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Male Infertility

Edited by
T. B. Hargreave

With 56 Figures

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Series Editor's Foreword

Twenty five years ago the subject of male infertility scarcely existed in urological practice. At that time semen analysis was a rather cursory microscopic test, the scope of endocrine and hormone tests was limited and debate about the relevance of a varicocele was only just beginning. Since then, progress has owed about as much to sociological change as medical progress. Acceptance that problems of fertility should be shared between partners is now no longer questioned and the willingness of both husband and wife to recognise this and to seek help has been an important change in clinical practice.

This book is concerned primarily with male infertility and it will be evident that this is now a substantial subject in its own right. However a need for a broad prospective in this subject is emphasised by a chapter on female assessment as well as reference to female aspects of infertility wherever relevant so that the reader remains properly aware that this subject is a problem for the couple.

Because our knowledge of this subject is changing so rapidly, Mr Hargreave has separated the contents into two parts. The first is concerned with basic biological problems and investigations with a full evaluation of current knowledge and methods. Also, details of new techniques that offer the prospect of real progress are a special attraction in this section.

In the second part of the book Mr Hargreave has encouraged his expert contributors to evaluate their subject both in terms of management and prognosis. It is a frustration to the clinician that so much of the work in male infertility comprises the choice of treatments that are very limited and their results unpredictable. Each topic in the second part of this book is dealt with in a very practical manner so that the authors succeed admirably in clarifying and not confusing the reader.

The infertile couple is anxious to know exactly what is the problem, whether or not it can be treated and what are the chances of achieving a pregnancy. Mr Hargreave has emphasised the management of the

couple and clinicians will appreciate this approach as well as the presentation of new data that offer important trends in diagnosis and treatment.

Edinburgh, April 1983 Geoffrey Chisholm

Preface

This book is intended to help the clinician in dealing with the infertile couple with an emphasis on the male factors. In the last 20 years there has been greater understanding of female infertility and it is now true to say that in most cases the problem can be defined and, in many, successful treatment given. The diagnosis of infertility on the part of the husband is imprecise and management usually ineffectual. There are two main problems that have led to this lack of progress: the first is that it has been traditional to assess husband and wife in separate clinics with the result that much of the published data about one or the other partner is meaningless if success is judged in terms of pregnancy. The second problem is that there are few tests that can be done on the husband that correlate with his subsequent fertility.

This book is divided into two parts. The first is devoted to investigation and basic science. In addition to an attempt to broadly cover the subject, special emphasis has been given to newer techniques of sperm analysis such as zona-free egg penetration. This latter test gives for the first time a method of measuring the ability of spermatazoa to fertilise and not only promises to give prognostic information about a man's fertility but may also allow the laboratory study of metabolic processes in sperm to be correlated with the ability to fertilise. There are also comprehensive chapters on recent advances in endocrinology and antisperm antibodies as these are areas where increased knowledge may soon lead to advances in therapy. Included in this section are guides to testicular pathology, chromosome analysis and psychiatric aspects of infertility.

The second part of the book is a problem orientated approach written by practising infertility clinicians. These sections are intended to provide quick reference as to what can be done now. Great care has been taken with the index in the hope that the busy clinician can quickly find a practical guide to the problem at hand as well as reference to the underlying science. Much clinical investigation of infertility remains to be done and if this book helps in any way to clarify the questions to be asked it will have been successful.

The chapter on AID and adoption is thought provoking; it remains to be seen whether the same problems of identity will afflict children born from AID as has happened with adopted children. In my experience most AID centres give very little information about donors and indeed it would be very difficult to recruit donors if the guidelines suggested by Dr. Triseliotis were followed; nevertheless this aspect of AID needs to be considered in any long term follow-up.

All the contributors to this book started by emphasising that rational management of infertility is only possible if the couple are dealt with together. The aim of the clinician should be to help the couple rather than to treat some abnormality. Most couples wish to know the prognosis quickly and do not want to waste years trying treatments that do not work. On the other hand the young couple must be protected from too quickly abandoning hope. The following extract from a press article conveys a patient's point of view:

> Yet there have always been men and women who have been unable for one reason or another to reproduce. As at the other end of the scale there are those who are superfertile. To my grandparents generation control of fertility was haphazard. Fortune dealt you a small or large family or none at all, and if children did not arrive this had to be accepted. Nowadays this is not so easily accepted and the infertile person is therefore encouraged to expect a cure. It is within this framework of belief that medicine operates. Infertility clinics are geared to success rates, i.e. pregnancies. They are therefore inclined to encourage patients to keep on attending. Inevitably patients themselves, driven by an intense desire to become pregnant support this approach. For some this may end in pregnancy. However, for many this is unrealistic and draws out the excrutiating period of hope and grief. Not knowing whether the future holds a child for you or not is like losing someone who is close to you. You don't know whether to believe he is dead or not so the future has no shape. The worst is expected, the best is hoped for, but neither can be assimilated until one becomes a fact. (Juliet Miller 1978. Quotation by permission of Juliet Miller and the Sunday Times).

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I would also like to thank Anna Paterson, Elizabeth Brunton and Ruth Bruce for secretarial help without which this volume would not have been possible. The figures and illustrations were prepared by William Hopkins, John Pizer and Lorna Gemmell of the Medical Illustration Department at the Western General Hospital.

I have received constant encouragement from Michael Jackson, Medical Editor and Roger Dobbing, Production Editor of Springer-Verlag and from our series Editor, Professor Geoffrey Chisholm. I owe special thanks to Jane Teather for her meticulous subediting.

Finally I would like to dedicate this book to my wife Molly who has had to put up with the manuscript about the house and has been so patient with me throughout this endeavour.

Edinburgh, March 1983 Timothy Bruce Hargreave

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Chapter 1

Human Infertility

T. B. Hargreave

Give me children or else I die.

Genesis, Chap. 30, Verse 1.

Importance of Fertility to Mankind Past and Present

Rachel's eloquent plea ringing through the centuries conveys clearly today the desperate hope of the infertile couple. It is interesting that some of the first biblical references to bodily disorders are to human infertility rather than ill health, e.g. Sarah, Rachel, Hannah and Elizabeth. In many societies the barren woman is condemned and childlessness may still be a conscious or unconscious reason for divorce.

The importance of fertility can be seen in the widespread existence of fertility rites. Often these rites are connected with the fertility of the land and thus symbolic or actual intercourse may be portrayed to ensure a good harvest. The May Day celebration of bringing home the may is of this type; a Maypole is erected and festooned and young girls dance around this phallic symbol. The converse is also true; the corn dolly made at harvest time would be kept to promote human fertility (Fig. 1.1). Another association with fertility in the fields is Mother Goddess symbolism (Neumann 1955). This ranges from crudely carved figures from the Palaeolithic period to images of the Mother and Child in ancient Egypt and the Virgin and Child in the Christian religion. Water has also been equated with fertility because of its power to regenerate barren land and thus wells and springs were commonly visited by infertile women. An example of this is the Derbyshire well-dressing ceremony, which is almost certainly a vestige of an ancient fertility rite. Many ancient gods have been depicted with an erect or large phallus e.g. Hermes in Greece, Osiris in Egypt, Frey in Sweden and the Cerne Abbas Giant cut into a Dorset hillside (Jensen 1963). In India sterile women used to visit the Temples of Siva where they would press their naked bodies against the huge phallus of the god's statue. The mandrake root taken by Rachel is one of the earliest fertility medicines but pigs' teeth, elephants' hair, frogs and spiders have all been

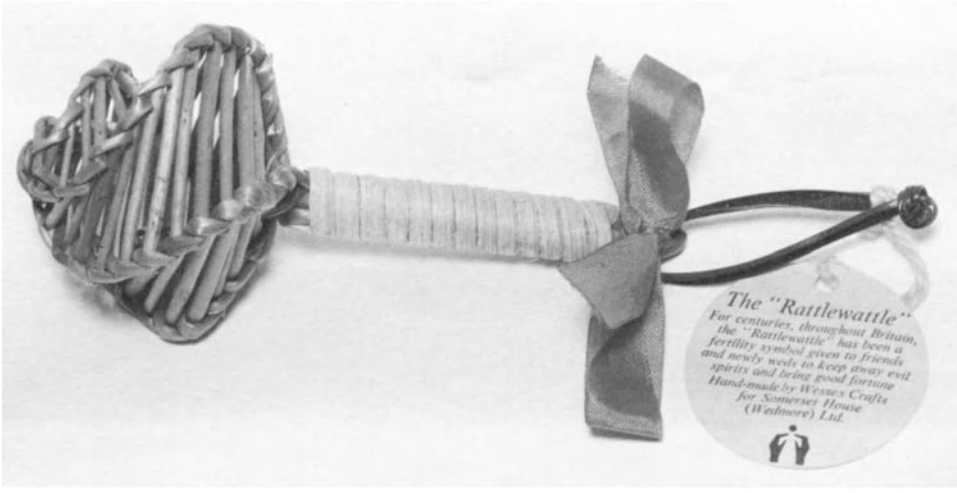

Fig. 1.1. Rattle Wattle—this variation of the traditional fertility symbol the corn dolly was purchased at a country fête in England in 1980

tried. In an old Hungarian custom the childless wife was struck with a stick which had been used to part mating dogs.

These rites demonstrate how very old and strong is the human desire for fertility. It is tempting to dismiss the problem of the infertile individual as irrelevant in the face of the world population explosion. This may be correct from the point of view of a politician allocating resources, but a doctor should always heed the patient's complaint. A strong argument for commitment to scientific research into human fertility is that better understanding may yield safer contraceptives.

Incidence of Involuntary Fertility Past and Present

In this book the word 'fertility' is used to mean biological fertility or the reproductive potential. This is generally accepted medical parlance although fertility is really a statistical concept with social relevance referring to the reproductive performance as measured in live births (Sauvy 1969), whereas the word 'fecundity' is more correct when talking of biological reproductive potential. The measurement of fertility is usually carried out by sociologists and statisticians and these differences in medical and sociological terminology have to be borne in mind when interpreting population data.

It is difficult to distinguish true biological involuntary infertility from voluntary or involuntary infertility secondary to socio-economic factors, because the latter have a much greater effect. We may gain some idea of the maximum fertility potential by examining statistics from two very special populations: the Hutterian Brethren Church, an anabaptist sect living in Canada, and the Cocos Islanders (Potts and Selman 1979). In both these societies a high value was set on children, contracep-

tion was rejected and a period of economic security of several decades allowed maximal child-bearing. In the Hutterites, late marriage, and in the Cocos Islanders abortion and abstinence by older people kept fertility below the theoretical maximum but these two populations represent the highest recorded values of human fertility (Fig. 1.2).

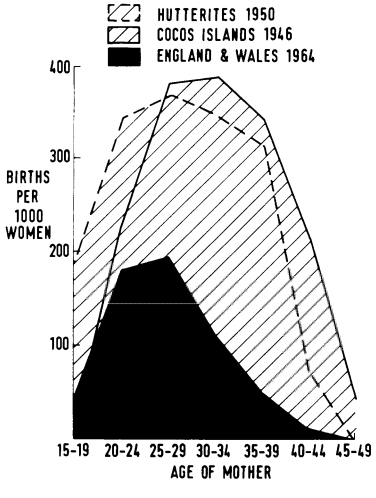

Fig. 1.2. Maximum human fertility. The Cocos Islanders and the Hutterites achieved the highest levels of human fertility ever recorded. (Adapted from Figs. 31 and 32 in *Society and Fertility*, Potts and Selman 1979 by permission Dr M Potts and MacDonald and Evans)

Eaton and Mayer (1953) have examined the records kept by the Hutterites. From 1880 to 1950 the population grew 19-fold from 443 to 8542. In the period 1946–1950 the total birthrate was 8.06 live births per woman. The average family size was 10, two-thirds of women having between 7 and 12 children, and only 3% remaining childless. By the age of 35, 96% of women had married and between 1875 and 1950 only one divorce and four separations were recorded. Pre-marital intercourse was censored by this strict religious sect and child-rearing in families encouraged by communal sharing of wealth. This childless figure of 3% probably represents true biological infertility for this population.

The age-specific fertility rates are compared for the Hutterites and Cocos Islanders with the UK in Fig. 1.2. It can be seen that any biological infertility is dwarfed by voluntary and involuntary infertility secondary to socio-economic factors. In the UK the annual birthrate between 1871 and 1880 was 35.5 per 1000, by 1901–1910 it had fallen to 27, by 1931–1940 it was below 15 and in 1977 it was 11.5. Women marrying in 1860 had an average of six live children whereas their granddaughters marrying in 1925 had an average of just over two, and it is generally accepted that this dramatic change was the result of deliberate restriction by married couples. This was achieved by late marriage, decreased exposure to intercourse and the use of contraception. There is no evidence that this was a reduction in biological fertility; if anything the change to modern living may have improved health and increased fertility.

In England and Wales the proportion of women with no children after 12 years of marriage fell from 13% for marriages in 1953 to 8% for marriages in 1960, while for

marriages in the inter-war years it had been over 15% and as high as 22% for lower-middle-class couples marrying in 1925. These high rates must have resulted largely from a conscious desire to avoid or postpone child-bearing. In the USA the proportion of married women remaining childless rose from 8% for those married in the latter half of the nineteenth century, to 15% in the first quarter of the twentieth century, and approached 20% between the two world wars. In the post-war years the childless population has decreased sharply but has risen in recent cohorts; this is possibly related to a shorter duration of marriage (Potts and Selman 1979).

In the UK approximately 8% of marriages are childless but not all of these are because of biological involuntary infertility. There is no epidemiological evidence of decreasing human biological fertility.

Psychiatric Aspects of the Therapeutic Approach

In infertility work, as with every other aspect of medicine, the clinician must have sympathy with the patient's problems. The interrelationship between hormonal fertility control and the state of mind of the patient are not yet understood but it is known that emotion or upset can alter hormonal mechanisms, as for example in the irregularities of menstrual cycles that may occur in female university students at exam times.

Sympathy of the clinician may have an effect on the result of treatment. One possible explanation for the different success rates between two hospitals following the same trial protocol was differing attitudes in staff running the clinics (Table 1.1).

Table 1.1. Results of a controlled trial of arginine treatment for male infertility. The same protocol was followed at two different hospitals. The difference in pregnancy rates between the two centres could be explained by psychological factors. (From Pryor et al. 1978, with permission of JP Pryor and the Editor of *British Journal of Urology*)

	Conception	Improved	Not improved
Centre A	6	12	24
Centre B	0	1	17

Ethics and Infertility Treatment

There are many ethical problems raised by the management of the infertile couple and the scientific investigation of human infertility. Usually the couple being investigated are in good health and any treatment given has to be weighed against

Table 1.2. Semen analysis results from 10 men with severely abnormal parameters who nevertheless reported their wives pregnant during the course of the investigation[a] (Infertility Clinic, University Department of Surgery/Urology, Western General Hospital, Edinburgh)

These figures are the best of three separate semen analyses				Elevated FSH levels indicate testicular damage
Volume ml	% Motility	Sperm density (millions/ml)	% Normal forms	FSH (Normal laboratory range 1.7–5.9 U/l)
7.0	1	1.0	Too few to evaluate	10.4
5.4	13	1.0	3	11.6
6.0	5	2.6	5	9.2
5.0	50	5.2	Not evaluated	4.8
1.5	8	5.25	14	3.5
3.0	0	7.3	5	11.3
3.4	50	8.0	26	7.2
1.5	70	8.8	20	3.8
2.8	50	9.5	32	10.4
3.0	60	16.6	45	6.3

[a] Whether or not the husband is the true father in every case is impossible to say—it is however unwise to counsel men as being totally infertile as long as some sperm are present.

any possible side effects. Apart from any legal considerations, it is therefore wise to submit protocols of any clinical investigation trials to an ethical committee. This raises a dilemma because some patients, when told that there is a trial, may lose faith in the planned programme of treatment. This has not been our experience.

Laboratory tests do not necessarily give good prognosis about fertility (Table 1.2). It is therefore unwise to advise a young couple in favour of either artificial insemination by donor (AID) or adoption too soon. There are many ethical problems in connection with AID and it should not be forgotten that the donors too have problems; for example, if later their own marriage should prove barren this could cause great mental anguish. The prospects of separating sperm by sex prior to AID also poses problems, particularly if this is to be done for commercial gain and not for genetic reasons. Another possible therapeutic approach is to add chemicals to seminal plasma prior to artificial insemination by husband (AIH) in the hope of improving sperm motility. This potentially valuable approach may also be dangerous because of possible mutagenic effects and will need careful laboratory work before use in humans. The ethical and legal problems of using AID semen to fertilise a donor ovum for implantation into a woman with bilateral tubal destruction and an azoospermic husband almost defy description, but the techniques now exist.

The newer laboratory tests involving assessment of the fertilising capacity of human sperm using in vitro penetration of animal eggs also pose problems. These tests have been extended to allow the resulting zygote to undergo division so that the chromosome complement of the sperm can be examined. There is disquiet in some centres about this type of testing and the morality of producing such a fusion.

When Should a General Practitioner Refer a Couple for Specialist Investigations?

Some guidelines for the general family practitioner as to when to refer a patient for specialist investigation are set out in Table 1.3. This assumes that facilities are available for simple semen analysis. If semen analysis is not available, it is usually reasonable to refer all couples who are worried, whatever their age, because there are now few who are unaware of the facts of life and even with the younger couple there is often a good reason for their worry.

