits the prohibited and the choice of the lesser harm. ART was not mentioned in the primary sources of Sharia. However, these same sources have affirmed the importance of marriage, family formation, and procreation (11). Adoption is not acceptable as a solution to the problem of infertility. Islam gives legal precedence to purity of lineage and the known parenthood of all children. The Quran explicitly prohibits legal adoption but encourages kind upbringing of orphans (17).

In Islam, infertility and its remedy with the unforbidden is allowed and encouraged. The prevention and treatment of infertility are of particular significance in the Muslim world. The social status of a Muslim woman, her dignity, and her self-esteem are closely related to her procreation potential in the family and in the society as a whole. Childbirth and rearing are regarded as family commitments and not just biological and social functions. As ART was not mentioned in the primary sources of Sharia, patients and Muslim doctors alike thought that by seeking ART for infertility treatment, they would be challenging God's will in trying to make a barren woman fertile and handling human gametes and embryos. ART was only widely accepted after prestigious scientific and religious bodies and organizations issued guidelines that were adopted by medical councils or concerned authorities in different Muslim countries that controlled practice in ART centers.

These guidelines that played a role in the change of attitudes of society and individuals in the Muslim world included Fatwa from Al-Azhar, Cairo (1980) (7), Fatwa from the Islamic Fikh Council, Mecca (1984), the Organization of Islamic Medicine in Kuwait (1991), Qatar University (1993), the Islamic Education, Science and Culture Organization in Rabaat (2002), the United Arab Emirates (2002), and the International Islamic Center for Population Studies and Research, Al Azhar University (14–19). These bodies stressed the fact that Islam encouraged marriage, family formation, and procreation in its primary sources. Treatment of infertility, including ART when indicated, is encouraged to preserve humankind within the frame of marriage in otherwise incurable infertility.

In family affairs, particularly reproduction, the decisions are usually taken by the couple. However, not uncommonly, the husband's decision is the dominating one. Today, the basic guidelines for ART in the Muslim world are: if ART is indicated in a married couple as a necessary line of treatment, it is permitted during validity of a marriage contract with no mixing of genes; and if the marriage contract has come to an end because of divorce or death of the husband, artificial reproduction cannot be performed on the female partner even using sperm cells from her former husband. The Shi'aa Guidelines have "opened the way" to third-party donation via Fatwa from Ayatollah Ali Hussein Khomeini in 1999. This Fatwa allowed third-party participation, including egg donation, sperm donation, and surrogacy. The Fatwa is gaining acceptance in parts of the Shi'ite world. Recently, there has been some concern about sperm donation among Shi'aa. All these practices of third-party participation in reproduction are based on the importance of maintaining the family structure and integrity among the Shi'aa family. They are allowed within various temporary marriage contract arrangements with the concerned donors.

Surrogacy is not permitted

Cryopreservation is permitted and the embryos may be transferred to the same wife in a successive cycle, but only during the validity of the marriage contract (7,8,16–18). The strict view is that marriage ends at death, and procuring pregnancy in an unmarried woman is forbidden.

Multifetal pregnancy, particularly high-order multifetal pregnancy (HOMP), should be prevented in the first place. Should HOMP occur, in spite of all preventive measures, then multifetal pregnancy reduction may be performed, applying the jurisprudence principles of necessity that permits the prohibited and the choice of the lesser harm. Multifetal pregnancy reduction is only allowed if the prospect of carrying the pregnancy to viability is small. It is also allowed if the life or the health of the mother is in jeopardy (16,20–22). It is performed with the intention not to induce abortion, but to preserve the life of remaining fetuses and minimize complications to the mother.

Embryo research for advancement of scientific knowledge and benefit of humanity is therefore allowed before 14 days after fertilization on embryos donated for research with the free informed consent of the couple. However, these embryos should not be replaced in the uterus.

Sex selection

The use of sperm sorting techniques or preimplantation genetic diagnosis (PGD) for non-medical reasons such as sex selection or balancing the sex ratio in the family is forbidden. However, universal prohibition would itself risk prejudice to women in many present societies, especially while births of sons remain central to women's well-being. Sex ratio balancing in the family is considered acceptable, for instance, where a wife had borne three or four daughters or sons and it was in her and her family's best interests that another pregnancy should be her last. Employing sex selection techniques to ensure the birth of a son or a daughter might then be approved to satisfy a sense of religious or family obligation and to save the woman from increasingly risk-laden pregnancies (18,19). Application of PGD or sperm sorting techniques for sex selection should be disfavored in principle, but resolved on its particular merits with guidelines to avoid discrimination against either sex, particularly the female child.

Postmenopausal pregnancy is now possible using an individual's own cryopreserved embryos or even oocytes and is possible in future cryopreserved ovaries.

JUDAISM

At Mount Sinai 3500 years ago, the "Torah" was given to Moses, who had to deliver it to the people of Israel. The Torah is viewed as a single divine text that includes moral values and practical laws. The first commandment out of the 10 in the Torah instructs the people to "be fruitful and multiply, fill the earth and subdue it" (20).

The Torah tells about the occurrence of infertility. The first known event of infertility was the case of Sarah, the matriarch, who suggested Abraham, her husband, to marry her their female servant in order to enable him fatherhood.

The second story was of Rachel, who used desperate measures. She declared to Jacob: "Give me children, otherwise I am dead." Rachel's next act was even more desperate. Reuven, the first-born son of Leah, brought to his mother some plants known to enhance conception: "dudaim" (21). Rachel begged her sister for the plants and made a deal: she would allow Leah to spend one night with Jacob in return for the plants. Leah's fifth-born son was the result of this deal. Rachel was finally "remembered" by God and she conceived and bore Joseph. She then stated: "God has taken away my disgrace."