Table 1.3. When to refer a man for further investigation assuming no abnormalities are found on history taking or physical examination and that facilities exist to carry out simple semen analysis (From Hargreave 1980, with permission of the Editor of *Modern Medicine*)

Wife and husband	Under 30	Trying for less than 2 years	Azoospermia	Refer Husband
			Sperm present	Try for 2 years
Wife and husband	Under 30	Trying for 2 years	Azoospermia	Refer husband
			Count < 40 millions/ml Motility < 60%	Refer husband and wife
Wife or husband	Over 30	Trying for less than 2 years	Azoospermia	Refer husband
			Count < 40 millions/ml Motility < 60%	Refer husband and wife
			Count > 40 millions/ml Motility > 60%	Refer wife
Wife or husband	Over 30	Trying for 2 years	Any value	Refer both

How to Organise a Clinic

There are two fundamental points to note when organising an infertility clinic directed at helping the husband.

1. The clinic should be run in conjunction with the gynaecological investigations of the wife.

2. The laboratory semen analysis service should be closely allied to the clinic to allow frequent contact between the technician concerned and the clinician in charge.

It is important that investigations should proceed speedily so that if an untreatable condition is discovered, the couple can be advised about alternatives quickly. In our practice the average age of the last 662 husbands seen was 30.5 years which leaves little time for adoption procedures or AID in the event of treatments failing. Time can be saved by administrative measures such as asking newly referred patients to bring semen samples either prior to or at their initial attendance and by having the seminology laboratory near the consulting area.

The clinic should also be organised in such a way that the couple can be seen together but with separate rooms for examination of one or other partner. If difficulties occur at any time during follow-up it is as well to see both partners. Certain tests may have to be organised in relationship to specific stages of the menstrual cycle and if possible clinics should be arranged so that there are at least two consultation sessions each week and at each session there is time available when patients may be seen without prior appointment. This greatly simplifies such diagnostic tests as plasma progesterone measurements or cervical mucus penetration tests and does not involve clerical staff in constant phone calls about altered menstrual dates.

Plan of Investigations

In most cases husband and wife should be investigated simultaneously, but it is sensible to start investigations of the couple with analysis of semen because even one analysis will determine in many cases whether there is a severe problem with the husband or not. This is of practical importance because in those centres without unlimited resources it is reasonable to defer the more invasive investigations of the wife in the event that the husband is azoospermic unless subsequently the couple decide in favour of help by way of artificial insemination by donor. It is surprising how difficult it is to obtain complete information about both partners even when this is established clinic policy (Table 1.4). However it can be seen from this table that azoospermia does not necessarily exclude tubal blockage in the wife and that in

Table 1.4. Results of investigations on 662 couples referred between 1978 and 1980

Best result from at least three semen analyses	Tubes patent; spontaneous ovulation	Tubes blocked	Lack of spontaneous ovulation	Wife's test not yet completed	Pregnancy	
					Before treatment	After treatment
Azoospermia	8	1	1	65[a]		
< 10 million/ml	23	4	6	45	4	13
< % 10 motility	11	2		9	1	3
Volume < 1.0 ml	3	2	2	7	1	1
Semen analysis adequate	92	53	42	146[b]	73	44

[a] These patients are not usually investigated unless the couple request AID
[b] Many of the wives were in the process of being investigated when this analysis was made

20% of cases where the best sperm density was less than 10 million/ml there were either tubal problems or ovulation problems in the wife as well. Our plan for investigation of the couple is shown in Fig. 1.3.

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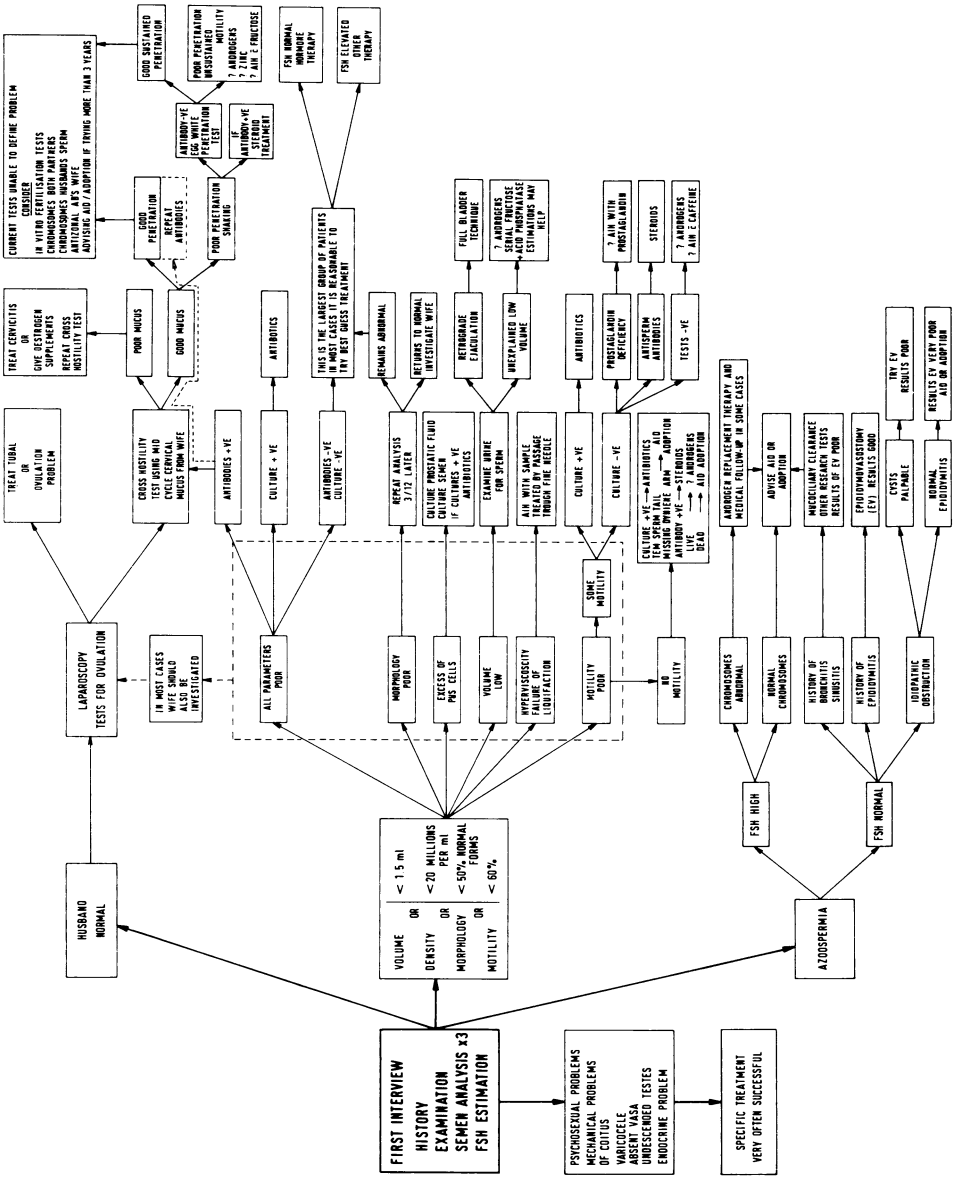

Fig. 1.3. Investigation of the husband of an infertile marriage

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PART I

INVESTIGATIONS

Chapter 2

History and Examination

T. B. Hargreave

History

Different clinics will have their own approach to the initial assessment of the infertile couple. It is our practice to ask patients to complete a questionnaire prior to their first clinic attendance (see Appendix, p. 28). We encourage both partners to attend during the initial interview. At this interview the questionnaire history is checked and additional points noted. The husband is then examined in the privacy of another room and will sometimes volunteer additional information. At the end of the consultation the couple are then told of the plan of investigation.

The purpose of this chapter is to amplify some aspects of history taking and physical examination.

Number of Years Trying for a Child

The couple should be asked for how long unprotected intercourse has taken place. In the younger couple (both partners under 25) where they have been trying for less than 2 years it is reasonable to defer all but the most simple investigation. If the wife has recently stopped oral contraception there may be a period of several months when cycles are anovulatory. A marriage should not be regarded as 'infertile' unless unprotected intercourse has taken place for 2 years, although investigations may begin immediately where one or other partner is over 30 years of age.

Previous Children

On occasion the male partner will only admit to a possible previous child in privacy, and such history must be recorded in confidence. A history of previous fertility does not necessarily exclude the male partner from further investigation; in our clinic 18% of such men had severe abnormalities on investigation (Table 2.1). However such a history may alter the priorities for testing.

Cases of secondary infertility within the current marriage should be distinguished from those involving another partner. When reporting the results of treatment for secondary infertility, these possibilities should be identified.

Table 2.1. Results of investigations from 65 couples where both partners were fully investigated and where the husband had fathered previous children (by his wife or by another partner)

	No. of couples	% of total
Wife ovulation or fallopian tube problems	15	23
Wife normal but husband had one of the following: Sperm density < 10 million/ml Antisperm antibodies (≥ GAT 1/125) Azoospermia	12	18
Tests on both partners within normal limits	15	23
Pregnancy either during investigation or within 2 years of follow-up	23	35

Sexual History

It is rare for infertility to occur as a result of psychosexual problems sufficiently severe to prevent intercourse; such problems appeared to account for two cases out of 662 couples seen at our clinic between 1978 and 1980. Indications of a possible psychosexual problem are:

1. It is very difficult to persuade the husband to attend the clinic;
2. The husband is unable or unwilling to produce semen samples for analysis;
3. An intact hymen is found on gynaecological investigation of the wife.

Problems with sexual intercourse such as premature ejaculation after vaginal penetration are common but these will not usually affect the chance of fertility. Psychosexual problems secondary to infertility are common if not universal. A more mundane problem is that either husband's or wife's work separates the couple or else shift work limits the opportunity for sexual intercourse. In most cases a knowledge of the fertile period is not essential for fertility and indeed if the wife counts the days too avidly this can result in impotence on the husband's part. In those cases where work causes separation it is important that the couple should be aware of the likely fertile time. The management of the orthodox Jew married to a wife with a short menstrual cycle has been clarified by Gordon et al. (1975).

The frequency of sexual intercourse is often assumed to be of great importance. This type of problem may be diagnosed if semen analysis is carried out: (a) after the couple's normal interval; and (b) after an interval of two to three days. In fact intercourse has to occur several times a day or less than three times a month before there is an appreciable delay in fertility (Table 2.2).

Table 2.2. Frequency of intercourse related to time taken to conceive (Yaukey 1961)

Reported coital frequency (times per month)	Months taken to conceive	
	Age at marriage	
	14–17	18–27
< 10	8.2	6.1
11–20	7.5	5.6
21–30	7.1	5.4
> 30	5.5	3.1

Occupational History

Hazardous Chemicals

In many cases there is a history of exposure to oil, tars or organic chemicals but most of these substances are far too widely used for their effect on fertility to be assessed. For example, systemically injected cadmium has been shown to damage the caput epididymis in rats (Gunn et al. 1963) and this effect can be counteracted by zinc and selenium (Gunn et al. 1968). Whether cadmium toxicity will affect human fertility has not been recorded and may seem irrelevant until the many uses of this metal are recognised (Table 2.3).

Table 2.3. Multitudinous uses of cadmium (Cadmium has been shown to damage spermatogenesis in laboratory animals; the effect on human fertility is unknown)[a]

Electronics	26.3%
Industrial fasteners	19.6%
Automotive parts	20.4%
Aircraft and aerospace	12.6%
Ordnance	5.6%
Hardware, e.g. keys	3.1%
Household appliances	2.3%
Ship building	2.5%
Other industrial uses	7.8%

[a] By courtesy of R Scott, Consultant Urologist, Royal Infirmary Glasgow

Alteration in semen analysis has been reported in lead workers (Lancranjan et al. 1975) who also found increased impotence in this group. Arsenic and zinc may also be toxic to sperm (Lindholmer 1974). Mercury poisoning manifest as acrodynia or pink disease in children may be associated with later subfertility.

Occasionally a specific factor comes under suspicion, e.g. Cannon et al. (1978) reported that 76 out of 133 workers at a plant producing the pesticide Kepone developed an illness characterised by nervousness, tremor, weight loss, clonus, pleuritic and joint pain and oligozoospermia. The seminal findings were only brought to light because of the thorough toxicity screen of the work force following hospital admission of patients with severe symptoms. In another factory in

California making DBCP, (1, 2-dibromo-3-chloropropane) a soil fumigant acting against nematodes, a number of cases of subfertility were reported by Whorton et al. (1977) and there seemed to be a correlation between semen analysis findings and exposure (Table 2.4). Whorton and Milby (1980) have followed up 21 of the men who were affected. Twelve were initially azoospermic and nine oligozoospermic. One year later, after stopping exposure, eight of the nine oligozoospermic men had improved and four wives were pregnant. None of the azoospermic men improved. One of the children born had multiple defects. Lantz et al. (1981) have also reported impaired semen analysis in workers exposed to DBCP with improvement following cessation of exposure.

Table 2.4. Comparison of non-vasectomised D.B.C.P. workers with very low (group A) and normal (group B) sperm counts[a] (From Whorton et al. (1977), by permission of D. Whorton and the Editor of the *Lancet*)

No. of subjects	Age (yr.)	Exposure time (yr.)	Sperm-count ($\times 10^6$/ml)	F.S.H. (mI.U/ml)	L.H. (mI.U/ml)	Testosterone (ng/dl)
A 11	32.7 ± 1.6[b]	8.0 ± 1.2[c]	0.2 ± 0.1[d]	11.3 ± 1.8[c]	28.4 ± 3.3[b]	459 ± 35
B 11	26.7 ± 1.2[b]	0.08 ± 0.02[c]	93 ± 18	2.6 ± 0.4[b]	14.0 ± 2.8[b]	463 ± 31

[a] All results given as mean ± SE of mean
[b] Difference between groups A and B significant at $P < 0.01$
[c] Difference between groups A and B significant at $P < 0.001$
[d] 9 workers without sperm, 2 with 1×10^6/ml

Other organic chemicals which have been shown to have toxic effects in animals, and may affect humans, are: ethylene oxide (sterilisation gas), α-chlorhydrin (discovered as part of a search for an antifertility drug), toluene diamine, certain organochlorines (e.g. DDT, dieldrin and pentachlorophenol wood preservative) organophosphates (agricultural insecticides derived from the organophosphate war gases) paraquat (herbicides) and carbamates (insecticides). The subject has been more extensively reviewed by Gomes (1977) and Mann and Lutwak Mann (1981) In most cases the hazard to humans is unknown but it is salutary to note that the wood preservative pentachlorophenol was actually detected in human semen by Dougherty and Piotrowska (1976); the particular hazard occurs because many of the above compounds are easily absorbed through the skin.

Another area of concern is dietary additives. In some countries hormones (particularly stilboestrol) are fed to cattle to promote growth and these may persist, resulting in ingestion of significant doses by humans. Workers in factories making these compounds may be at risk.

The sensitivity of the germinal epithelium to mutagenic chemicals has long been realised and recently the 'mouse sperm test' has been developed for the rapid screening of potential mutagens and carcinogens (Topham 1980).

Hazardous Environments

Certain working environments may impair fertility, e.g. boiler rooms or other very hot places. One occupation that has come under some suspicion is that of fighter pilots who, because of their warm survival clothing and lack of G-suit protection in the testicular area, may sustain testicular damage (Jequier 1980).

There is also an impression among some clinicians that long-distance drivers may be at risk possibly because sitting in a heated lorry cab allows high scrotal temperatures to develop, and if so this is similar to the situation of paraplegic men in wheelchairs (see Chap. 15).

Cancer Chemotherapy

Now that effective chemotherapy is available for some malignant disease, attention is being focused on criteria other than survival rates. One area of concern is the subsequent reproductive ability of children treated for leukaemia, nephroblastoma and other formerly fatal childhood cancers; also of concern is the reproductive ability of young adults treated for Hodgkin's disease, leukaemia, Burkitt's lymphoma, and testicular malignancy.

The potentially devastating effect of chemotherapy was highlighted by Chapman et al. (1979) who reported on gonadal function in 74 men who received M.V.P.P. (nitrogen mustard, vinblastine, procarbazine and prednisolone) for Hodgkin's disease. All patients were rendered azoospermic with a raised FSH level following therapy and only four patients showed evidence of renewed spermatogenesis despite follow-up of up to 62 months. In addition 25 men (46%) reported a persistent long-term reduction in libido. In considering the counselling of young adult men about to start chemotherapy several factors need to be borne in mind:

1. Is Spermatogenesis Normal Prior to Therapy? There is evidence that a significant percentage of men may have impaired spermatogenesis before treatment is begun (Bracken and Smith 1980; Sanger et al. 1980). Thachil et al. (1981) found that 22 out of 42 (52%) of men with germinal testicular tumours had a sperm density of less than 20 million/ml. The cause of this damage is not known. Possible factors are: the general systemic upset associated with many cancers; the effect of anaesthetic and operation if semen analysis is carried out after operation but before chemotherapy; and finally in some cases of testicular neoplasm, an associated history of bilateral testicular undescent. In view of the compromised fertility of many patients prior to chemotherapy great care must be taken before ascribing damage to a particular drug.

2. Is the Proposed Regime Known to be Damaging? The current evidence is that most of the alkylating agents, including chlorambucil, melphalan, cyclophosphamide, nitrogen mustard and disulphide have been shown to damage human fertility (Thachil et al. 1981; Schilsky et al. 1980) and many other effective chemotherapeutic agents are suspect; thus the effects of *cis*-platinum are as yet unknown. Methotrexate given for psoriasis produces oligozoospermia which improves when the drug is stopped (Sussman and Leonard 1980). In the light of current knowledge it is wise to counsel patients that the regime is likely to damage fertility and that sterility may be permanent.

3. Is There Likely to be Any Recovery? The results reported by Chapman et al. (1979) suggest that sterility may be permanent. In our experience of severe subfertility secondary to other factors the time to recovery of normal spermatogenesis may be as long as 3 years. Many studies do not continue follow-up of

fertility for this length of time although since the patient is usually continuing to attend hospital this should be possible.

4. Is Sperm-Banking Appropriate? Usually, the news that a young man has malignancy is devastating and treatment quickly follows. There is thus little time for the impact of possible future sterility to be absorbed. In some cases sperm-banking may not be possible because of poor semen, and even in those cases where it is apparently possible, there may be more subtle defects in the spermatozoa not readily detected by current laboratory techniques. Thus even in good cases the chances of subsequent pregnancies resulting from the stored samples may not be good. There is no doubt, however, that there may be considerable psychological benefit from the knowledge that samples are stored. If sperm-banking is undertaken, samples should probably not be used until the maximum at-risk time for tumour recurrence has passed; in most cases 1–2 years.

5. What Advice About Post-treatment Contraception? It is usually undesirable for a pregnancy to occur during the first year following chemotherapy because this is the maximum at-risk time for tumour recurrence. The couple may choose to continue contraceptive use or alternatively advice may be given in the light of findings on semen analysis.

6. Is There Any Measure that Will Prevent Damage? Glode et al. (1981) reported an animal model where mice can be protected from cylcophosphamide-induced testicular damage by [D-Leu^{6}]des-Gly-$NH_2{}^{10}$ pro-ethylamide GnRH, a synthetic analogue of gonadotrophin-releasing hormone. This substance blocks the pituitary–gonadal axis resulting in depressed androgen synthesis as well as spermatogenesis. Whether this can be safely applied to humans and whether there will be time for this sort of treatment to work before chemotherapy must commence is still unknown.

Chemotherapy given before the onset of puberty may be less damaging. Shalet et al. (1981), have recently reported on 44 boys who had evidence of preserved Leydig cell function using the hCG stimulation test following combined chemotherapy. Some of these boys have subsequently undergone normal pubertal development.

Irradiation

Male infertility following irradiation was first reported in prison volunteers (Thorsland and Paulson 1972). Post-irradiation infertility is now usually encountered after treatment for cancer but may occasionally be an occupational hazard. Such infertility is seldom reversible (Lushbaugh and Casarett 1976). Azoospermia has also been reported following I-131 therapy for thyroid cancer (Handelsman et al. 1980).

Drugs Associated with Infertility

Table 2.5 lists drugs that may interfere with spermatogenesis and Table 2.6, antihypertensives which may result in impotence. It is uncommon for a young man

attending an infertility clinic to be on long-term medication; probably the most commonly encountered drugs are sulpha drugs given to control colitis or long term anticonvulsants.

Table 2.5. Drugs causing inhibition of spermatogenesis

1. *Cytotoxic Drugs*
   Busulphan
   Chlorambucil
   Vincristine
   Cyclophosphamide
   Melphalan
2. *Anabolic Steroids*
3. *Cimetidine*
   Thought that it may competitively inhibit androgens.
4. *Sulphasalazine*
   Reported to cause oligozoospermia (reversible on discontinuance of the drug). This could be due to antifolate activity inhibiting maturation and perhaps antiprostaglandin activity inhibiting sperm motility (Toovey et al. 1981)
5. *Spironolactone*
   Tends to antagonise the action of androgens in tissues
6. *Propranolol*
   May inhibit spermatozoal motility
7. *Opiates*
   These may result in elevation of prolactin levels and consequent inhibition of fertility
8. *Nitrofurans*
   These may immobilise sperm and were used by Albert et al. (1975) to wash out the vas after vasectomy!
9. *Niridazole*
   An antischistosomal drug works by inhibiting spermatogenesis in the gonads of the schistosome
10. *Colchicine* (Merlin 1972)
11. *Ethanol*
    This may cause a relative deficiency of vitamin A
12. *Marijuana*

Table 2.6. List of antihypertensives causing impotence

Type of Antihypertensive	Drug name	Method of causing impotence
Ganglion blocker	Guanethidine	Erectile failure; failure of ejaculation
Ganglion blocker	Bethanide	Erectile failure; failure of ejaculation
Ganglion blocker	Debrisoquine	Failure of ejaculation
Centrally acting (enzyme inhibitor)	Methyldopa	Erectile failure; failure of ejaculation; reduction in libido
Centrally acting	Clonidine	Erectile failure; failure of ejaculation; reduction in libido
Central and peripheral depressant action	Reserpine	Reduction in libido; failure of ejaculation
Predominantly peripheral effect	Prazocin	Reports of sexual dysfunction rare
β-Blocker	Labetalol and Propranolol	Few isolated reports of sexual dysfunction
Diuretic	Spironolactone	Erectile failure; tends to antagonise actions of androgens in tissues

Smoking

There is evidence that cigarette smoking impairs spermatogenesis. Viczian (1969) reported an increase in the number of abnormal morphological forms of spermatozoa in smokers compared with non-smokers and we have confirmed these findings (Table 2.7).

Table 2.7. Morphological analysis of spermatozoa in smokers and non-smokers. Samples were analysed from infertile men in groups matched according to sperm density. Men with varicocele, undescended testis or any history of exposure to dangerous chemicals or other noxious environments were excluded from the analysis (From Evans et al. (1981) by permission of the Editor of the *Lancet.)*

Sperm density	Non-smokers No. of men	% Normal forms ± SE	Smokers No. of men	% Normal forms ± SE
<20	3	48.7 ± 3.2	3	43.7 ± 5.47
20–40	5	61.2 ± 2.73	3	45.3 ± 1.51
40–60	12	54.3 ± 2.06	14	55.1 ± 2.73
>60	11	59.6 ± 2.06	13	53.4 ± 1.55

Disease Association

Primary testicular failure may occur after orchitis secondary to mumps, tuberculosis, gonorrhoea, brucellosis, typhoid, influenza, undulant fever, smallpox and syphilis. There may also be a direct depression of spermatogenesis in diabetes and renal failure. Secondary testicular failure may occur after hypothalamic pituitary diseases such as chromophobe adenoma, astrocytoma, hamartoma, teratoma and sarcoidosis. In most of the above there will be evidence of androgen deficiency. The following congenital disorders are also associated with infertility: Kartagener's syndrome, cystic fibrosis, prune belly syndrome and coeliac disease.

Many febrile illnesses may cause temporary or permanent subfertility e.g. malaria, filariasis, Bornholm's disease, meningococcal meningitis, glandular fever, atypical pneumonia, enteric fever, undulant fever, sandfly fever, amoebiasis, schistosomiasis kala azar and lymphogranuloma venereum. Reduced semen density measurements have recently been reported in patients with sickle cell disease (Osegbe et al. 1981).

Physical Examination

The husband should be examined in a warm room both standing erect and lying on the examination couch. Clothes should be removed to enable accurate assessment of the endocrine status and build. It is often convenient to apply simple objective tests at the time of physical examination, e.g. the testicular size can be measured with an orchiometer and testicular venous return assessed by Doppler analysis.

General Examination

Observation is made of the body configuration and any deposition of fat over the hips or gynaecomastia is noted. In Klinefelter's syndrome the limbs may be disproportionately long in relation to the trunk but in many cases there are no obvious clinical features (Fig. 2.1). In other chromosomal disorders there may be associated skeletal deformity (Fig. 2.2). A tall stature and immature physique may suggest an endocrinological factor resulting in delayed puberty but usually such patients have sought advice earlier and come to the infertility clinic with the diagnosis already made.

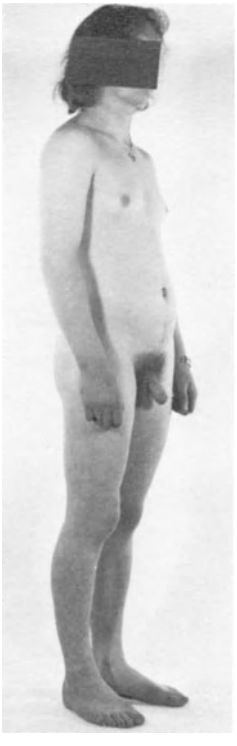

Fig. 2.1. Klinefelter's syndrome

Gynaecomastia

Gynaecomastia is rare in a general infertility clinic but may be seen in association with hyperprolactinaemia. Thorner et al. (1977) reported the clinical findings in 17 cases of male hyperprolactinaemia; gynaecomastia if present was slight, although one third of cases had galactorrhoea; 90% were impotent with loss of libido, lack of erection and in some cases diminished semen volume. The testes were either normal in size or small but were usually soft. The body fat distribution usually resembled that of a female.

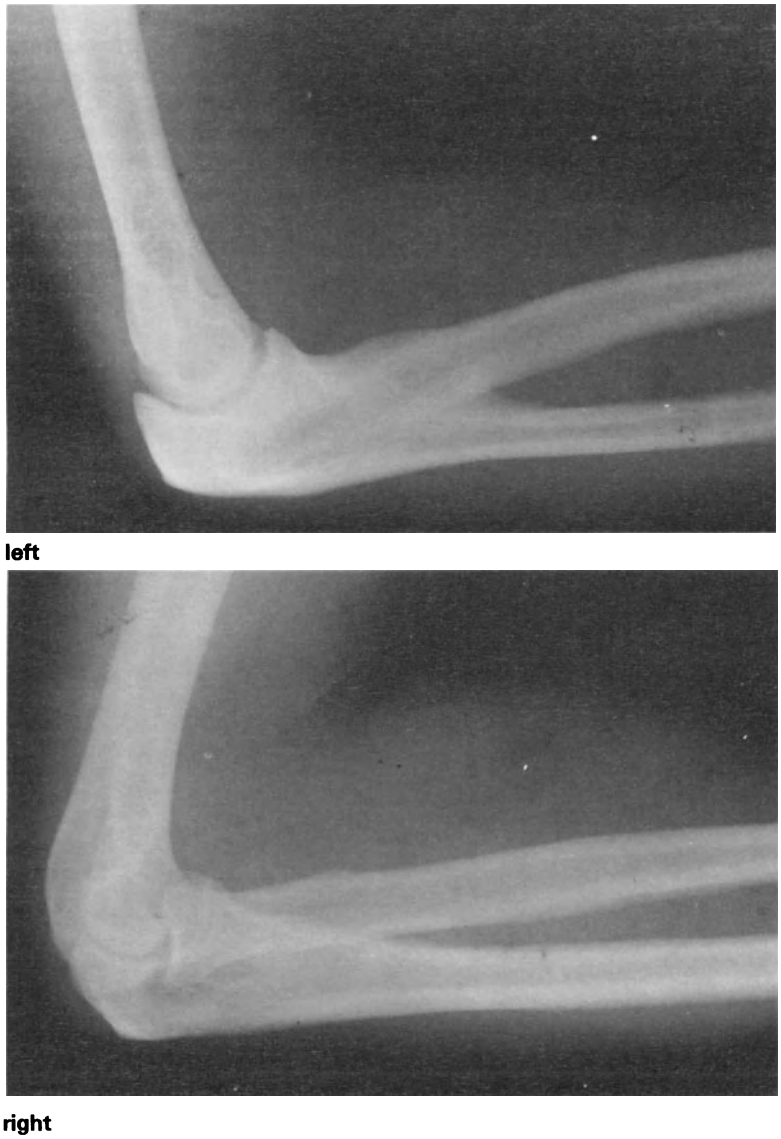

Fig. 2.2. Fusion of radius and ulna noted on clinical examination in a man with trisomy 8 who presented with an infertile marriage. (Chandley et al. 1980)

Body Hair Distribution

Body hair is extremely variable, depending on both genetic and hormonal factors. In cases of delayed puberty there may be scanty body hair as well as tall stature. Body hair would appear to be a dihydrotestosterone-mediated secondary sex characteristic whereas pubic and axillary hair are probably related to testosterone or oestradiol secretion.

Examination of the Penis

There is some variation in penile size and contrary to popular belief this rarely influences fertility. Penile deformity during erection may occur as a result of Peyronie's disease or because of inadequate surgical correction of chordee associated with hypospadias. It is difficult in most clinics to create circumstances where a natural erection may be observed but usually the couple will have given an history of difficulty with intercourse. The degree of penile deformity during erection can also be assessed pre-operatively by asking the patient to take Polaroid photos of himself while at home. Infertility secondary to Peyronie's disease is uncommon because this condition has a maximal incidence at 40 years by which time the issue of fertility has usually been resolved. Penile deformities secondary to hypospadias are more common and the extent of the deformity may not be evident from clinical examination of flaccid penis. It is wise in such cases to believe the patient and to assess the extent of the deformity by artificial erection with saline infusion in the operating theatre prior to surgical correction.

The foreskin should always be retracted as simple problems such as meatal stricture or phimosis can impair fertility by making ejaculation ineffective or intercourse painful and in any case should be corrected to ensure efficient voiding of urine. Previous urethroplasty may result in defective ejaculation because the reconstructed urethra consists of a skin tube with no muscle coat. (see Chap. 14)

Examination of the Testes

The size and consistency of the testes should be assessed. The size may be defined as large, medium or small, but it is more accurate to use an orchiometer. The bulk of the testes is composed of seminiferous tubules and it is therefore not surprising that there is good correlation between the measure of testicular size and spermatogenesis as measured by the Johnsen Score (Table 2.8).

Table 2.8. Relationship between testicular size and spermatogenesis. Numbers represent numbers of men (Pryor 1980)

Testicular length (cm)	Mean Johnsen score		
	<2.0	2.1–8.0	>8.1
<3	14		
3–4	18	21	12
4–5	18	17	33
>5	4	16	57

There are racial differences in testicular size and this is presumably due to differences in body weight. In orientals the mean testicular weight at autopsy of 100 individuals varying in age from late teens to 70s was: right testis 10 g (SE ± 0.3), left testis 9.4 g (SE ± 0.3) (Change et al. 1960). Comparable data for 140 caucasians gave the following measurements: right testis 21.6 g (SE ± 0.4), left testis 20.4 g (SE ± 0.5) (Olesen 1948). Some of the differences may be due to a Y-linked gene (Short 1979).

Epididymis

Both epididymes are palpable as soft tissue adjacent to the testes. A soft cystic swelling in the head of the epididymis in conjunction with azoospermia is indicative of obstruction. A hard epididymis may be associated with a past history of venereal disease, but despite the prevalence of gonorrhoea in the UK (Fig. 2.3) this is a rare finding, presumably because primary treatment is effective. This is not the case in some countries, where many patients with obstructive azoospermia give a history of past gonococcal epididymitis; this may explain regional differences in the results of epididymo-vasostomy. Painless craggy swelling of the epididymis may indicate tuberculous disease of the urinary tract.

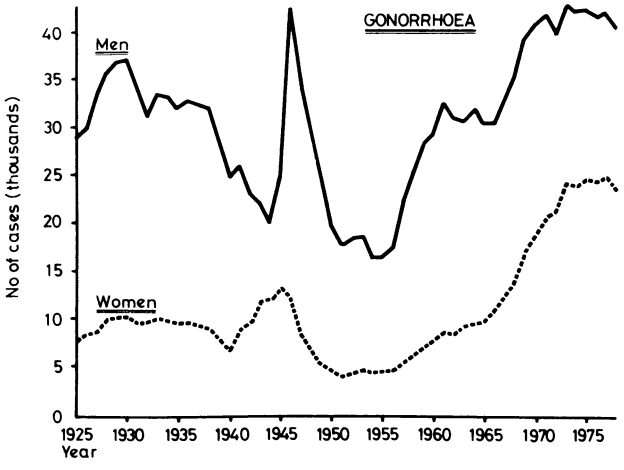

Fig. 2.3. Prevalence of gonorrhoea in the UK. Figure reproduced by permission of the Academic Department of Genitourinary Medicine, Middlesex Hospital, the Communicable Surveillance Centre and Communicable Diseases (Scotland) Unit, and the Editor of the *British Medical Journal*

Vas Deferens

Both vasa should be palpable. Bilateral absence of the vasa accounts for 9% of our cases of azoospermia or 1% of the last 662 cases we have seen. This is similar to the incidence of 2% in 5000 cases reported by Amelar et al. (1975). Unilateral absence of a vas is much rarer but is often associated with renal abnormality or an ectopic kidney on the same side. If unilateral absence is found it is reasonable to investigate the urinary tract with intravenous urography to define any urological abnormality.

Hernial Orifices

The skin over the hernial orifices should be carefully examined for scars because the patient may be unaware of herniorrhaphy in infancy.

Prostate

In most cases examination of the prostate does not reveal any pathological condition. It is standard practice in many clinics to perform prostatic massage and to examine prostatic fluid for pus cells. A tender prostate may indicate prostatitis and in such cases bacteriological studies and a white cell count should be performed on the semen sample and antibiotic treatment should be considered.