The commandment and the knowledge that the soul enters the body at 40 days of gestation enable, in the Jewish tradition, the use and promotion of fertility treatments when needed. Thus, ovulation induction and all ARTs are permitted. The orthodox Jewish legal system supports the constant questioning process that takes place when a new situation arises. Therefore, Orthodox Jews, who are about 15% of the inhabitants in Israel, consult with their Rabbi to get his advice on what treatment they should take for their specific problem, as well as if a treatment has already been suggested and discussed with them by their physician (4). The Rabbi's views on the suggested treatment may not always coincide with those of the physician. A Jewish couple will definitely ask for the Rabbi's advice when it comes to deciding to accept gamete donation or surrogacy. Selective reduction is acceptable, as the goal is to enhance the possibility of life as determined by doctors. Embryo research to promote life is acceptable. Therapeutic cloning is acceptable and even obligatory in order to do research that could promote life-saving treatment (e.g., stem cell and cellular replacement therapy).

However, there are some controversial issues. Among them, spilling of seed in vain is forbidden. Some Rabbis insist that the husband should not ejaculate to provide a semen specimen. Therefore, knowledge about sperm quality will come only via sperm sampling extracted from the vagina following intercourse. Collection of sperm for a therapeutic procedure will be permitted by collection of sperm following intercourse performed with a condom without antisperm agents (a medical condom). The Talmud (the written instructions collected over the years from verbal tradition) specifically forbids "cutting the sperm ducts." Therefore, most of the Rabbis will not permit biopsy from the testes in cases of azoospermia, but would prefer a trial of aspiration from the testes. Only when aspiration results in no sperm would they allow the performance of a biopsy (22).

Gamete donation is another debatable issue. There is no clear announcement from Rabbinic authorities. Most Rabbis will not allow sperm or egg donation. It is not considered adultery, but is strongly discouraged. However, if the Rabbi is convinced that this will be the only way to enable the couple to achieve parenthood, some Rabbis will agree to it. In this way, the couple has to search for the "right Rabbi"; namely, the more open-minded one.

If donor sperm is allowed, and since Jewishness is conferred through the mother's side, most conservative Rabbis will prefer non-Jewish donor sperm in order to prevent adultery between a Jewish man and a Jewish woman and to prevent future genetic incest among the offspring of anonymous donors.

It is well known that the ancient Israelis were divided into three sections according to the functions their antecedents fulfilled in the Temple: Kohen, Levi, and Israel. The Kohanim were the more prestigious people who were eligible to enter the Holy of Holies in the Temple and bless the present prayers. Since the destruction of the Temple, the blessing of the Kohanim entered as part of the prayer in the Synagogue. When an orthodox Jew who is a Kohen needs to use donor sperm, he has the right to perform gendering on the resulting embryos to have a girl. In this way, he will not be consciously disgraced by risking the prayers with a blessing given by his virtual son from the donor sperm who is not a Kohen. This issue of gendering for this religious indication was decided by the Israeli Ministry of Justice in 2006. The Israeli Ministry of Health also permits gendering in couples who have at least four children of the same sex, once a national committee for sex selection for non-medical reasons has approved it in light of decided guidelines.

Recently, some Rabbis have decided to permit egg donation with oocytes of non-Jewish donors. Again, the reason is to prevent incest among the offspring. They overcame the problem of conferring the Jewishness through the mother's side by deciding that the Jewish religion can be conferred by the religion of the parturient.

Surrogacy is still debatable. For the Rabbis who allow it, single Jewish women are preferred as surrogates, both to avoid the implications of adultery for married surrogate women and to confer Jewishness through a Jewish woman's gestation of the fetus.

In summary, the procedures that are currently used in ART are not universally permitted in the three religions: Christianity, Islam, and Judaism. Table 74.1 shows the procedures and their availability according to different religions.

CURRENT PERMISSIONS FOR ART IN DIFFERENT COUNTRIES

The availability of different procedures of ARTs varies from country to country according to the relationships between the religion, laws, and traditions in each place, as is shown in Table 74.2.

Table 74.1 The permitted procedures of assisted reproduction technologies in the different religions

	IUI	IVF/ICSI	PGD	Surrogacy	Gamete donation	Fetal reduction
Christianity						
Catholic	No	No	No	No	No	No
Greek Orthodox	Yes	No	No	No	No	No
Protestant	Yes	Yes	No	No	No	No
Anglicans	Yes	Yes	No	No	No	No
Coptic	Yes	Yes	Yes	No	No	No
Judaism	Yes	Yes	Yes	Yes	Yes[a]	Yes[b]
Islam	Yes	Yes	No	No	No	Yes[b]

[a] Some Rabbis will agree to sperm donation from a non-Jewish donor and/or egg donation from a divorced woman.
[b] In cases with risk to the mother's health.
Abbreviations: IUI, intrauterine insemination; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; PGD, preimplantation genetic diagnosis.

Table 74.2 Procedures that are not permitted in some European countries

Forbidden procedures	Countries	Limitations
Access to assisted reproductive technologies	France	Singles and lesbians
Sperm donation	France	Singles and lesbians
Oocyte donation	Germany, Italy, Norway	
TESE/PESA	The Netherlands	Limited to only two clinics; since 2007, as part of a research program
PGD	Germany, The Netherlands	Permitted only in PB, except for one center (Maastricht)—BRCA
Surrogacy	Germany, Norway, Spain	—
Embryo freezing	Italy[a], Germany	

[a] Recently, the Italian court instructed the enabling of the freezing procedure.
Abbreviations: BRCA, breast cancer mutation; TESE, testicular sperm extraction; PB, polar body; PESA, percutaneous epididymal sperm aspiration; PGD, preimplantation genetic diagnosis.

This diversity of permissions for reproductive treatments causes patients to search for their desired treatment outside the boundaries of their home country. Thus, a huge movement of "cross-border reproductive care," which is also referred to as "medical tourism," started to be established (23).

With globalization, doctors and patients alike are moving around to different parts of the world; it is now not uncommon for physicians to have to provide medical services to patients with ethical precepts that are different from their own. However, conscientious objection to offer certain required treatments to patients by their physicians should not deprive them from the right of being referred to other physicians who would provide such treatment. It therefore becomes mandatory to be aware of various religious perspectives on various practices.

The main desired treatments in this cross-border reproductive care are mainly gamete donations, gendering, surrogacy, and treatments in postmenopausal and homosexual patients. In recent years, this phenomenon has increased tremendously. For this reason, more and more professional meetings, by-laws, and instructions are being presented by professional societies, governments, parliaments, and courts in different parts in the world.

EPILOGUE

On October 14, 2010, the Nobel Assembly in Sweden announced that the 2010 Nobel Prize in Physiology or Medicine was awarded to Dr Robert G. Edwards for his achievements in IVF. The Roman Catholic Church reacted to the announcements with a couple of declarations. Monsignor Carrasco de Paula said that the decision of the Nobel Prize Committee was completely wrong. Without the achievements of Dr Edwards, there would not have been the huge trade of millions of oocytes, nor full freezers of embryos, nor surrogates waiting for them (24).

In addition, other prominent persons from Catholic Universities and Catholic members of the European Parliament have criticized the choice of Dr Edwards, utilizing the same arguments (25).

Benagiano et al. (26) tried to combat these arguments with the following facts. First, by analyzing the treatment given to achieve the pregnancy of Louise Brown, only one oocyte was collected in order to achieve one embryo, which was transferred to the uterus. This was the original target of Dr Edwards. Not only this, but Dr Edwards always argued in his presentations in professional meetings for reducing intense ovarian stimulations, which indeed were

the ideological and professional basis for the "soft" ovarian stimulation of today. The fact that many embryos are created with IVF treatments was a later phenomenon, when centers experienced the emerging interest of improving the rates of pregnancies achieved. The second argument, is that theology cannot decide when the interaction between oocyte and spermatozoon is considered as human being. Certainly, when a "human lifeform" becomes a "potential human person," he or she must be protected (27).

In conclusion, the diversity in the approaches of the different religions to the definition of a "human being" is still a valid argument for endless debates. However, the strength of the technology of extracorporeal procreation, with all of the problematic questions that have been raised, and the strong natural desire for having offspring demand all involved parties to continue enabling those couples who need our help, and to give them our support and aid.

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REFERENCES

1. Steptoe PC, Edwards RG. Birth after reimplantation of human embryo. *Lancet* 1978; 12: 366.
2. Singer P, Wells D. *In vitro* fertilization: The major issues. *J Med Ethics* 1983; 9: 192.
3. Abou Abdalla M. The Vatican view on human procreation: Religious perspectives of ethical issues in infertility and ART. In: *Infertility and Assisted Reproduction*. Rizk B, Garcia-Velasco J, Sallam HN, Makrigiannakis A (eds). Cambridge, U.K.: Cambridge University Press, 2008, pp. 741–5.
4. Silber SJ. Infertility, IVF and Judaism: Religious perspectives of ethical issues in infertility and ART. In: *Infertility and Assisted Reproduction*. Rizk B, Garcia-Velasco J, Sallam HN, Makrigiannakis A (eds). Cambridge, U.K.: Cambridge University Press, 2008, pp. 728–31.
5. Serour GI. Islamic perspectives of ethical issues in ART: Religious perspectives of ethical issues in infertility and ART. In: *Infertility and Assisted Reproduction*. Rizk B, Garcia-Velasco J, Sallam HN, Makrigiannakis A (eds). Cambridge, U.K.: Cambridge University Press, 2008, pp. 737–40.
6. Iglesias T. *In vitro* fertilization: The major issues. *J Med Ethics* 1984; 10: 32–7.
7. Mills JE. Some comments to Iglesias's paper, "*In vitro* fertilisation: The major issues". *J Med Ethics* 1986; 12: 32–5.
8. Dunstan GR. The moral status of the human embryo: A tradition recalled. *J Med Ethics* 1984; 10: 38.
9. Dunaway M. *What is the Orthodox Church? A Brief Overview of Orthodoxy*. Ben Lomond, CA: Conciliar Press, 1995.
10. Mohler A. Christian Morality and Test Tube Babies, Part II. [Available from: http:// www.albertmohler.com].
11. Serour GI. Ethical considerations of assisted reproductive technologies: A Middle Eastern perspective. *Opin Middle East Fert Soc J* 2000; 5: 1–13.
12. Bishop Gregorios HG. The Christian opinion in *in vitro* fertilization and embryo transfer. In: *Bisphoric of Higher Studies*. Cairo, Egypt: Coptic Culture and Scientific Research Publications, 1988.
13. Gad El-Hak AGH. *In vitro* fertilization and test tube baby. *Dar El Iftaa Cairo Egypt* 1980; 1225: 3213–28.
14. Gad El Hak AGE, Serour GI, eds. Some gynecological problems in the context of Islam. *The International Islamic Center for Population Studies and Research*. Cairo, Egypt: Al Azhar University, 2000.
15. Serour GI. Attitudes and cultural perspective on infertility and its alleviation in the Middle East area. In: Vaycnna E (ed.), *Current Practices and Controversies in Assisted Reproduction. Report of WHO Meeting*. Geneva, Switzerland: WHO Press, 2002, pp. 41–9.
16. *Sura Al Bakara* 2: 185, Holy Quran.
17. *Sura Al Ahzab* 32: 4–5, Holy Quran.
18. Scrour GI. Transcultural issues in gender selection. In: Daya S, Harrison R, Kampers R (eds). *Recent Advances in Infertility and Reproductive Technology. International Federation of Fertility Societies (IFFS) 18th World Congress on Fertility and Sterility*. Montreal, Canada: Elsevier, 2004.
19. Serour GI. Family and sex selection. In: Healy DL (ed.). *Reproductive Medicine in the Twenty-First Century. Proceedings of the 17th World Congress on Fertility and Sterility*. Melbourne, Australia: The Parthenon Publishing Group, 2002, pp. 97–106.
20. *Genesis* 1: 28.
21. *Genesis* 30: 14.
22. Schenker JG. Assisted reproductive practice: Religious perspectives. *RBM Online* 2005; 3: 310.
23. Pennings G. International evolution of legislation and guidelines in medically assisted reproduction. *RBM Online* 2009; 21: 159.
24. ANSA News Agency, Nobel al padre della fecondazione *in vitro*, 2010. [Available in the website of the journal of Toscanaoggi: www.toscanaoggi.it].
25. Casini C. Nobel medicina Ci offende come membri della famiglia Umana. 2010. [Available from: http:// www.toscanaoggi.it].
26. Benagiano G, Carrara S, Filippi V. Robert G Edwards and the Roman Catholic Church. *RBM Online* 2011; 22: 665.
27. Ford NM. *The Prenatal Person: Ethics from Conception to Birth*. Oxford, U.K.: Blackwell Publishing, 2002.