Varicocele

This condition, which may account for one of the largest potentially treatable groups of patients, is discussed in Chap. 11. Examination for varicocele is more reliable if the patient is standing erect but still there may be a discrepancy between the clinical findings of different physicians. In view of this variability of clinical examination and the conflicting results of surgery there is a need for objective non-invasive methods to assess varicocele. Special tests are described below.

Doppler Analysis

This technique is simple and can be performed at the time of physical examination; each Doppler assessment takes approximately 5 min. The apparatus is portable and little skill is required to obtain reproducible results. In most cases one side of the scrotum will act as a control, the difference between the two sides indicating that the apparatus is working. The examination is performed with the patient standing erect breathing quietly and also during a Valsalva manoeuvre. The resulting activity graphs can be described as: (1) no activity; (2) activity during Valsalva only; (3) intermittent activity not related to Valsalva and not increased by Valsalva; (4) intermittent activity increased by Valsalva; and (5) continuous activity (Fig. 2.4). The results of Doppler analysis do not however always correlate with

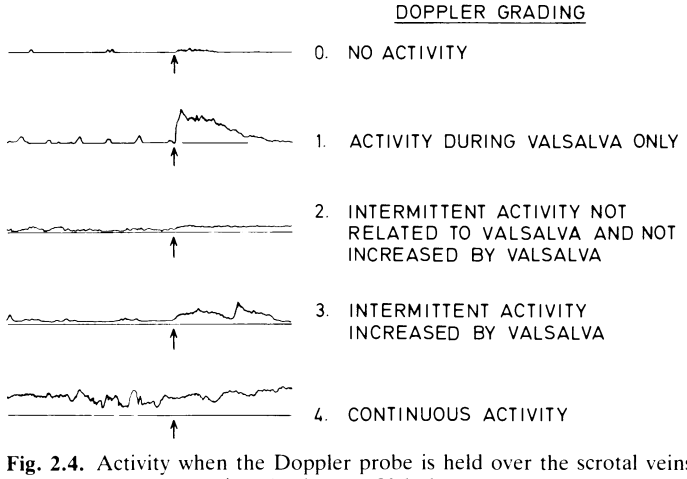

Fig. 2.4. Activity when the Doppler probe is held over the scrotal veins above the testis. The arrow indicates when the patient begins the Valsalva manoeuvre

either clinical examination or venography (Hirsh et al. 1980). There is thus need for further assessment of whether Doppler findings are a useful prognostic index as to who will respond to ligation.

Thermography (section by Mr. W. F. Hendry)

Thermography provides a measurement of scrotal surface temperature, and in the absence of subcutaneous fat provides an accurate assessment of the temperature of the underlying testes. Kormano et al. (1970) used thermography to show that varicocele increased the temperature of the left side of the scrotum, and Comhaire et al. (1976) showed that thermographic abnormality correlated well with the presence of internal spermatic vein reflux demonstrated by retrograde caval venography. Thermography is non-invasive and can be repeated as often as necessary.

Clinical Studies of Thermography We have studied 12 fertile control subjects (successful AID donors) and 40 subfertile males with possible varicocele, clinically and by scrotal thermography. The scrotal temperature was measured with the Rank Thermographic System or the 680 AGA Medical Thermovision, after 10 min equilibration at an ambient temperature of 19°C (Hendry et al. 1973; Jones and Hendry 1979). The subject stood in front of the thermography camera with legs slightly apart and penis held out of the field of view. The anterior view was supplemented by an underside or posterior view obtained with the aid of a narrow polished aluminium mirror supported at an angle of 45° beneath the scrotum.

The results in ten of the fertile control subjects with no clinical evidence of varicocele are shown in Table 2.9. The maximum temperature difference between the two sides was 0.3°C. Two of the fertile control subjects had obvious varicoceles and their scrotal temperatures were as follows: *Subject 1*: 29.8°C (right) and 34.1°C (left) on anterior view, 30.4°C both sides on underview; *Subject 2*: 31.8°C both sides anterior view, 35°C both sides on underview. In the subfertile patients, the thermographic readings were considered to be abnormal if the scrotal temperature exceeded 32°C, or if there was a difference between the two sides of more than 1°C. The results for 12 infertile men with equivocal varicoceles on clinical examination are shown in Table 2.10. Thermographic abnormality was demonstrated in five patients, four of whom had scrotal temperatures above 32°C; the abnormality was only detected on the underview in one patient (Fig. 2.5), and was bilateral in one

Table 2.9. Scrotal thermographic findings in 10 fertile control subjects with no clinical evidence of varicocele (range and mean)

	Temperature	
	Anterior view	Underview
Right side	29.5°C–31.2°C (mean 30.4°C)	29.6°C–32.2°C (mean 30.7°C)
Left side	29.5°C–31.5°C (mean 30.5°C)	29.6°C–32.2°C (mean 30.7°C)

Maximum difference between the sides 0.3°C

Table 2.10. Results of scrotal thermography in subfertile males; numbers represent number of patients

Type of patient	Number	Temperature > 32°C		Temperature difference >1°C		Normal
		Left	Right			
Equivocal varicocele seen on clinical examination	12	4	1	4		7
Possible bilateral varicocele	7	4	4	1		3
Possible residual or recurrent varicocele 3 months after surgical ligation	21[a]	8	8	L > R 6	R > L 6	7

[a] Five produced pregnancies after reoperation

case. In one patient the temperature of left side was 31.5°C, and that of the right side was 29°C, giving an abnormal temperature differential of 2.5°C. All five patients with thermographic abnormalities had ligation of varicocele, while the seven patients with normal thermogams were not operated on.

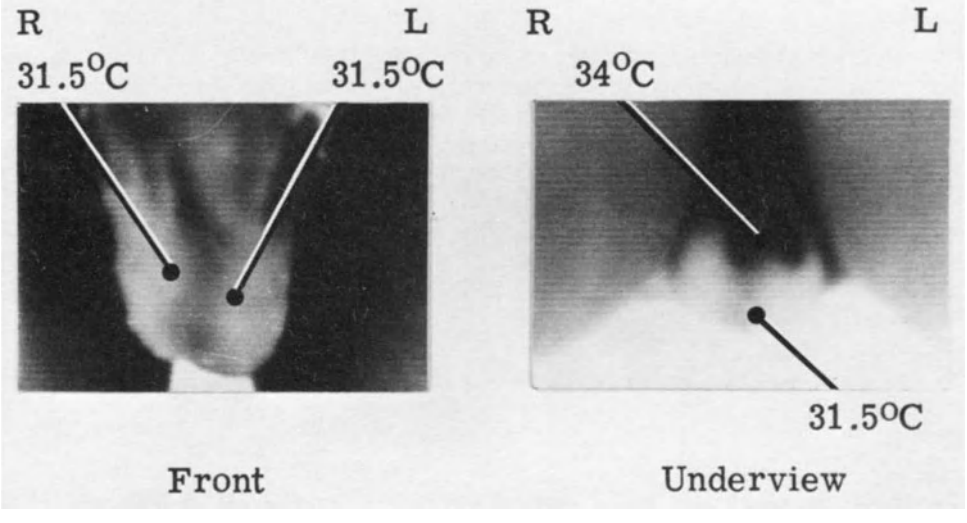

Fig. 2.5. Anterior and underview thermograms in subfertile male with doubtful clinical findings: normal anterior findings, but markedly raised temperature on left side posteriorly

Seven infertile patients had possible bilateral varicoceles; elevated temperatures on both sides of the scrotum were observed in four cases, although in only one was there a temperature difference between the two sides of more than 1°C. All four patients had bilateral ligation of varicoceles (See Fig. 2.6). In the remaining three patients the thermographic findings were normal and no surgery was done (Table 2.10).

Table 2.10 also shows the results in the remaining 21 infertile patients who had had a varicocele ligated 3 months or more previously, and in whom there was doubt on clinical examination as to whether or not the varices had gone completely. Seven were normal and no further surgery was considered. In 14 patients, however, there

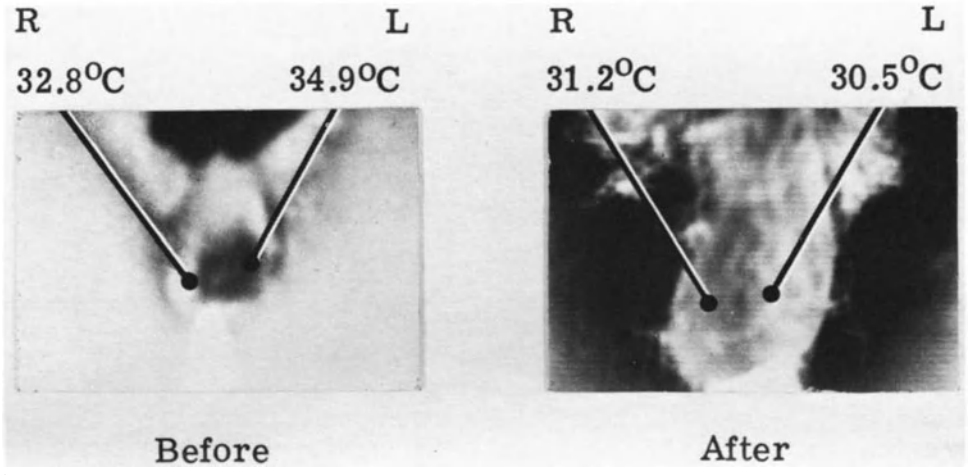

Fig. 2.6. Bilateral varicoceles, before and after surgical correction

were persistent thermographic abnormalities in the scrotum. In six patients the left side was more than 1°C warmer than the right, indicating persistent left-sided varicocele. In a further six patients, the *right* testis was warmer than the left, probably indicating unrecognised bilateral varicoceles that were only partially corrected by ligation on the left side only. Following reoperation on left, right or both sides, the thermographic findings were re-checked in five patients: they were normal in all cases (Fig. 2.7). In these cases improvement in sperm count did not occur until the varicocele was completely corrected, despite supplementary medical therapy (see Fig. 2.8). Five pregnancies were produced followed reoperation on these residual or recurrent varicoceles.

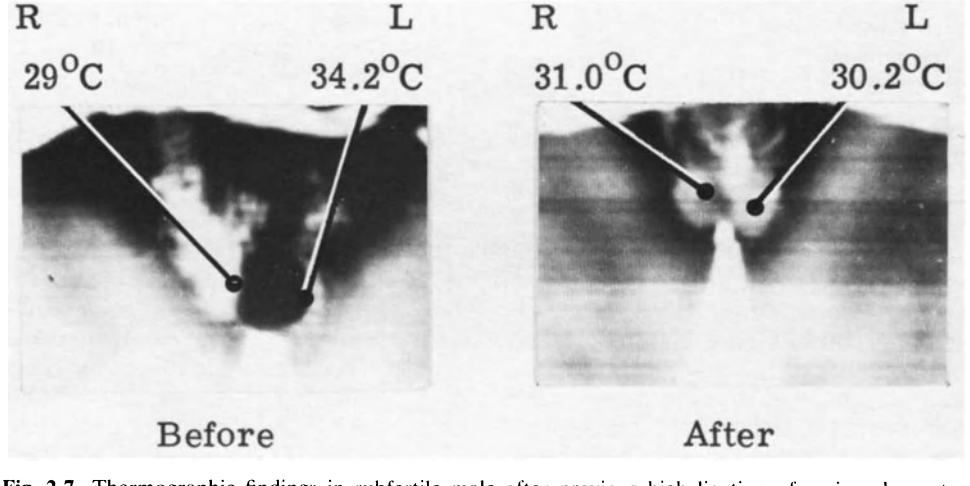

Fig. 2.7. Thermographic findings in subfertile male after previous high ligation of varicocele: note persistence of raised temperature on left side, corrected by reoperation

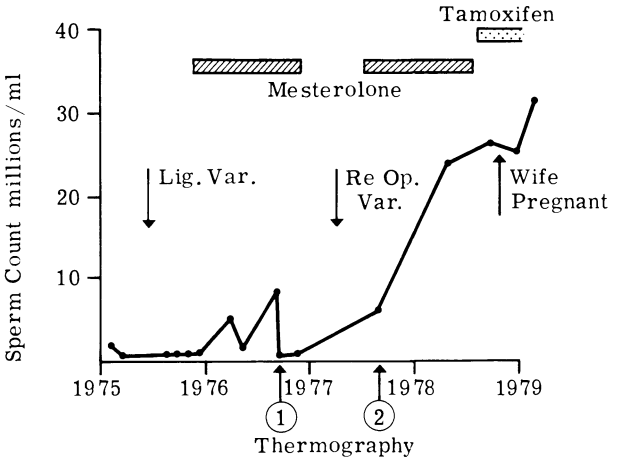

Fig. 2.8. Subfertile male who had abnormal thermogram (*left* 32.5°C; *right* 30.9°C) after ligation of varicocele, subsequently corrected (28.8°C and 28°C) by reoperation. Note that sperm count did not improve to satisfactory levels, even with medical therapy, until the thermographic abnormality was corrected

Conclusion from Clinical Studies

Scrotal thermography offers a painless, non-invasive objective test which can confirm or refute the diagnosis of varicocele associated with significant alteration in scrotal temperature. This information is of real help to the surgeon who must decide whether to recommend an operation and whether to undertake either unilateral or bilateral surgery. It also provides an objective test for the critical assessment of the results of surgery and routine post-operative use to detect unsatisfactory results has resulted in a reoperation rate of 5%.

The results shown in Table 2.9 are roughly in accordance with those reported for 171 normal men by Fornage and Lemaire (1978) who found mean (±SD) values of 29.33°C ± 0.18°C; we found a rather broader range of normality, and believe that our upper limits of normality of 32°C or a differential of more than 1°C between the two sides are reasonable though a little conservative.

The observation of varicoceles with grossly abnormal thermographic findings in two of our fertile control subjects was of considerable interest, and confirmed that varicocele does not necessarily interfere with fertility. However, some clinicians regard the mere existence of a varicocele as an indication for surgery even if other findings are normal.

Clinical diagnosis of the presence and extent of varicocele is imprecise, whereas thermography can provide accurate objective evidence of disturbed scrotal temperature which may be important in a subfertile man with evidence of impaired spermatogenesis. The results of surgery are not uniformly good, and improvement in semen quality and resulting pregnancy in the spouse may not occur until the temperature abnormality has been corrected, which may require reoperation. We believe that thermography adds a degree of precision to the diagnosis of varicocele and that this is of considerable value in the management of the subfertile male. With the advent of liquid crystal contact thermography this test may become universally available.

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Appendix

Infertility Questionnaire in use at the Western General Hospital, Edinburgh. The questionnaire is sent to the couple before their first consultation. It has been used by more than 1000 couples and so far only 2 have failed to complete the questions. This failure was because of poor literacy. The layout is designed to show relevant answers in the right hand margin which helps to identify problems quickly.

Infertility Clinic
University Department of Surgery Urology
Western General Hospital
Edinburgh.

Answer question by ticking box YES—∅
NO—○

Please could you and your wife carefully answer these questions together. I realise that this is a long questionnaire but remember we are trying to help you and this has been designed to give us all the information about your case so that we can give you our best possible advice. Please take some time and try to answer all the questions very carefully. When you come to the Out-patient clinic *bring this questionnaire with you and give it to me when I see you. DO NOT* give it to the nurse or receptionist.

T. B. HARGREAVE
Consultant Urologist

Mr. & Mrs. .....
Address .....
.....
Husband's Christian Name .....
Wife's Christian Name .....
How long have you been married or living together? .....
How long have you been trying for a child? .....
(Please be as accurate as you can when answering this question)
If you used contraception, please say what method and for how long .....
.....
Do you have any children by each other? → YES—○
NO—○
Ages of children .....

HUSBAND
(1)

Please state your age ☐☐

Date of birth ☐☐☐☐☐☐

Please could you state your country of birth .....

If you do not live in the UK please state the country in which you live .....

PREVIOUS MARRIAGE

Have you been married before? ——→ YES——○ NO—○

Have you any children by someone else? ——→ YES——○ NO—○

Ages of children .....

If you have been married before were you trying to have children during that marriage ——→ YES——○ NO—○

FAMILY HISTORY

Have you any brothers or sisters? ——→ YES——○ NO—○

BROTHERS

Ages	Married (Yes/No)	Nos. of children if any
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

SISTERS

Ages	Married (Yes/No)	Nos. of children if any
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

Have any of your relatives been attending hospital because of infertility problems? ——→ YES——○ NO—○

If YES please give details if known .....

.....

.....

.....

Is there any illness in your family (e.g. tuberculosis or heart disease)? ——→ YES——○ NO—○

If YES please give details .....

.....

.....

HUSBAND
(2)

HEALTH RECORD

Have you ever had any serious illnesses? ——→ YES——○ NO—○

If YES please give details as far as you know them .....

.....

Have you ever had any of the following? If you were under 10 years of age write 'AS CHILD'.

Mumps ——→ YES——○ NO—○ If YES at what age .....

Measles ——→ YES——○ NO—○ If YES at what age .....

Chickenpox ——→ YES——○ NO—○ If YES at what age .....

Malaria ——→ YES——○ NO—○ If YES at what age .....

Typhoid ——→ YES——○ NO—○ If YES at what age .....

Dysentery ——→ YES——○ NO—○ If YES at what age .....