Risk and safety management in assisted reproduction technology

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INTRODUCTION

Risk and safety management has become a science in its own right that today accompanies most modern enterprises. Medicine is no exception (1,2). Risk and safety management aims not at eradicating errors and mistakes—humans make mistakes and always will do—but at managing mistakes so that their most dreadful consequences can be foreseen and avoided (1). This is accomplished by proper understanding, anticipation, and implementation of targeted measures—defenses. Understanding risk and safety management ultimately aspires to developing a safety culture that accompanies all medical teams—in this case, assisted reproduction technology (ART). In this endeavor, on should largely count on education as a privileged vector for inoculating the desired safety culture deep into the daily activities of our working groups (3).

SAFETY MANAGEMENT SYSTEMS

Hazards and risks

Hazards are circumstances that constitute a potential source of danger. For example, in mountain climbing, the mountain itself is a hazard (Figure 75.1). Mountains being what they are, slipping while climbing can have far more dreadful consequences than if the same occurs in low lands. In this example, the mountain is the hazard that impacts on the consequences of other events, such as possibly slipping. This example illustrates the fact that hazard is a parameter that cannot be mitigated. Hazards are inherent to given processes and have to be dealt with.

Distinct from existing hazards, a risk is the possibility that something having unpleasant consequences happens. In our mountain climbing example, slipping is a risk. This risk—its practical consequences—is impacted by the prevailing hazard—the mountain. Indeed, slipping on top of a mountain can have consequences that are influenced by the nature of the hazard—the height of the mountain and the nature and proximity of its cliffs, among other factors. Mitigating the risk—reducing the chances that the risk materializes and/or reducing its consequences—will aim at altering the chance of slipping and avoiding what may ensue—a dramatic fall. However, as was said, hazards—the mountain in our example—have to be accounted for. Hazards impact on risks, but stand as facts that cannot be altered.

Defenses are measures that aim at preventing risk from materializing and/or reducing the severity of their consequences (4). In the case of mountain climbing, roping is an effective defense against the consequences—a possible fatal fall—that slipping may have (Figure 75.1). In the example given, we see that choosing the proper defense—here roping—implies an intimate understanding of both the risk and prevailing hazard, which modulates the seriousness of the risks (4). Ultimately, defenses ought to be judiciously chosen for not interfering too much with the task to be performed—here mountain climbing—and yet being as effective as possible for avoiding the most serious consequences—here a fatal fall (4).

Hazards in ART

When performing surgery, such as an oocyte retrieval in ART, the inherent hazard is linked to the fact that a needle penetrates the patient's natural protections against bleeding and infection, the protective layers of the body (5). By deliberately entering a needle into the pelvis for the purpose of retrieving oocytes, one confronts a hazard that is inherent to the measure taken. There are no other ways of performing oocyte retrievals, however, so entering a needle into the pelvic cavity cannot be avoided in ART. Just like the mountain is a hazard that needs to be taken as a fact, inserting needles into the pelvic cavity is a hazard inherent to the oocyte retrieval process itself (6). One can only mitigate the risk of a catastrophic hemorrhage that might result from vascular damage by either preventing it happening or proactively managing its consequences if it does happen. We will conduct several risk analyses for the three categories of risk that exist in ART (5). The objective is to show how proper understanding of risks helps with deploying the best defenses for avoiding possible catastrophic consequences and thereby practicing safe medicine.

Know your risks in ART

Risks are dynamic processes generally evolving toward increasingly serious consequences (7). Risks are commonly mapped on a severity versus likelihood diagram (Figure 75.2). Slipping on a mountain can have increasingly serious consequences depending on the proximity to the cliff and/or the ability to stop the slipping process early. Likewise, a hemorrhage is a dynamic process that will evolve from a minor self-contained event—the incident—to a catastrophic, possibly fatal accident. On the risk diagram, the consequences of the risk will move toward the less likely and more severe as we progress down and to the right (8). Clinical management ought to maintain the course of risk complications within the green or possibly yellow parts of the diagram. In this analysis, one should

Figure 75.1 Hazards, risks, and defenses in the mountain climbing example.

emphasize the fact that adverse events such as post-oocyte retrieval hemorrhage cannot be avoided, no matter how careful one is. Hence, the protection against catastrophic outcome is not being careful in preventing hemorrhage occurring, but rather proper handling if it occurs. Patients, their spouses, and the whole team need to be trained to react accordingly, as here the ultimate safety—avoiding dreadful consequences—resides in managing hemorrhages, not preventing them.

The dynamic characteristics and the ability to detect the occurrence and progression—symptoms, laboratory findings, etc.—are specific for each risk. In the case of post-retrieval hemorrhage, one relies on symptoms such as pain, dizziness, and so on. In case of deep veno-thromboembolism (DVT), however, there are no announcing symptoms. Understanding the dynamics of each risk is therefore crucial for preventing catastrophic consequences. Post-oocyte retrieval hemorrhages can be followed clinically—symptoms exist—whereas avoiding DVT ought to revolve entirely on prevention in predefined high-risk patients. In the latter case, one solely relies on screening and initiating preventive treatment in identified high-risk women. The challenge therefore is to identify the patients who is at higher risk for DVT, knowing that if this identification fails, there are no symptoms to count on. Management of these two risks—hemorrhage and DVT—is therefore drastically different because the dynamics of these risks differ. Hence, as demonstrated for hemorrhage and DVT, each risk must be identified, understood, and its dynamic known. This is indispensable in order to adequately and effectively position protective measures—the defenses—while minimizing possible interferences with the process itself.

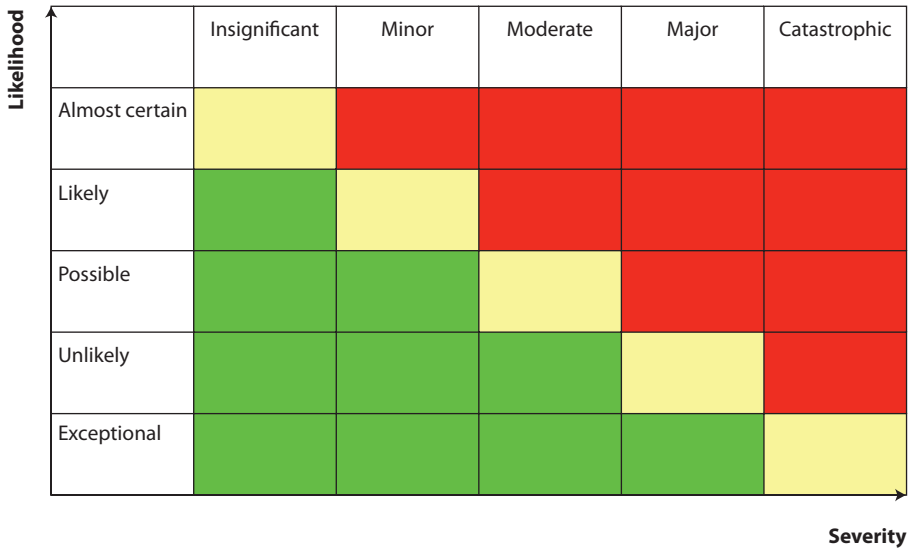

Figure 75.2 Know your risk. The consequences of any given risk result from a dynamic process that can be plotted on a likelihood versus severity diagram. In the case of post-oocyte retrieval hemorrhage, a slight increase in intrauterine bleeding (incident) may progress toward a dramatic, uncontrolled, possibly fatal hemorrhage, an unlikely but most severe event. The diagram serves to plot the parameters—symptoms and findings—that help recognize progression of the risk toward its lower right corner.

Safety management systems in ART

A safety management system (SMS) is a formalized system of management of safety issues that has been rendered mandatory in aviation by its international supervising organization. Four sections are recognized in an SMS: (i) the definition of safety policies and objectives; (ii) safety and risk management, assessing all identified risks, knowing their characteristics, and adopting adequate defenses; (iii) safety assurance; and (iv) safety promotion. The safety level accomplished in the airline industry is such that SMS as it stands should inspire the development of safety systems that are adapted to the various segments of medicine. However, despite being inspired by the accomplishments achieved in aviation, this should be adapted to the specifics of the various segments of medicine, as copycat models simply will not work, considering the amount of differences between the two industries.

RISKS IN ART

Three categories of risks are recognized in ART: operational risks, functional risks, and personal risks (Figure 75.3).

Operational risks

Operational risks are linked to the procedure undertaken (i.e., oocyte retrieval). These risks are modulated by the hazard that consists of inserting a needle—for oocyte retrievals—into the pelvic cavity. We typically distinguish the risks of hemorrhage and of infection.

The risk of infection, post-ART tubo-ovarian abscess (TOA), is modulated by a new hazard—the now frequent presence of endometriomas. Recent data have indeed pointed to the risk that ovarian surgery decreases ovarian reserve to the point of compromising responses to controlled ovarian stimulation (COS) and, in turn, ART outcomes. This has led to the now generalized practice of performing oocyte retrievals while endometriomas are in place, a hazard known to impact on the risk of TOA complications. Patients need to be made aware of this risk, including its possible late occurrence after ART (9). Indeed, the primary defense against severe complications of TOA, such as ovariectomies, resides not in avoiding them, but rather in proper and prompt management by a team that is skilled in the art of managing such complications if they happen (9).

Functional risks

Functional risks are possible adverse consequences of ART directly linked to the effects—hence, "functional"—of treatment used for inducing COS. First among these is the risk of ovarian hyperstimulation syndrome (OHSS) when the desired effect of treatment—multifollicular ovulation—is exceeded (10). OHSS is a dreadful complication of ART that leads to a possibly fatal outcome. It is understood today that OHSS is not directly linked to multiple follicular development per se, but rather stems from an effect of human chorionic gonadotropin (hCG)—administered for triggering ovulation—on a large cohort of ovarian follicles (10). Today, it is possible to nearly eradicate OHSS by refraining from using hCG in women who are at risk of OHSS, and rather reverting to gonadotropin-releasing hormone agonist for triggering ovulation, together with deferred embryo transfer.

Personal risks

Personal risks regroup possible adverse consequences encountered in ART that stem from various personal predispositions. These include three general categories of risks:

- Veno-thrombo embolism (VTE) risk. Certain individuals are at increased risk of having intravascular clotting processes when exposed to hormonal imbalances such as those encountered in ART, notably elevated estradiol levels. Women whose personal or family history is positive for past VTE episodes ought to be investigated with the objective of initiating protective measures—low-molecular-weight heparin treatment—during the course of ART and possibly pregnancy (11).
- Genetic risk. Certain genetic disorders associated with infertility can have dreadful consequence for the future child. An example of this is given by the possible *FRAXA* pre-mutation of the *FMR1* gene. In women, this disorder is known to cause premature ovarian failure

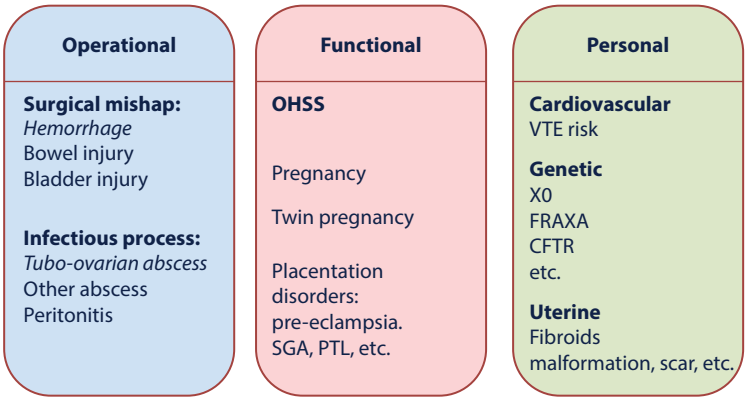

Figure 75.3 Three types of risk in assisted reproduction technology. *Abbreviations*: OHSS, ovarian hyperstimulation syndrome; PTL, preterm labor; SGA, small for gestational age; VTE, venous trombo embolism.