Hepatitis ——→ YES——○ NO—○ If YES at what age .....

Bronchitis ——→ YES——○ NO—○ If YES at what age .....

Asthma ——→ YES——○ NO—○ If YES at what age .....

Sinus trouble ——→ YES——○ NO—○ If YES at what age .....

Chest trouble ——→ YES——○ NO—○ If YES at what age .....

Stomach ulcer ——→ YES——○ NO—○ If YES at what age .....

Duodenal ulcer ——→ YES——○ NO—○ If YES at what age .....

Long term bowel trouble ——→ YES——○ NO—○ If YES at what age .....

Epilepsy ——→ YES——○ NO—○ If YES at what age .....

Diabetes ——→ YES——○ NO—○ If YES at what age .....

High blood pressure ——→ YES——○ NO—○ If YES at what age .....

Heart problems ——→ YES——○ NO—○ If YES at what age .....

HUSBAND
(3)

Cancer → YES—○ If YES at what age .....
NO—○

Radiotherapy → YES—○ If YES at what age .....
NO—○

Have you ever been in to hospital? → YES—○
NO—○
If YES please give details as far as you know them .....
.....

Have you ever had any other tropical disease, e.g. Filiaris → YES—○
NO—○
If YES please give details .....
.....
.....

If you had mumps or measles did this affect your testicles? → YES—○
NO—○
If YES please give details .....
.....
.....

Have you ever suffered from any illness associated with shivering (rigors) and/or high fever? → YES—○
NO—○
If YES please give details of severity and length of illness
.....
.....

Have you ever had an operation? → YES—○
NO—○
If YES please give details (e.g. mastoid operation when a child)
.....
.....

Have you ever had a hernia operation (even if done when you were a baby)? → YES—○
NO—○
If YES please give details .....
.....

Have you ever had any injury or operation to the penis or testicles?

Circumcision → YES—○
NO—○

Other operations on penis → YES—○
NO—○

Varicocele operation
(varicose veins near testicles) → YES—○
NO—○

Vasectomy → YES—○
NO—○

Biopsy of the testicle → YES—○
NO—○

Other operations or injuries to the testicles (please give details) → YES—○
NO—○

.....
.....

HUSBAND
(4)

Has there been any recent change in the size of your testicles? ——→ YES—○ NO—○
If YES please give details .....
.....

What sort of underwear do your normally wear?
Y fronts ——→ ○
Boxer shorts ——→ ○
Other ——→ ○

Have you ever had any venereal disease? ——→ YES—○ NO—○
If YES please give details .....
.....

If you have had venereal disease please could you state what treatment you received
.....
.....

Have you ever had any discharge from the penis? ——→ YES—○ NO—○
If YES please give details .....
.....

Is your weight Steady ——→ ○
Gaining ——→ ○
Losing ——→ ○

Are you taking any of the following:
Medicine, drugs, sleeping pills, herbal remedies or vitamin pills? ——→ YES—○ NO—○
If YES please give details (even if not prescribed by doctor) .....
.....

Have you ever had to take a long course of tablets or medicine for more than 2–3 weeks? ——→ YES—○ NO—○
If YES please give details .....
.....

Do you smoke now? ——→ YES—○ NO—○
If YES please say how many cigarettes or how much tobacco smoked each day
For how many years have you smoked? .....

Have you ever smoked in the past? ——→ YES—○ NO—○
If YES please say how many cigarettes smoked per day .....
If YES please say how many years since you stopped smoking .....

Do you ever drink alcohol (beers, wines, spirits) ——→ YES—○ NO—○
If you have answered YES please could you complete the following questions.
When was the last time you had an alcoholic drink?

REMEMBER WE ARE TRYING TO HELP YOU, SO PLEASE ANSWER CAREFULLY.

HUSBAND
(5)

Within the last 7 days	
8 days–14 days	
15 days–21 days	
21 days–1 month	
within the last 2 months	
within the last 3 months	
within the last 6 months	
within the last year	

Starting with yesterday I would like you tell me exactly what you had to drink over the past 7 days. Please be exact, e.g. 2 cans of Carlsberg Special Brew Monday lunch time.

Monday _____
Tuesday _____
Wednesday _____
Thursday _____
Friday _____
Saturday _____
Sunday _____

Was that typical of what you have been drinking recently? ——→ YES—○ NO—○

If NOT typical please say why .....

.....

Have you ever had a problem with drinking too much in the past? ——→ YES—○ NO—○

HUSBAND
(6)

If YES please give details .....
.....

Are you in the habit of taking very hot baths? ——→ YES——○ NO—○

Are you in the habit of taking sauna baths? ——→ YES——○ NO—○

Please state your shaving habits
More than twice a day
Twice a day
Once a day
Every other day
Less than every two days

LIVING AND WORKING ENVIRONMENT

Do you live in a built up area, e.g. town or city, or in the country?

Built up area ——→ ○
Country ——→ ○

Please state how far your home is from a busy main road, e.g. town high street, 'A' road, motorways or any other heavily used road

Less than 50 yards ——→ ○
(e.g. two houses' width)
More than 50 yards ——→ ○

Is your place of work, e.g. factory, office, farm, shop etc. in a built up area or in the country?

My place of work is in the country ——→ ○
My place of work is in a built up area ——→ ○

OCCUPATIONAL HISTORY

What is your present job/occupation? .....
.....

Please could you indicate the exact nature of your work, eg. Office work, Factory work, Manual work, Driving, etc.
.....
.....

Do you have to work night shifts? ——→ YES——○ NO—○

If YES please say whether it is constant, regular or occasional
.....

Please list your previous jobs. Work systematically from leaving school ensuring that no periods are omitted. In the case of working abroad, record which country. For seamen put 'at sea'

Job *Details of type of job*

1 .....
2 .....

HUSBAND
(7)

3 .....
4 .....
5 .....
6 .....
7 .....
8 .....
9 .....
10 .....
11 .....
12 .....
13 .....
14 .....
15 .....

Have you ever been exposed to very high temperatures, e.g. furnaces, boilers etc.? ——→ YES——○ NO—○

If YES please give details .....
.....
.....

Do you or have you ever worked with any of the following chemicals:

Lead ——→ YES——○ NO—○

Cadmium ——→ YES——○ NO—○

Mercury ——→ YES——○ NO—○

Cyanide ——→ YES——○ NO—○

Arsenic ——→ YES——○ NO—○

Herbicides ——→ YES——○ NO—○

Pesticides ——→ YES——○ NO—○

Fungicides ——→ YES——○ NO—○

Dyestuffs ——→ YES——○ NO—○

Vinyl chloride ——→ YES——○ NO—○

Plastics ——→ YES——○ NO—○

Oil ——→ YES——○ NO—○

HUSBAND
(8)

Tars → YES—○ NO—○

Radioactive substances → YES—○ NO—○

Other chemicals (solvents etc.) → YES—○ NO—○

If you have answered YES to any of the above could you please give details of where and when you were exposed to the chemical

.....

.....

.....

Have you at any time ever been overcome by chemical or poisonous fumes or exceeded permitted radiation doses? → YES—○ NO—○

Have you ever been treated in the factory or company sick bay because of poisonous chemicals or fumes or radiation? → YES—○ NO—○

Have you ever been admitted to hospital because of poisonous chemicals or fumes or radiation? → YES—○ NO—○

Have you ever changed your job because of poisonous chemicals or fumes or radiation? → YES—○ NO—○

If you have answered YES to any of the above four questions please give details .....

.....

.....

Details of clinic attendance

Have you ever attended any clinic or had previous treatment for infertility in the past? → YES—○ NO—○

(Please give details even if you have been seen at this hospital before).

If YES please give name of the doctor and the address of the clinic.

.....

.....

.....

What is the name and address of your family doctor?

.....

.....

Any other information you feel may help .....

.....

.....

THIS QUESTIONNAIRE HAS MORE QUESTIONS THAN MOST DOCTORS HAVE TIME TO ASK AND IS DESIGNED TO HELP US FIND THE PROBLEM IN YOUR CASE QUICKLY. PLEASE HELP US TO HELP YOU BY ANSWERING CAREFULLY AND FILLING IN THE BOXES CORRECTLY. YES—⊘ NO—○

WIFE
(1)

Please state your age in years ☐☐

Date of birth ☐☐☐☐☐☐

Please could you state your country of birth .....
If you do not live in the U.K. please state the country in which you live .....

PREVIOUS MARRIAGE

Have you been married before? ——→ YES—○ NO—○

Have you any children by someone else? ——→ YES—○ NO—○

Ages of children .....

FAMILY HISTORY

Have you any brothers or sisters? ——→ YES—○ NO—○

BROTHERS

Ages Married (YES/NO) Nos. of children if any

.....
.....
.....
.....

SISTERS

Ages Married (YES/NO) Nos. of children if any

.....
.....
.....
.....

Have any of your relatives been attending hospital because of infertility problems? ——→ YES—○ NO—○

If YES please give details if known .....
.....
.....

Is there any illness in your family (e.g. tuberculosis or heart disease)? ——→ YES—○ NO—○

If YES please give details .....
.....

HEALTH RECORD

Have you ever had any serious illnesses? ——→ YES—○ NO—○

WIFE
(2)

If YES please give details as far as you know them .....
.....
Have you ever been into hospital? → YES—○ NO—○

If YES please give details .....
.....
Have you ever had any of the following? If you were under 10 years of age write 'AS CHILD'.

Mumps → YES—○ NO—○ If YES at what age .....
Measles → YES—○ NO—○ If YES at what age .....
Malaria → YES—○ NO—○ If YES at what age .....
Typhoid → YES—○ NO—○ If YES at what age .....
Dysentery → YES—○ NO—○ If YES at what age .....
Bronchitis → YES—○ NO—○ If YES at what age .....
Asthma → YES—○ NO—○ If YES at what age .....
Sinus trouble → YES—○ NO—○ If YES at what age .....
Chest trouble → YES—○ NO—○ If YES at what age .....
Chickenpox → YES—○ NO—○ If YES at what age .....
Hepatitis → YES—○ NO—○ If YES at what age .....
Epilepsy → YES—○ NO—○ If YES at what age .....
Diabetes → YES—○ NO—○ If YES at what age .....
High blood pressure → YES—○ NO—○ If YES at what age .....
Heart problems → YES—○ NO—○ If YES at what age .....
Cancer → YES—○ NO—○ If YES at what age .....
Radiotherapy → YES—○ NO—○ If YES at what age .....

WIFE
(3)

Have you had any other tropical diseases, e.g. Filiaris? ——→ YES—○ NO—○

If YES please give details .....

.....

Have you ever had a surgical operation? ——→ YES—○ NO—○

If YES please give details (e.g. mastoid operation when a child, appendix operation, etc.)

.....

Are you taking any of the following:

Medicine, drugs, sleeping pills, herbal remedies or vitamin pills? ——→ YES—○ NO—○

If YES please give details (even if not prescribed by a doctor) .....

.....

Have you ever had to take a long course of tablets or medicine for more than 2–3 weeks? ——→ YES—○ NO—○

If YES please give details .....

.....

Do you smoke now? ——→ YES—○ NO—○

If YES please say how many cigarettes or how much tobacco smoked each day

.....

For how many years have you smoked? .....

Have you ever smoked in the past? ——→ YES—○ NO—○

If YES please say how many cigarettes smoked per day .....

If YES please say how many years since you stopped smoking .....

Do you ever drink alcohol (beers, wines, spirits)? ——→ YES—○ NO—○

If you have answered YES please could you complete the following questions.

When was the last time you had an alcoholic drink?

Within the last 7 days	
8 days–14 days	
15 days–21 days	
21 days–1 month	
within the last 2 months	
within the last 3 months	
within the last 6 months	
within the last year	

WIFE
(4)

Starting with yesterday I would like you to tell me exactly what you had to drink over the past 7 days. Please be exact, e.g. 2 cans of Carlsberg Special Brew Monday lunch time.

Monday
Tuesday
Wednesday
Thursday
Friday
Saturday
Sunday

Was that typical of what you have been drinking recently? → YES—○ NO—○

If NOT typical please say why .....

.....

Have you ever had a problem with drinking too much in the past? → YES—○ NO—○

If YES please give details .....

.....

OCCUPATIONAL HISTORY

What is your present job/occupation? .....

Please could you indicate the exact nature of your work, e.g. Office work, Factory work, Manual work, Driving, etc.

.....

REMEMBER WE ARE TRYING TO HELP YOU, SO PLEASE ANSWER CAREFULLY

WIFE
(5)

Do you have to work night shifts? → YES—○ NO—○

If YES please say whether it is constant, regular or occasional.

.....

Do you or have you ever worked with any of the following chemicals:

Lead → YES—○ NO—○

Cadmium → YES—○ NO—○

Mercury → YES—○ NO—○

Cyanide → YES—○ NO—○

Arsenic → YES—○ NO—○

Herbicides → YES—○ NO—○

Pesticides → YES—○ NO—○

Fungicides → YES—○ NO—○

Dyestuffs → YES—○ NO—○

Vinyl chloride → YES—○ NO—○

Plastics → YES—○ NO—○

Oil → YES—○ NO—○

Tars → YES—○ NO—○

Radioactive substances → YES—○ NO—○

Other chemicals (solvents etc.) → YES—○ NO—○

If you have answered YES to any of the above could you please give details and if possible the name of the chemical.

.....

.....

If you have answered YES to any of the above please state whether at any time you have been overcome by chemical or poisonous fumes or exceeded permitted radiation doses → YES—○ NO—○

WIFE
(6)

If YES please give details .....
.....

Please list your previous jobs.. Work systematically from leaving school ensuring that no periods are omitted. In the case of working abroad, record which country.

Job. Details and type of job

1. .....
2. .....
3. .....
4. .....
5. .....
6. .....
7. .....
8. .....
9. .....
10. .....
11. .....
12. .....
13. .....
14. .....
15. .....
16. .....
17. .....
18. .....
19. .....
20. .....

GYNAECOLOGICAL AND MENSTRUAL HISTORY

Please state your age in years when your periods began ——→ ☐☐

Are your periods now regular? ——→ YES——○ NO—○

If your periods are regular please state the average length of your menstrual cycle, e.g. 28 days .....

Date of first day of last period .....

Date of first day of period before last .....

For how many days do you lose blood? .....

Do you lose blood between periods? ——→ YES——○ NO—○

If YES please give details .....
.....

Do you suffer any pain during your periods? ——→ YES——○ NO—○

WIFE
(7)

If YES please give details .....
.....

Have there ever been any suspected pregnancies, miscarriages or abortion *by your present husband?* ——→ YES——○ NO—○
If YES please give details .....
.....
.....

Have you ever had a pregnancy, miscarriage or abortion *by someone else*? ——→ YES——○ NO—○
If YES please give details .....
.....
.....

Have you ever had any venereal disease? ——→ YES——○ NO—○
If YES please give details .....
.....
.....

Have you suffered from any vaginal infections–
a) within the last 3 months ——→ YES——○ NO—○
b) at some time in the past ——→ YES——○ NO—○
If YES please give details .....
.....
.....

Have you ever had a gynaecological operation ——→ YES——○ NO—○
(e.g. D & C, biopsy of cervix etc.)
If YES please give details .....
.....
.....

Have you ever had peritonitis? ——→ YES——○ NO—○
If YES please give details .....
.....

Have you at any time taken oral contraceptive pills (The Pill)? ——→ YES——○ NO—○
If YES please say—
1) For how many months and years you were on the pill

☐☐ YEARS ☐☐ MONTHS

2) How many months and years since you stopped taking the pill

☐☐ YEARS ☐☐ MONTHS

WIFE
(8)

PREVIOUS CHILDREN

If you have had a previous child was this a normal vaginal delivery? ——→ YES——○
NO—○

If you have answered NO please give details, e.g. birth by caesarean section.

.....

.....

Details of clinic attendance

Have you ever attended any clinic or had previous treatment for infertility in the past? ——→ YES——○
NO—○

(Please give details even if you have been seen at this hospital before).

If YES please give name of the doctor and the address of the clinic.

.....

.....

.....

What is the name and address of your family doctor? .....

.....

.....

REMEMBER WE ARE TRYING TO HELP YOU, SO PLEASE ANSWER CAREFULLY

HUSBAND AND WIFE

SEXUAL HISTORY

How often on average do you have intercourse (number of times per week)? → ☐☐

Does shift work or absence from home interfere with opportunities to have sex at the fertile time? → YES—○ NO—○

If YES please give details .....

.....

.....

Do you have any physical difficulties with the sex acts that would prevent a conception, e.g. pain during intercourse sufficient to prevent penetration? → YES—○ NO—○

If YES please give details .....

.....

.....

Do you have any emotional difficulties which prevent you having sexual intercourse often enough? → YES—○ NO—○

If YES please give details .....

.....

.....

HUSBAND

Do you get satisfactory erections of the penis for sexual purposes? → YES—○ NO—○

If NO please give details .....

.....

.....

Do you get erections of the penis at other times, e.g. in the mornings? → YES—○ NO—○

Do you get satisfactory ejaculation of sperms? → YES—○ NO—○

WIFE

Do you experience pain during sexual intercourse? → YES—○ NO—○

If YES is the pain sufficient to make you stop having intercourse on occasions? → YES—○ NO—○

Do you have to use any additional lubricants during sexual intercourse → YES—○ NO—○

If you have answered Yes you may wish to know that most lubricants including KY Jelly will kill sperm and make conception less likely.

Please add any other information you feel may help

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Chapter 3

Psychological Aspects

J. Stephen Bell

Introduction

It is well recognised that patients attending infertility clinics generally experience feelings of frustration and inadequacy, and frequently display signs of emotional distress. Historically, childlessness has always been recognised as a problem, whether as a misfortune or even as an offence (Carr 1963). Likewise, it has long been suspected that infertility may, in some cases, have a psychogenic basis. The question of the relationship between psychological variables and infertility is nevertheless a complex one, and the literature, although extensive, is speculative, anecdotal and contradictory. Few studies meet adequate methodological standards. Nevertheless, any consideration of the relevance of psychological factors to infertility management must address the question of psychogenic infertility.

Psychogenic Infertility

'Psychogenic infertility' refers to a state where psychopathology is thought to play a part in the aetiology of childlessness. Thus, the fertility of psychiatric patients has received considerable attention. Although there is no consistent evidence that those with neurosis and personality disorder have reduced fertility (Mai 1972), some major psychiatric disorders, such as schizophrenia, manic-depression, depression and anorexia nervosa, seem to be associated with decreased fertility. Psychotics do seem to have reduced fertility compared with the general population (Mai 1972); this statistic is largely due to the low fertility of schizophrenics (Kendell 1975). The picture is complicated because psychotropic drugs may themselves influence fertility. Amenorrhoea in women, and depressed testosterone levels in men, have been reported in patients taking phenothiazines (Beumont et al. 1974); and diminished sexual activity, possibly as a consequence of increased prolactin levels, is also reported (Meltzer 1980). Nevertheless, menstrual disorders are common in schizophrenic women even if they are not receiving medication (Beumont 1979).

In depressive illness, secondary sexual dysfunction is common. Lower LH secretion in depressed post-menopausal women has been reported (Altman et al. 1975).

The syndrome of self-induced weight loss, anorexia nervosa, is seen particularly in adolescent girls, but is by no means infrequent in young women. It may also occur, though rarely, in males (Dally and Gomez 1979). Amenorrhoea is essential for the diagnosis. Whilst this can be as a result of weight loss, menstruation typically stops when the patient begins to diet, at least in primary anorexia nervosa. In a proportion of patients, amenorrhoea precedes weight loss, and may be the first symptom. Between the restoration of weight and the return of menstruation there is usually a gap of several months. In the rarer male cases there is clinical evidence of hypogonadism. The endocrinology of anorexia nervosa has recently been discussed by Beumont (1979).

Although gross psychopathology is rare in the majority of patients attending the infertility clinic (Berger 1974), the above observations are important because they confirm an association between psychopathology and infertility and suggest the possibility of both a psychosomatic mechanism and a behavioural one (i.e. via the sexual relationship) mediating psychogenic infertility.

Numerous investigators have attempted to correlate measures of mood and personality traits with infertility among patients attending infertility clinics. The considerable psychoanalytic literature on infertility is rich in speculation. Noyes and Chapnick (1964) in their review of the literature found 'no conclusive evidence ... that a specific psychological factor can alter fertility in the normal infertile couple', and the ability of some women to conceive despite severe neurotic or psychotic illness, revulsion for sexual activity or overt reluctance to become pregnant also supports the view that the search for a single personality-related or attitudinal dimension characterising infertile individuals is probably misguided. Notions such as Deutsch's (1945) typology of infertile women are firmly in the realms of conjecture. Many of the experimental studies are methodologically inadequate: none are longitudinal, few meet adequate diagnostic criteria; and psychological assessment has often relied on measures of dubious validity and reliability and been carried out only after lengthy medical investigation. The majority of these studies have looked at so-called normal or idiopathic infertility, and compared individuals (usually women) in this category with controls. It has frequently been assumed that these individuals will show more psychological disturbance than those in whom an organic factor has been identified. It is apparent that the absence of a clear anatomical or physiological cause is not sufficient reason for assuming the presence of psychogenic factors (Mai et al. 1972a) since these authors and others (Seward et al. 1965) found no differences between diagnostic subgroups in the psychological variables measured. It is salutary to note that the number of women thought to be psychosomatically infertile has decreased with improvements in diagnostic techniques (McGuire 1975). Furthermore, it is not unreasonable to suppose that without a definite explanation for their inability to achieve a pregnancy many couples will continue to hope for a miracle and pursue investigations for an indefinite period, thus being unable to grieve (Menning 1975). It has been suggested that the longer a couple remains infertile, the more likely they are to develop secondary psychological problems (Debrovner and Shubin-Stein 1976).

Some studies have produced positive results: infertile couples were found to show more signs of depression and low self-esteem than controls (Platt et al. 1973),

and infertile women have been reported to show more evidence of disturbance of sexual identity and problems of sexual adjustment (Mai et al. 1972b), although these latter authors found no evidence of the supposed association between lack of orgasm capacity and infertility. Nevertheless, the nature of these established relationships is unclear, for it is possible that psychological disturbance might have resulted from the stress of involuntary childlessness rather than played a part in its aetiology. Only prospective studies could resolve this question, and these would be difficult to arrange.

Much speculation about the supposed effects of emotional tension on infertility has been based on the frequently reported association between adoption and conception. Recent studies, however, although still open to some criticisms of the methodology (Mai 1971), suggest that adoption in fact has no effect on conceptive capacity.

Stress has been shown to lower testosterone levels (Kreuz et al. 1972), and it has been suggested that it may be associated with a decreased sperm count (Mehan 1976, cited in Berger 1977). In women, there are numerous reports of amenorrhoea during stressful experiences such as wartime (Kistner 1972). Clinical evidence of such psychophysiological endocrine reactions among patients attending infertility clinics is anecdotal. Thus, although a psychosomatic mechanism associated with infertility is possible (Seibel and Taymor 1982), there is little firm evidence of stress-induced infertility, except in some cases of amenorrhoea. Our knowledge of psychophysiological endocrine reactions is extremely limited.

Psychological factors may still play an aetiological role in infertility by means of a *behavioural* mechanism, i.e. through interference with the sexual relationship. Psychosexual problems may masquerade as cases of infertility (Elstein 1975). Vaginismus, impotence, retarded ejaculation, ejaculation prior to intromission and infrequent intercourse have all been cited in this respect. Excessive frequency of intercourse or masturbation, so that only poor semen specimens are produced at the time of coitus, has also been reported (Dubin and Amelar 1972). Steele (1976) reported sexual or marital problems in 165 (37%) of 500 couples complaining of infertility. In a study of 1294 consecutive cases of male infertility, Dubin and Amelar (1972) reported 69 cases (5.5%) where sexual problems were considered to be the primary cause of the couple's infertility. Amelar et al. (1977) suggest that male sexual dysfunction may be the primary causative factor or an important contributing factor in almost 10% of involuntarily childless marriages. Jeffcoate (1975) estimates that 5% of women seeking advice on sterility have not consummated their marriage. The number of problems detected will depend upon a number of factors including the comfort of the clinician in asking detailed questions about sexual behaviour (Burnap and Golden 1967), and how extensive an assessment of sexual function is made.

Psychological Factors and Infertility Management

It will be clear from the above that greater theoretical understanding of the relationship between psychological factors and infertility must await detailed longitudinal studies. Nevertheless, the practical significance of the literature is more immediately apparent: since psychological disturbance is not uncommon among infertile patients, adequate clinical management of the infertile couple must

take into account psychological factors. For the purpose of this discussion, these will be considered firstly in relation to the initial assessment, secondly to ongoing investigation and treatment, and thirdly to the termination of treatment.

The Initial Interview

Masters and Johnson (1970) state that a basic premise of their therapeutic approach to sexual dysfunction is that: 'There is no such thing as an uninvolved partner . . .' and that 'the marital relationship is considered as the patient'. These comments might equally apply to the management of infertility, for such a unified approach facilitates assessment for, and compliance with, treatment, as well as alleviating emotional distress (Taymor 1978). A joint approach, however, does not imply that all interviews, particularly on such matters as current sexual behaviour, should be carried out with both partners present; attenders at infertility clinics are a compliant group, who may, when seen jointly, present a 'good' picture and withhold information, sometimes in an attempt to protect their partners from upset (Taymor and Bresnick 1978). Both joint and individual interviews are therefore required, and it may be advantageous to have a pair of interviewers, male and female, to facilitate joint involvement.

At the initial assessment, an attempt should be made to discover psychological factors which may be contributing to the infertility, or indeed other problems masquerading as cases of infertility (Elstein 1975). One aim should therefore be to screen out those cases of emotional problems or marital conflict, to which a child is sought as a solution. One of the dangers of an infertility clinic is that its manner of functioning places upon its patients an implicit expectation of achieving a pregnancy (Berger 1974). Yet clinical experience shows that this does not always accurately reflect patients' own wishes: privately they may express ambivalence about, or even rejection of, parenthood. Attendance at the clinic may be more a reflection of social pressure than of individual motivation, or may even indicate a wish for a socially acceptable formula with which to answer the questions of friends and relatives. It can also represent a disguised request for help with marital, sexual or family problems (Berger 1977). Unnecessary investigations may therefore be avoided if these questions concerning motivations for, and decision making about, parenthood are initially raised and explored by the physician, who must indicate that a decision to remain childless can be a valid choice which will receive suitable support. Discussion of these issues will give some indication of the couple's ability to communicate, and will form part of the necessary evaluation of the emotional adjustment of the individuals and the stability of their relationship.

As discussed previously, Dubin and Amelar's figures suggest a small but significant incidence of sexual problems in infertility clinic patients. Thus, a detailed and thorough sexual history should be taken by the clinician, since patients are reluctant to volunteer information about their sexual difficulties. This assessment must go beyond a simple request for an estimate of the frequency of intercourse and a general question about whether any problems are encountered. Each partner should be asked, individually, specific questions about interest, arousal, vaginal entry, ejaculation, and any variations in sexual behaviour or responsiveness at different stages of the menstrual cycle, for problems may be confined to mid-cycle (Drake and Grunert 1979). This information may be of diagnostic significance and will also serve as a baseline against which to measure

subsequent change. Such dysfunctions that are discovered may well respond to short-term therapeutic intervention, as described by Masters and Johnson (1970) and Kaplan (1974). It is to be hoped that a member of the infertility team will have received some training in this approach, and in marital counselling generally.

Investigation and Treatment

Menning (1975) argues persuasively that infertility is a complex life crisis—psychologically threatening and emotionally stressful. Anxiety, frustration and fears of inadequacy are likely to be present in the majority of couples seeking help for an infertility problem (Berger 1977). Paradoxically, current improved diagnostic and therapeutic measures, whilst holding out greater promise for couples, may well increase the stress upon them, by dictating a lengthy medical regimen at a time when they are extremely vulnerable.

Many authors have commented upon the emergence of marital and psychiatric problems during the course of treatment. Additionally, couples with previously satisfactory sexual relationships may develop secondary sexual dysfunctions as a result of the pressures and anxieties arising out of their infertility (Elstein 1975). Basal temperature-taking may lead to disruption of spontaneous lovemaking by timing of intercourse for the fertile period; spontaneity may also be disrupted by suggested coital positions and frequency; post-coital tests may trigger impotence; and goal-orientation may impair sexual responsiveness (Debrovner and Shubin-Stein 1976; Bullock 1974; Kaufman 1969). While it is reasonable to suppose that many such difficulties resolve when the stresses are over, others may require definitive therapy (Elstein 1975): although not of primary aetiological significance, they may serve to maintain the problem.

It has been suggested that much could be done during treatment to alleviate emotional distress (McGuire 1975). Many infertility patients have fears about the process of becoming pregnant and about what their treatment will involve. Banks et al. (1959) advocated the use of audio-visual group therapy as part of an educational programme to facilitate understanding of the various diagnostic and therapeutic procedures. Many couples report difficulties associated with the regimentation of fertility studies, and it is important that they are therefore placed in context (Debrovner and Shubin-Stein 1976). A recent study of patients undergoing laparoscopy (Wallace 1980) revealed a variety of fears, emotional distress, and a lack of knowledge in many individuals, together with a widespread desire for more information, suggesting the possibility that postoperative problems in emotional and social adjustment might be prevented or alleviated by more thorough preparation. A number of authors, for example Menning (1977), have recently published short guides for couples on the subject of infertility, which may serve as useful adjuncts to the information provided verbally by the clinician. It is important that the patient should be prepared for, and reassured about, emotional responses to infertility, as well as informed about medical aspects.

The use of various kinds of group therapy has been recommended by numerous authors, for example Wilchins and Park (1974). Although there have been few attempts to systematically assess the impact of this approach, it would seem to offer a valuable means of decreasing the infertile couple's considerable sense of isolation (Menning 1975; Burnage 1977) and facilitating open discussions between marital partners, by providing an environment for the release of pressures and frustrations.

It may be valuable for couples to be given an estimate of the length of time likely to be involved (Hutcheson 1976). 'Breathers' in treatment may be worthwhile, but at the same time overlong treatment may delay both coming to terms with childlessness and decisions about career and life plans, so that reasonable time limits must be placed on investigations. Behrman and Kistner (1975) feel that a prognosis is in itself important to the psychological well-being of the couple. Lenton et al. (1977) report that most of their patients felt that if they had been given a more realistic prognosis they would have been better able to adapt to the situation and to counter pressures from family, friends and workmates.

Again, the importance of treating the couple as a unit must be emphasised. Open communication can provide a means of ventilating feelings, alleviating guilt and anger, and reducing the impact of infertility on the marriage as a whole. If the couple can communicate freely and have adequate information, they may be more able to cope with the stresses of the investigation, and not feel victims of it. In view of patients' diffidence in disclosing problems which may develop, the clinician must be prepared to take the initiative by routinely enquiring about how the couple are coping emotionally. In this way, permission is given for feelings and fears, such as concern about loss of femininity or masculinity, to be expressed. Although it is understandable that the doctor does not wish to precipitate distress by probing too deeply, patients themselves often regret the lack of opportunity to discuss their worries, but feel that they do not have the right to trouble a busy doctor with them. Lack of confidence in the handling of emotional problems can make the doctor reluctant to deal with psychological sequelae of illness (Maguire 1976). This could be overcome, however, by good liaison with a consultant in this area or, ideally, by the attachment of such a specialist to the infertility team.

Results of Investigations

Reactions to the results of investigations may be varied. Berger (1974) reports periods of depression and impotence in a high proportion of males upon being told they were azoospermic. In a later publication he reports a 63% incidence of transient (lasting less than 3 months) impotence in a group of azoospermic men in the period following the discovery, with feelings or signs of anger towards the men in a high proportion of their wives (Berger 1980). Feelings of guilt are common in both males and females, and not only in the partner with the diagnosed organic problem (Bell 1981). Ford et al. (1953) also found psychological disturbance in many patients whether organic pathology was present or not.

Since fear of being 'exposed' as the infertile partner may be present at the beginning of the investigation (Christie 1980) counselling should be a part of management, starting from the time of first attendance rather than only when results are known and problems have resulted.

Where the couple fall into the 'normal' or 'idiopathic' infertility category, with no positive results from investigations, the clinician must avoid falling into the trap of diagnosing psychogenic infertility by exclusion. To suggest this aetiology to the couple may add to existing feelings of guilt and inadequacy. To further counsel that the 'normal infertile' woman with a career should perhaps consider giving this up (Christie 1980) is premature. Although anecdotal reports of pregnancy following such action do exist, not only has this never been systematically investigated, but it may also serve to further increase the woman's preoccupation with achieving

pregnancy, and decrease the available range of alternative life plans if the infertility continues.

Termination of Treatment

There is broad agreement that there are four stages in the reaction of couples to their infertility: shock, protest, despair and resolution (Renne 1977b). Such a sequence is, of course, similar to the grief reaction which follows bereavement. Although some authors have described a characteristic time scale for this sequence (Nijs and Rouffa 1975), the individual couple should not be expected to complete the process according to any schedule (Menning 1975). The mechanism of denial, for example, seems to be necessary for some patients to cope with the initial shock. Many patients will become preoccupied with events in the past which they may link with their infertility (Berger 1974); some patients fail to move beyond the stage of anger about the injustice of the problem. The process of adaptation may take considerable time, and it is important that the patients' reactions to infertility should be explored and discussed. Grief over infertility is grief over the loss of a potential child and is thus often idealised, so that even when a pregnancy does eventually ensue, this does not guarantee that there will be no problems of adjustment (Menning 1977).

Little is known about the long-term impact of infertility on the couple (Bierkens 1975), a number of authors variously reporting that the marriages of childless couples are happier (Renne 1977a), more likely to end in divorce (McGregor et al. 1970), or more symbiotic (Stauber and Schulz-Ruhtenberg 1979). The attempt to discover the *nett* effect of childlessness is arguably not, however, the most helpful approach, since the consequences could be diverse in different contexts (Bierkens 1975). Further research, looking in particular for predictors of response, is needed.

It has been a central theme of this chapter that psychological factors and their appropriate clinical management must be considered for all patients throughout investigation and treatment, and not only when organic factors have been excluded or treatment terminated. There is evidence that some problems could be avoided or attenuated by this approach. Nevertheless, infertility counselling has historically been concerned mainly with those couples for whom treatment has proved unsuccessful, and it is indeed important to assess what can be accomplished for this group. The success of the clinic should be judged by its ability to maximise the quality of life of all its patients, not only by the pregnancy rate achieved.