(12). In the next generation, the *FRAXA* pre-mutation can transform into a full-blown mutation, which carries the risk of severe mental retardation in boys (fragile X) (12). Premature ovarian weakness therefore warrants testing the *FMR1* gene, calling for *ad hoc* preimplantation or prenatal testing in positive findings. Several genetic risks have to be screened for in case of male factor infertility as well, notably the *CFTR* gene.

- Uterine risk. Constitutional (uterine malformation) or acquired conditions (large fibroids or past uterine surgery) can be associated with unwanted—possibly catastrophic (uterine rupture)—consequences during pregnancy. Proper counseling and precautions need to be implemented before undertaking ART.

QUALITY CONTROL AND ASSURANCE

Simply put, enacting a quality control system—ISO 9001, Six Sigma, or others—in any industrial activity consists of reviewing the sum of processes undertaken, describing them in detailed documents, and subsequently ensuring that directives are followed. This can be summarized by the simple formula: "say what you do and do what you say."

Practically, ISO 9001 is the quality control system most commonly chosen in ART. It implies creating a core management document, which contains all the necessary standard operation procedures (SOPs) that describe each and every step of what is done practically. Detailed SOPs describe, for example, the set of measures that are taken for selecting between different treatment steps and protocols based on the prevailing circumstances. For example, as ART outcomes decline with age, dealing with women whose ovarian response to COS is insufficient will differ depending on age. One will likely not pursue further ART treatments once it has been documented that a patient in the older age group had an insufficient response to COS. Conversely, *ad hoc* SOPs will describe that a different management should be applied when a seemingly similar event—poor response to COS—is encountered in a frankly younger patient.

Enacting a quality control system of ISO 9001-type controls for practice drifts over time, which could ultimately alter ART outcomes. If changes need to be enacted in ART management, new SOPs are prepared, distributed internally, and finally enacted. Later, outcomes—pregnancy rates and other relevant outcome parameters—can be assessed in order to determine the possible impact that the enacted change may have had. In case of a negative impact, it is easy to notice it and, if need be, to revert to the prior, possibly more effective process. By the nature of ART results—they hover between 0% and 100%, generally in the middle—ART is prone to fluctuations in outcomes, making it difficult to determine whether such changes in results are due to chance or a change having occurred in a given process. By its thorough documentation, a quality control system of the ISO 9001 type allows us to rapidly account for possible practice changes. In many countries, including France, supervising bodies mandate that a quality control system is enacted for certifying ART programs (13). Most often, ART programs have chosen ISO 9001 as their quality control system.

LESSONS FROM AVIATION

Checklists

Everyone knows—and it is an emblematic figure of aviation—that pilots run checklists before performing crucial steps of their flights. This has been an unavoidable approach taken in order to ensure that no crucial steps and/or actions are overlooked at a time when the workload may be significant in the cockpit. Historically, it is the introduction of more complex airplanes—the Boeing B-17, to be specific—that led to the generalized introduction of formal checklists in aviation.

The soundness and efficacy of checklists as safety measure have been widely recognized and, in recent years, exported to other industries, including medicine (14). The mounting awareness and arising concerns about medical errors and their sometimes dreadful consequences have sparked efforts for introducing *ad hoc* checklists in the highest-risk segments of the medical environment. This has notably included operating rooms (ORs), intensive care units (ICUs), and delivery rooms (DRs). Insurance carriers and hospital administration have deployed remarkable efforts for introducing and enforcing the use of checklists in medical institutions, notably in ORs, ICUs, and DRs.

Checklists introduced at long last in medicine—the Boeing B-17 is a World War II-era bomber—have curbed the unacceptable series of mega-mistakes (notably, the infamous "triple-W"—the irrecoverable wrong patient, wrong organ, and wrong side errors). Once these achievements are accomplished, however—indispensable as they are—checklists do little for reducing the larger part of medical errors, which occur in the doctor's office. These revolve around making the wrong decision for undertaking a non-ideal treatment at a non-optimal time. Hence, once checklists are introduced—and they certainly need to be—it is important to go beyond that and address the root causes of medical errors that originate in the doctor's office. Education, as discussed below, is one effective vector for bringing about a safety culture in the doctor's office.

From airmanship to medicalship

Airmanship is a word inspired from the seminal concept of seamanship that has long existed and inspired sailors. Like seamanship—the art of mastering navigation while taking all factors into account—airmanship describes the skill of mastering all that matters for the safe and efficient conduction of flights.

A similar concept has been long awaited in medicine. "Medicalship" is the word coined for similarly defining the art of managing medicine as a global—series of strings—rather than "in slices" juxtaposition of independent steps simply added one after the other. Procedures have implications that carry far beyond the limits of the procedure itself. For example, what are the therapeutic options for a

woman suffering from infertility and endometriosis? Will this woman undergo surgery for removing her endometriotic lesions, or rather revert to ART? In which order will this be done? Knowing that surgery favors natural conception but not ART outcomes, one will enquire before offering surgery about her ovarian reserve and the spouse's sperm. Does she have time for attempting to conceive naturally after surgery if it is performed? Likewise, is the sperm quality compatible with natural conception? In the risk–benefit equation pertaining to surgery for endometriosis, the chance of conceiving naturally after surgery is the benefit that compensates for the cost and risk of surgery. If the chance of conceiving naturally cannot be met because there is no time for waiting for natural conception—perhaps due to impaired ovarian reserve—or the sperm is suboptimal, the risk of surgery is not balanced out. In these cases, surgery should not be opted for. This example illustrates how medical measures intricate themselves into one another and should be looked at as series of linkages—medicalship—rather than the isolated steps of "by-slices" medicine.

It is an active part of safety management in medicine to ensure that procedures are proposed in a medicalship-inspired philosophy and spirit. Medical procedures—diagnostic measures, treatment processes, and surgical procedures—need to be assessed dynamically as strings of mutually dependent procedures, rather than taken in isolation.

By-procedure operation versus resilience

Airlines have championed the concept of "by-procedure" operation. If weather conditions at the destination are below a minimum, approaches to landing are simply not flown and flights are diverted to alternative airports. Most often, little is left to interpretation, and pilots simply follow procedures. Moreover, when an approach to landing at a destination cannot be flown due to adverse conditions and the alternative airport is of no commercial interest for passengers, airlines may set internal procedures and simply cancel the flight. In this case, internal procedures (cancelling the flight) complement or supersede regulatory procedures (flying to an alternative airport).

The remarkable safety levels of airline operations—the safest mode of transportation—is, to a great part, dependent upon the by-procedure mode of operation that has ruled the airline industry. One requirement for relying on by-procedure operation, however, is the repetitive nature of these operations. This typically applies to airline flights. Very little is left to the unknown. However, this is not necessarily the case for all procedures in medicine, nor is it the case for certain non-airline aviation operations.

Non-repetitive tasks simply cannot rely on by-procedure operation alone. This notably includes certain air and medical operations (15). For example, search and rescue air operations in mountainous terrain or high seas are too varying in nature and by essence not repetitive enough to be conducted on a by-procedure basis. Different from airline operations, search and rescue sorties engaged for salvaging endangered human life will have to count on the crew's resilience as much as its adherence to procedures. Such operations generally take place in bad weather, because this is precisely when accidents occur. Search and rescue missions rely at times on the crew's ability to improvise based on past experience and immediate analysis of the unique circumstances prevailing in each mission—a skill that is identified as resilience. Often, the circumstances prevailing in search and rescue missions are such that airline pilots would simply call off the flight. But in search and rescue missions, this might equate to death for the endangered mountaineers or seamen in distress. While search and rescue missions are not always possible, crews will nonetheless strive to achieve their utmost in order to deliver the impossible, always pushing the limits further. Predictably, search and rescue operations do not have the safety records of airlines, but do remarkably well in view of the circumstances, a fact that stands to inspire medicine as a whole.

Not all helicopter operations rely primarily on resilience like the extremes encountered in search and rescue missions, as discussed above. Supply to offshore drilling platforms, for example, is conducted with near-airline repetition and essentially on a by-procedure basis. If the weather is bad, supply will wait until the next day. Logically, helicopter operations to offshore platforms are accomplished with near-airline safety records. We see therefore that helicopter operations as a whole encompass a span of activities ranging from the extreme in search and rescue missions that call for unrestrained resilience, to near-airline, by-procedure operation in the case of supplies flown to offshore platforms. In that sense, helicopter operations—less known to the lay public than airlines—are better models for medicine. Indeed, medicine also includes a comparable diversity that ranges from extreme missions—surgery for cancer "all over"—to routine, by-procedure operations. In this spectrum of diverse medical operations, ART occupies the position of airline-like, by-procedure operations and should therefore achieve optimal safety records. While resilience can become handy in certain difficult, unpredictable circumstances, it should be sparsely and judiciously used in ART, which is in essence a by-procedure activity. We can see from the discussion above how ART should strike out as a reliable and efficient by-procedure operation that is capable of achieving near-airline safety records.

Differences between medicine and aviation

Passengers and patients

In the safety realm of aviation—SMS and quality control—passengers do not actively partake in the process. In aviation, passengers might as well be sandbags, as solely their mass is taken into account when conducting weight and balance calculations. This is not the case for patients, who cannot be ignored, as they clearly participate and can influence the whole safety process.

In aviation, only the crew is taken into account when assessing workload patterns encountered during the

successive segments of flights, ensuring that a ceiling—excessive workload—is not exceeded. Typically, it is before initiating approaches to landing that the workload is at its highest for the crew, leading to the risk of exceeding an acceptable and safe ceiling. Awareness of this process allows the crew to take specific measures to prevent reaching this ceiling, such as through anticipation.

In ART operations, one can easily understand that too many oocyte retrievals falling on a given day may lead to an excessive workload for certain team members (clinicians, biologists, etc.) (Figure 75.4). The approach that needs to be undertaken in order to prevent this from happening will possibly include cycle synchronization with timely use of oral contraceptive (OC) or other measures in order to even out the number of ART cases conducted each week and to set it to a level that is acceptable for the whole group.

In ART, however, it is not just the medical team who can be put under an excessive workload—patients may be as well. If patients are overwhelmed (e.g., by too much information given at the same time) mistakes will occur (i.e., treatment errors). Clearly, patient mistakes can impact on the overall safety of the whole ART process. Patients are prone to encounter an excessive workload at times in the ART process that is different from the medical team. For example, patients could not care less about the number of retrievals performed on a given day. They only have one on that day—their own—and that is all that counts for them. Days before the retrieval, however, patients are given slews of information and lists of safety-inspired recommendations that may be excessive if not adequately planned. For example, before retrievals take place, you want to be sure that the patient's spouse will take his wife home after the retrieval and stay with her for the whole first night. Recognizing the importance of this safety measure highlights how and particularly when patients have to be made aware of it. The best time for reminding patients of this measure is on the day of ovulation triggering, early enough for action to be taken and not too long before the retrieval day so as to incur the risk of the recommendation being forgotten. Awareness of the patient workload (Figure 75.4) is therefore a crucial safety step that is as important as the workload of the medical team. In the defense deployed against hemorrhage risks, the spouse is a key element in the whole safety link. Misinformation or information provided at an erroneous time deprives the patient of a key safety feature if, ultimately, the spouse is not with the patient during that first night. Safety of the whole process indeed includes the presence of the spouse for intervening—returning to the hospital—if need be. We see therefore that proper safety operation of outpatient procedures such as ART implies mastering the workload pattern—What? How much/many? When?—of both the medical team and patients. Each may encounter their limit with an excess workload—the safety ceiling. However, this will likely happen at distinct times in the ART process for patients and the medical team. Contrary to what prevails in aviation—passengers might as well be sandbags—patients need to be included as active partners in the workload analysis of an ART operation and therefore in the whole of safety management.