Couples must at this stage evaluate their original goals and consider alternatives: adoption or fostering, AID or remaining childless. It is, however, widely accepted that before embarking on such options some resolution of the emotional reactions to infertility must have taken place. It has, for example, been proposed that non-resolution of such feelings may be a major cause of difficulties in adoptive placements (Kent and Ritchie 1976; Kirk 1964; Lawder et al. 1969; Schwartz 1966).

Decision-making about alternatives such as AID is an area where counselling of some kind is generally indicated and it has been suggested that in fact psychiatric referral should be routine in such cases (Berger 1977). Certainly it may be valuable that such an option is available and the counsellor who is not also responsible for the couple's medical treatment may be in a better position to evaluate the couple and help them plan for the future (Taymor and Bresnick 1978). Regrettably, there is virtually no scientific evidence as to how couples cope with donor insemination

and although in general favourable results are reported, the reliability of these data is in doubt. For the moment, then, there is little information to guide the selection of recipients, and a comprehensive follow-up study is required. Some broad guidelines have, however, been suggested (Kerr and Templeton 1976).

Implicit in the argument that psychological sequelae of infertility should be detected is the notion that effective measures exist to deal with them. There is now some evidence that counselling may be helpful in facilitating emotional adjustment and improving quality of life in both male and female infertile patients (Wilchins and Park 1974; Bresnick and Taymor 1979), although much remains to be done in adequately assessing its efficacy. In particular, which patients require in-depth counselling, and what is the most appropriate form for this to take, are questions meriting further research. Self-help groups such as Resolve in the United States (Menning 1977), and the National Association for the Childless (318 Summer Lane, Birmingham B19 3RL) in Britain, may also be able to offer valuable services in providing telephone counselling, support groups and public education, although it would be unfortunate if responsibility for the provision of such counselling were to be neglected by clinics providing infertility treatment.

Conclusions

Although it seems that the concept of psychogenic infertility may have a rather more limited application than once was thought, and although much basic research remains to be carried out, there can be no doubt that psychological factors must routinely be taken into account in the management of the infertile couple. Infertility is a complex phenomenon and psychological variables may be relevant to the aetiology, maintenance and sequelae of the problem. Psychological factors may co-exist with physical pathology, and should thus be considered in all patients, not only in those with no demonstrable organic disease. Reliable methods of measuring changes in psychological and social adjustment have been developed, so that both an appropriate methodology for assessing problems, and means of dealing with them, now exist. The clinical management of the infertile couple can only be enhanced by their application.

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Chapter 4

Seminology

T. B. Hargreave and S. Nillson

Semen Analysis

The intention of this chapter is to describe tests that are in routine use so that intelligent communication can take place between the seminology laboratory and the clinician. Full details of the various laboratory techniques have been published elsewhere; particularly recommended is the 1980 laboratory manual for the examination of human semen and semen–cervical mucus interaction, which may be obtained from the Special Programme of Research and Development and Research Training in Human Reproduction, World Health Organisation, 1211 Geneva 27 Switzerland. A fuller laboratory manual has been published by Hafez (1977).

Semen may vary qualitatively and quantitatively with age, illness, season and sexual activity. In addition to these real variations there are problems because techniques of analysis are imprecise allowing variations of up to 25% between laboratories. These problems are compounded by the fact that many of the time-honoured semen tests show little or no correlation with subsequent fertility. The traditional method of testing has been to report total numbers of sperm or total motility and while these bulk measurements may show marked differences when large populations are assessed (Macleod 1951) it is difficult to prognosticate about an individual (Table 4.1). Normal values are often based on the results of examinations of sperm samples from men with proven fertility requesting sterilisation; data not necessarily giving the appropriate information, since the child-producing period might be several years past. On the other hand, childlessness depends on the wife in at least 50% of infertile marriages. In consequence it has been difficult to define the minimum standards of fertile sperm characteristics.

Recently there has been a trend to make selective measurements, i.e. the best that an individual spermatozoon or a selected population from the semen sample can do. The three areas of testing that show promise are: (a) penetration tests, (b) photographic detection of corkscrew progressive motility, and (c) zona-free egg penetration tests (Chap. 5). These tests, as well as being research tools, are now being introduced into clinical practice because the results appear to correlate with subsequent fertility.

Table 4.1. Effect of viral infection (hepatitis or mononucleosis) on spermatogenesis in one patient (From Macleod (1964), by permission of Professor J. Macleod and the Editor of the *International Journal of Fertility*)

Date 1961	Volume cc.	Count/cc. (millions)	Total count (millions)	Quality %	Activity	Oval %	Large %	Small %	Taper %	Amorph. %	Double Heads	Immature %
28.1	4.0	79	316	3	75	65	14	3	7	10	1	1.0
31.1	3.7	60	222	3	85	69	16	3	3	5	4	0.6
3.2	4.2	35	147	3	75	73	8	2	5	9	3	1.0
6.2	3.2	60	192	3	90	72	4	5	2	13	4	0.8
11.2	4.0	70	280	3	75	76	2	9	3	4	3	0.6
14.2	3.2	65	208	3	70	74	6	10	1	5	3	0.2
17.2	3.0	58	174	3	70	67	6	19	7	9	2	1.4
The patient was admitted to hospital on 21 Feb and discharged on 3 Mar												
14.3	3.8	80	304	3	55	46	12	7	22	12	1	4.0
17.3	2.5	101	253	3	55	33	12	9	26	17	3	8.0
20.3	3.0	10	30	2+	30	47	7	17	13	12	4	9.0
23.3	2.8	15	42	2	20	44	5	11	12	21	7	17.0
29.3	3.0	10	30	2+	30	35	7	12	19	25	2	10.0
31.3	2.6	3	8	2+	60	55	7	11	8	15	4	2.5
3.4	5.0	6	30	2+	60	40	18	8	3	29	2	1.0
6.4	5.1	12	61	3−	55	57	12	5	4	19	3	0.5
16.4	3.0	14	42	3−	65	70	15	8	4	1	2	1.0
19.4	4.0	14	56	3	65	68	11	2	7	10	2	1.0
26.4	3.2	58	186	3+	75	79	6	2	5	7	1	0.0

Variations in Semen Characteristics

Seasonal Variation

The human reproductive system is the result of millions of years of evolution and it is thus worth noting that man, the continuous breeder, differs from most non-mammalian vertebrates and other animals whose gonads regress seasonally (Lofts 1978). The question remains whether there is any seasonal variation in man; the evidence suggests that there may be some variation in sexual activity through the year (Fig. 4.1) and there may be seasonal variation in seminal analysis data (Tjoa et al. 1982)

Variation with Age

Macleod (1951) found that the ejaculate volume and sperm density remain remarkably constant through the reproductive years though there is a tendency for sperm motility to decrease in men over 40.

Variation with Illness

Table 4.1 illustrates the effect of a viral illness on seminal measurements (Macleod 1964). Most illness has a depressant effect on spermatogenesis.

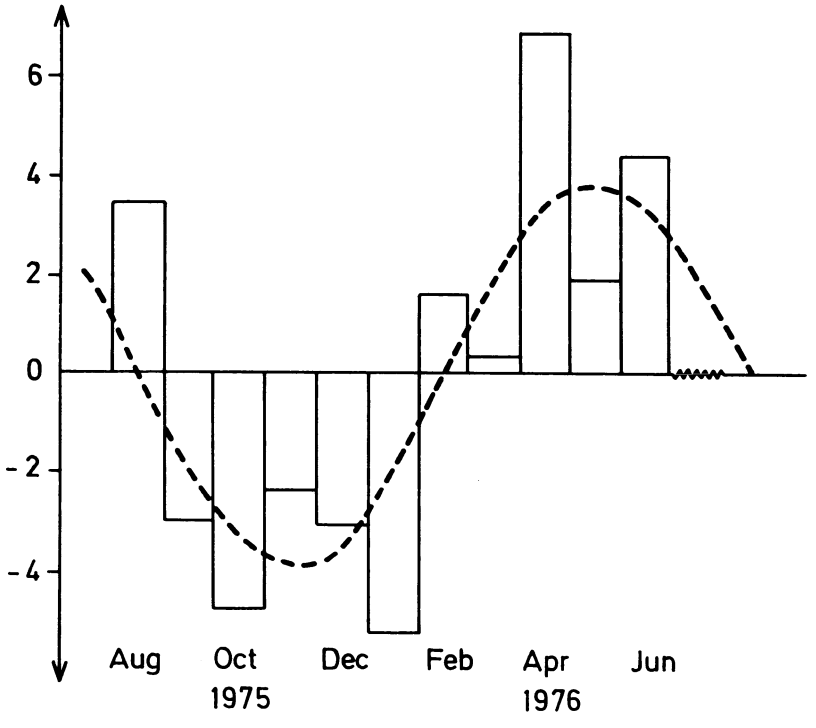


Fig. 4.1. Deviations from the annual mean and the associated expected distribution of condom retrieval per month (Titmar 1978)

Day-to-Day Variation

Table 4.1 also illustrates the day-to-day variation in seminal measurements: the average sperm density before a viral illness was 62 million/ml but individual readings varied from 79 million/ml to 35 million/ml. It is unlikely that more than 20% of this variation was accounted for by errors inherent in the method of laboratory analysis.

Sexual Abstinence

In a population of semen donors seen at the Sahlgren Hospital, Gothenberg, a study was carried out on the donors providing three or more ejaculates collected after an abstinence from 1 to 5 days (Fig. 4.2) and the effect of the abstinence period on semen characteristics was calculated. The semen volume and total number of spermatozoa increased significantly with abstinence up to 3 days; thus semen analysis is best carried out after a 3-day period of abstinence.

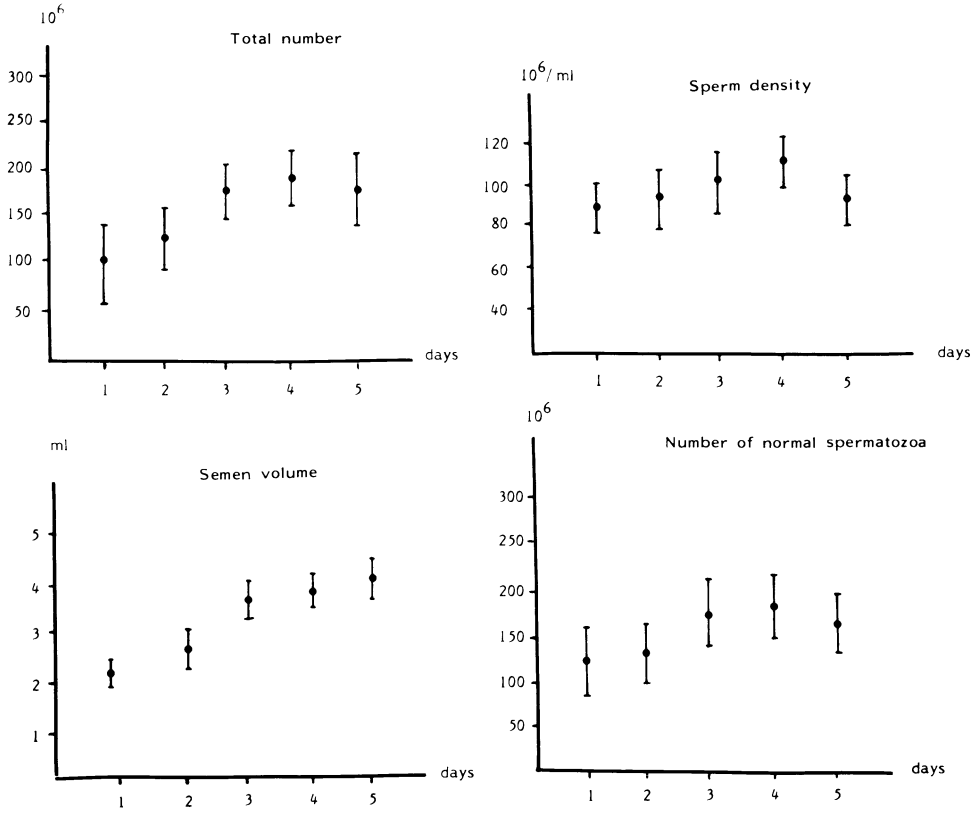


Fig. 4.2. Regression of semen characteristics as related to length of sexual abstinence (mean ±2SE). Observations from artificial instimation donors seen at the Sahlgren Hospital, Gothenburg, Sweden.

Collection of Semen Samples

The patient should be given clearly written instructions about the production and delivery of samples.

Three samples should be collected at weekly or longer intervals. An alternative is to test two samples 3 weeks apart and if they do not correspond, to test a third sample 8 weeks later.

If possible there should be a room near the laboratory where samples can be produced and this is particularly useful when patients come a long distance or in cases where the initial motility of previous samples is poor. Many patients, however, prefer to produce a sample at home and this is acceptable provided that the delivery to the laboratory is within 2 h of ejaculation.

Samples should be collected in a glass container with a wide neck and screw cap. If plastic containers are to be used they should be tested, as plasticisers can be spermicidal. If glass containers are re-used all detergent should be thoroughly washed off. The patient should be instructed to make sure that the container is properly closed before bringing the sample to the laboratory.

Samples should be collected by masturbation if possible. In some cases samples cannot be produced in this way and coitus interruptus is used; this often makes the evaluation misleading as the first sperm-rich fraction of the ejaculate may be lost.

If the semen sample is seen to contain an excess of cellular debris or organisms the patient should be asked to produce another sample on another day shortly after voiding urine as this will help reduce any urethral contamination.

Rubber stoppers on the collection bottles and rubber condoms are spermicidal and should not be used.

Samples may be conveniently kept at body heat, e.g. in a trouser pocket, until they are within the laboratory. Extremes of temperature should be avoided, particularly reduced temperature.

Split Ejaculate

It is sometimes necessary to collect split ejaculates as the first part of the sample is relatively rich in spermatozoa compared with subsequent fractions (Tauber et al. 1975). This can be done by mounting containers on a carrying frame or by numbering them and taping them together. This technique may take some practice on the part of the patient before suitable samples are produced but usually such patients are highly motivated and prepared to make the effort.

Seminal Volume

The volume is measured using a pipette and a graduated centrifuge tube. If the volume is less than 1 ml it is worth repeating collections after the patient has been instructed to collect the whole sample into the container and to prevent leakage by carefully closing the top. Low volumes are sometimes associated with retrograde ejaculation or a deficiency in the seminal vesicles and prostate. A congenital absence of these structures or gonadotrophin deficiency is a much rarer cause. A volume of greater than 6 ml may indicate an undue period of abstinence and it is again worth repeating a sample with instructions to the patient to have intercourse 4 days before producing the sample.

Liquefaction

If the sample can be produced at the laboratory or handed in immediately it is then possible to assess liquefaction. In this case, the sample should be examined for the presence of a coagulum. This will normally completely liquefy within 20 min; failure to do so may indicate prostatic disease. If the couple's infertility appears to be due to sperm being trapped by persistant coagulation of semen, it is helpful to carry out AIH after the sample has been thinned by passage through a fine needle.

Colour and Odour

The specimen is normally a whitish-grey colour. Yellowish colour and the presence of a bad odour in a fresh sample may indicate infection.

Viscosity

Usually the sample can be drawn up into the pipette when measuring the sample volume. If the sample is hyperviscid this may not be possible. In such cases further examination may be difficult without reducing viscosity. It is obviously desirable to estimate the motility on the untreated sample as high viscosity with poor motility may respond to treatment by AIH. The viscosity can be reduced temporarily by passing the sample through a fine needle (gauge 23) and this may give sufficient reduction to allow sperm density estimation to proceed.

pH

(The measurement of pH is only useful if the sample is fresh and should be estimated immediately after liquefaction.) It is usually done using a pH paper (range 6.6–8.0). Normal semen pH is within the range 7.2–7.8. Inflammatory disorders of the prostate or seminal vesicles may alter the pH outside these limits.

Motility

A drop of semen is covered with a coverslip and examined immediately. Ideally the microscope plate should be at 37°C. Overall motility is calculated by counting motile and immotile sperm in ten separate random fields away from the edge of the coverslip. The percentage of motile spermatozoa is calculated from the mean percentage motility for all fields counted. Semiquantitative motility is determined by grading the motility according to the following scale:

0 = No progression
1 = Weak forward progression
2 = Moderate forward progression
3 = Active forward progression

Normally, 60% of spermatozoa show grade 2–3 motility for the first 3 h after ejaculation. There is evidence that high motility is associated with improved conception rates and vice versa (Nilsson et al. 1982). In Nilsson's study there was a 22.4% conception rate following donor insemination with samples containing 55% or more motile spermatozoa but much lower conception rates associated with poorer motility.

Motility assessment using the above techniques is very subjective and recently there has been renewed interest in objective measurement (Table 4.2), but unfortunately few centres apply such techniques to all routine samples.

Debris

Any cellular or bacterial debris should be noted. The amount of contamination from the distal urethra is significantly reduced when the full urinary bladder is emptied before production of sperm samples. This procedure is necessary if the sample is to be transported for 1 h or more, otherwise bacterial contamination may alter semen parameters.