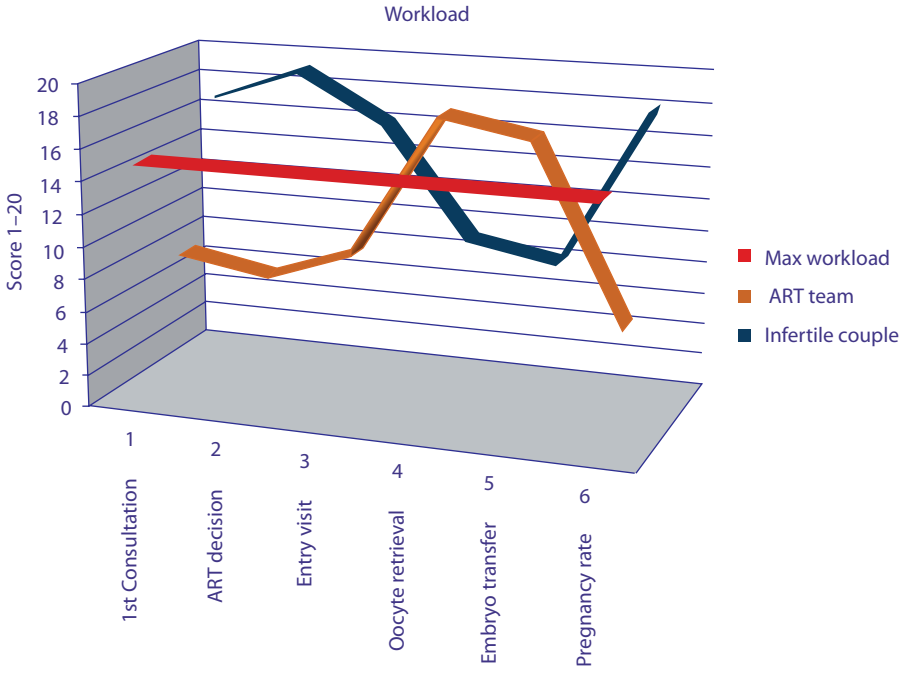

Figure 75.4 Workload pattern throughout the ART process. Workload increases at certain times in the ART process, and these increases are different for the various members of the medical teams. Cycle synchronization—using OC or other means—can help with avoiding reaching an excessive workload for team members (i.e., too many retrievals on a given day). Planning information that is given to patients can help with preventing them reaching an excessive workload and becoming overwhelmed by too much information being given at a specific time. *Abbreviation*: ART, assisted reproduction technology.

EDUCATION CONCEIVED AS "SAFETY INSIDE"

Safety and, in particular, a safety culture cannot be force-fed to people who have long been managing their work operations individually with limited concerns for outside inputs into safety management. The perfect vector for inoculating a safety culture in medical operation is education (3), which can dispense new knowledge items laced with related pertinent safety issues. This is what we identify as education conceived as "safety inside," by analogy to a certain microprocessor found "inside" computers of all kinds and makes.

CONCLUSION

Safety management is a science that has taken medicine by storm under the impetus of insurance companies and hospital administration. ART, a highly repetitive by-procedure operation, is no exception. The nature of ART as generally conducted in healthy individuals should be an example of ultimate safety achievement.

REFERENCES

1. de Ziegler D, Gambone JC, Meldrum DR, Chapron C. Risk and safety management in infertility and assisted reproductive technology (ART): From the doctor's office to the ART procedure. *Fertil Steril* 2013; 100: 1509–17.
2. Meldrum DR, de Ziegler D. Introduction: Risk and safety management in infertility and assisted reproductive technology. *Fertil Steril* 2013; 100: 1497–8.
3. de Ziegler D, de Ziegler N, Sean S, Bajouh O, Meldrum DR. Training in reproductive endocrinology and infertility and assisted reproductive technologies: Options and worldwide needs. *Fertil Steril* 2015; 104: 16–23.
4. Boothman RC, Imhoff SJ, Campbell DA, Jr. Nurturing a culture of patient safety and achieving lower malpractice risk through disclosure: Lessons learned and future directions. *Front Health Serv Manage* 2012; 28: 13–28.
5. Schenker JG, Ezra Y. Complications of assisted reproductive techniques. *Fertil Steril* 1994; 61: 411–22.
6. Nardelli AA, Stafinski T, Motan T, Klein K, Menon D. Assisted reproductive technologies (ARTs): Evaluation of evidence to support public policy development. *Reprod Health* 2014; 11: 76.
7. Guo J, Meng F, Ma N et al. Meta-analysis of safety of the coadministration of statin with fenofibrate in patients with combined hyperlipidemia. *Am J Cardiol* 2012; 110: 1296–301.
8. Schulman S, Spencer FA. Antithrombotic drugs in coronary artery disease: Risk benefit ratio and bleeding. *J Thromb Haemost* 2010; 8: 641–50.
9. Villette C, Bourret A, Santulli P, Gayet V, Chapron C, de Ziegler D. Risks of tubo-ovarian abscess in cases of endometrioma and assisted reproductive technologies are both under- and overreported. *Fertil Steril* 2016; 106: 410–5.
10. Messini CI, Daponte A, Anifandis G, Mahmood T, Messinis IE. Standards of care in infertility in Europe. *Eur J Obstet Gynecol Reprod Biol* 2016; 207: 205–10.
11. Nelson SM. Venous thrombosis during assisted reproduction: Novel risk reduction strategies. *Thromb Res* 2013; 131(Suppl 1): S1–3.
12. Streuli I, Fraisse T, Ibecheole V, Moix I, Morris MA, de Ziegler D. Intermediate and premutation FMR1 alleles in women with occult primary ovarian insufficiency. *Fertil Steril* 2009; 92: 464–70.
13. Harper J, Geraedts J, Borry P et al. Current issues in medically assisted reproduction and genetics in Europe: Research, clinical practice, ethics, legal issues and policy. *Hum Reprod* 2014; 29: 1603–9.
14. Denham CR, Sullenberger CB 3rd, Quaid DW, Nance JJ. An NTSB for health care: Learning from innovation: Debate and innovate or capitulate. *J Patient Saf* 2012; 8: 3–14.
15. Skinner H. Bad medicine: Resilience. *Br J Gen Pract* 2017; 67: 13.

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