Table 4.2. Objective methods of assessing spermatozoal motility

Method	Comment	Reference
Darkfield cinematography	Used for evaluating sperm from trout in water but involved excess time	Schlenk and Kahmann (1935)
Impedance change	Used for ram, bull and monkey sperm	Rothschild (1949)
Centrifugation and measurement of optical density	Depends on different sedimentation rates between living and dead sperm. Special apparatus required means this technique has not been widely used except in veterinary practice	Atherton (1975)
Laser	Measurement is made of the Doppler shift after submitting sample to light from a low power laser. Apparatus not yet truly reliable but this is potentially a quick bench top method	Dubois et al. (1975)
Photographic assessment of sperm tract	Permanent record produced allows objective comparison of different samples from same patient before and after treatment. If colour filter is used and eosin added additional information can be collected. These techniques although time-consuming are used in many centres because it is usually possible to modify an existing microscope	Janick and Macleod (1970) Phillips (1972) Makler (1979)
Computer analysis of video signals	Can possibly be confirmed with electronic density measurements and sperm morphology estimation (image processing) and allows rapid objective measurement of bulk sperm parameters	Jecht and Russo (1973)

Agglutination

This is frequently seen and may occur as a result of bacterial or immunological problems. The percentage of spermatozoa showing spontaneous agglutination should be estimated microscopically in ten separate fields; a reading of greater than 10% is abnormal. Agglutination may be tail to tail (common), head to head, tail to head, or mixed (common); or there may be aggregation to cellular debris. The latter is not usually indicative of antisperm antibodies.

Vital Staining

It is helpful to stain samples with initial motility of less than 60% with 1% eosin in distilled water and then to counterstain with 10% nigrosin in distilled water. The percentage of living sperm is estimated in ten separate fields. By this technique, necrospermia (rare) may be distinguished from immotility of spermatozoa; it also provides a check on the accuracy of motility assessments. This is particularly helpful where the motility estimation is suspect and where the patient is unable to deliver samples to the laboratory within the 2 h time limit.

Sperm Density

The most commonly used method for the determination of sperm density is the haemocytometer chamber; this is most suitable for samples with low counts or where a few estimations are being done at one time. Where many samples are to be estimated a Coulter counter will save time but allowance must be made for inaccuracy when density is less than 10 million/ml.

The haemocytometer method is described in detail as it is the method most commonly used. The sperm density is first estimated at the time of the initial motility assessment; 40 spermatozoa per field (40 × objective) is roughly equivalent to 40 million/ml. Depending on the initial assessment the well-mixed sample is diluted 1:10, 1:20, 1:50 or 1:100 with a solution containing 50 gm sodium bicarbonate, 10 ml 35% formalin and 5 ml aqueous gentian violet and distilled water to a volume of 1000 ml. The dilution should be carried out using automatic micropipettes fixed for 10, 50, 500 or 1000 μl. A drop of the diluted well-mixed sample is transferred to a Neubauer chamber and covered with a cover slip. The chamber is placed in a moist environment for 15 min. to allow cells to sediment and then counted under a light microscope at a magnification of 100× or 400×. For greater accuracy, counts should be made at two dilutions and the difference in readings should not exceed 20% when sperm density is great; however, in view of the limited value of sperm density in our laboratory estimations are carried out on one dilution only.

The Neubauer chamber has a grid which contains a number of large squares as shown in Fig. 4.3.

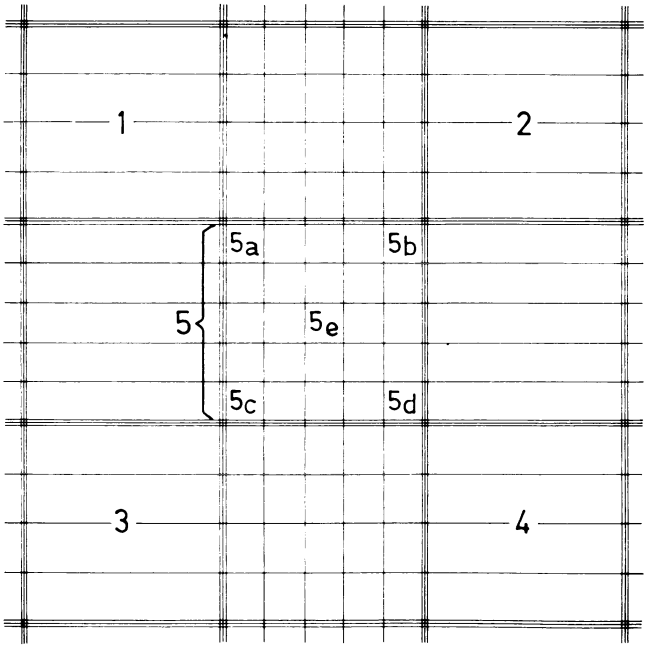


Fig. 4.3. The Neubauer Counting Chamber

Accurate Counts. Spermatozoa are counted in all the squares in square 5. The number counted is multiplied by 10 000 (the larger squares hold a volume of 10^{-4} ml of fluid between the coverslip and the haemocytometer) and by the dilution factor to give the density in million/ml.

Quick Counts. A less accurate but quicker method is to count five smaller squares 5a, b, c, d, e, and to multiply the result by 50 000. This method may be the only practical method if the density is high.

Low Density. In this case the five larger squares are counted—squares 1–5—and the multiplication factor 20 000 is used.

There is much controversy about normal values. We regard a density of less than 10 million/ml as almost certainly abnormal, less than 20 million/ml as probably abnormal and greater than 60 million/ml as probably normal. It can be seen from Fig. 4.4 that there is a chance of pregnancy even when the sperm density is less than 10 million/ml. However, too much emphasis is placed on sperm density estimation and it is to be hoped that newer tests, e.g. zona-free egg penetration, may give better prognosis for fertility.

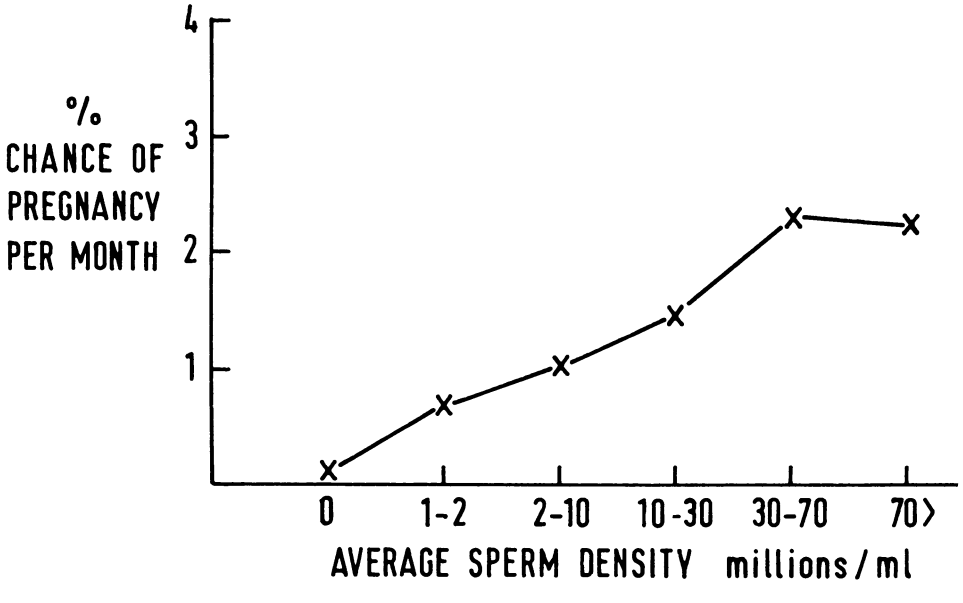


Fig. 4.4. Subsequent pregnancies related to semen density measurements. 566 couples are included in this analysis with a total follow up of 1120 years.

Presentation of Density and Total Count Data

Most authors give the sperm concentration (million/ml) without reporting the volume. However, the semen volume is not controlled by any homeostatic mechanism and can vary from 2 ml to 6 ml in normal healthy men. With increasing age there is a reduction in semen volume and that is also the case if the time of abstinence is shorter than 3–4 days. It is common that a semen sample of 5.8 ml with 19 million spermatozoa per ml is classified as 'oligozoospermic' while a semen sample with 2.7 ml and 41 million spermatozoa per ml will be classified as 'normal'. The total sperm count is the same (110 million) in the two samples. Reports should contain information about the total number of spermatozoa in the ejaculate.

Morphological Estimation

Morphological estimation of human spermatozoa is complicated by the fact that there is great natural variation in shape. This is a difference from all other animals, except the gorilla; usually, spermatozoa are remarkably uniform. This natural variation in shape makes it difficult to say which forms are associated with infertility and which are normal variations. Possibly in vitro fertilisation tests will help to clarify this question by showing which sperm are capable of penetration.

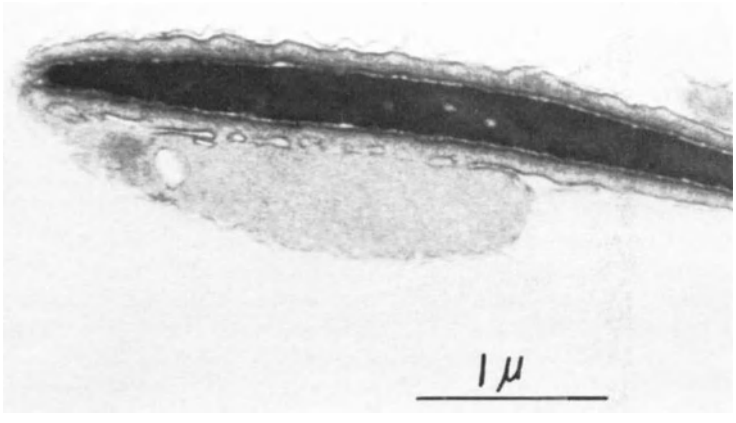
If the sperm density is greater than 10 million/ml a smear is prepared direct from a fresh sample; if the density is less, the sample is centrifuged at 2000 rpm for 15 min and then a smear is made. The slide is stained using the Papanicolaou method (Appendix 4.1) and 100 sperm are classified into the following groups; normal, large oval heads, small oval heads, tapering heads, double heads, amorphous heads, tail defects and cytoplasmic droplets. If there are many immature cells or leucocytes these can be better distinguished using Bryan/Leishmann stain (WHO 1980).

Ultrastructure

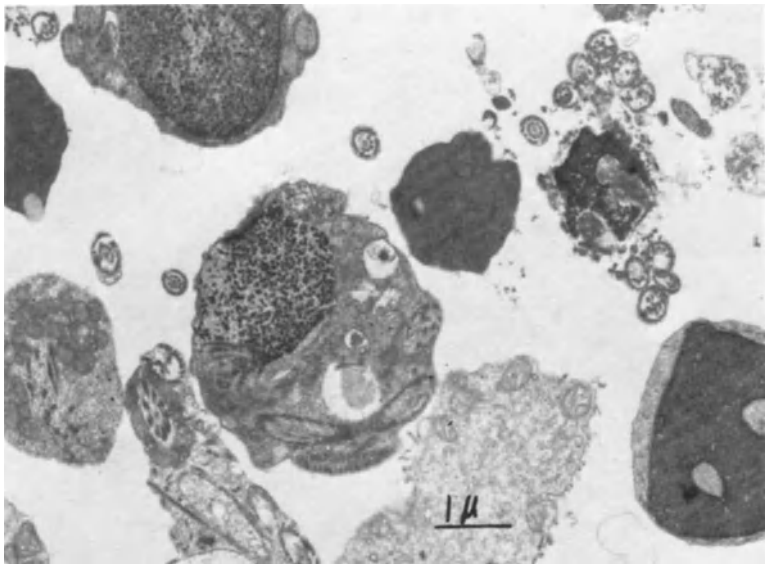
With a light microscope a magnification of 1000× or more can be obtained giving a good resolution. However more detailed examination with transmission electron microscope or scanning electron microscope may reveal unsuspected abnormalities.

Electron microscopy has been used in the veterinary field for some time and many specific abnormalities have been noted. Abnormalities of the sperm head in bulls, with a knob head (Fig 4.5a) and in some cases a decapitated sperm have been described by Blom and Birch (1970). Additionally, a decapitated-sperm-type defect with separation of the spermatozoa into loose tails and heads has been described in the Guernsey bull. This defect causes congenital sterility (Hancock and Rollinson 1949). Similar defects have been recognised occasionally in the spermatozoa of infertile men, where there was dislocation of the heads and tails (Fig. 4.5b) (Aughey and Orr 1979).

The transmission electron microscope has been used for the study of normal human spermatozoa (Pederson 1969) and more recently in studying specific abnormalities (Ross et al. 1971). Lacy and colleagues (1974) have suggested the use of the scanning electron microscope (SEM) to assess surface texture, shape and size



a



b

Fig. 4.5. Electron microscopy studies of sperm. **a** Knob head deformity of bull spermatozoa (Blom and Birch 1970) **b** Decapitated spermatozoa from an infertile man (Aughey and Orr 1978)

of the head and in particular the insertion of the tail into the post-acrosomal cap. Other examples of sperm dysgenesis, such as mitochondria in the region of the head or multiple tails, can also be observed. Recently it has been found possible to obtain satisfactory preparations for scoring of spermatozoal morphology using the SEM. This involves deposition of a known concentration of spermatozoa onto a filter and the use of critical point drying and staining of the filter. (Liakatas et al. 1982) Excellent photographs are obtained allowing detailed assessment of surface morphology (Fig. 4.6). The use of the back scattering facility allows assessment of the nucleus and special stains can be used to demonstrate mitochondria and enzymes in the acrosomal cap. In the future, computerised image processing may. allow further automation and refinement of these techniques.

Fig. 4.6. Scanning electron micrograph of human spermatozoa using a new preparation technique. The examination of the filtered sample allows preparation of an even film allowing accurate scanning of abnormalities. (Photo by courtesy of Dr. A. E. Williams, Teaching and Research Centre, Western General Hospital, Edinburgh)

Selective Tests

Information from semen analysis may be used in two ways. The first is to define an abnormality known to be associated with sterility so that precise treatment may be given; this ideal situation is only encountered in cases of azoospermia associated with hypogonadotrophism. The second is to define an abnormality known to be associated with subfertility or sterility so that the couple may be given an accurate prognosis. Traditional practice has been to advise the husband in the light of results of estimation of sperm volume, motility, density and morphology with particular reliance on sperm density and often without knowledge of the results of the wife's tests. The difficulty of giving accurate prognosis based on conventional semen measurements is increasingly appreciated and in fact the duration of involuntary infertility of the couple often gives a more reliable guide to prognosis (Fig. 4.7). In view of the poor prognostic value of conventional semen analysis and the fact that specific abnormalities are not defined there is a need for better tests of the sperm function. The semen measurements described above are made on the total sample whereas the following tests detect the capabilities of the best spermatozoa in the sample. There are three types of tests coming into clinical use:

1. Mucus penetration tests
2. Photographic motility estimation
3. Zona-free egg penetration

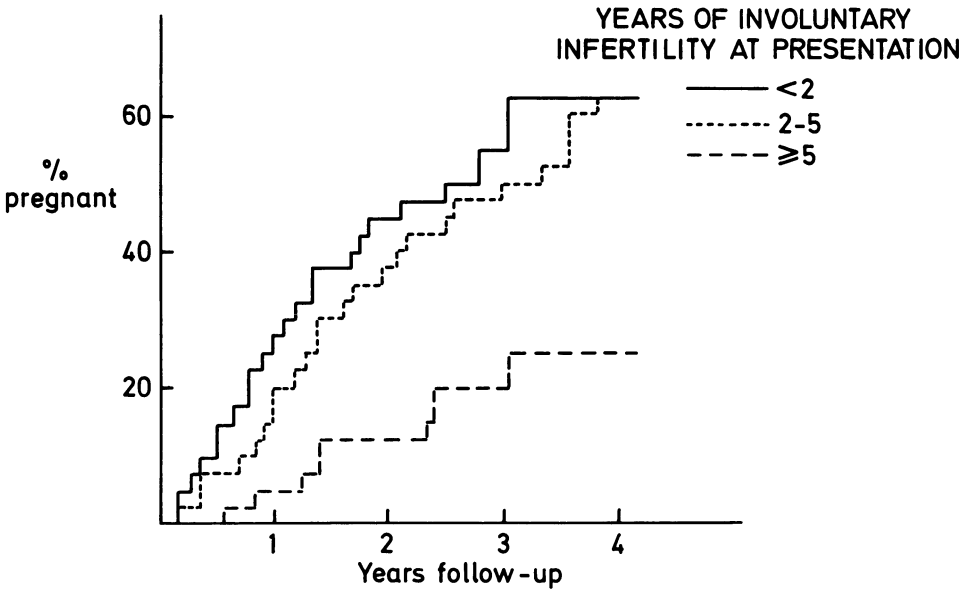


Fig. 4.7. Analysis (Life table) of the number of pregnancies following the first visit to the infertility clinic. The 517 couples included in this analysis are separated into 3 groups according to the number of years of involuntary infertility before their first attendance. Data from Infertility Clinic, Western General Hospital, Edinburgh by courtesy of Dr. R. Elton, Department of Medical Computing and Statistics Unit, Edinburgh University

The latter test has immense clinical and research possibilities and is discussed in Chap. 5. Mucus penetration tests are not new but have always been subject to the logistical difficulties of obtaining human cervical mucus. The search for human cervical mucus substitutes has included animal mucus, egg white and synthetic migration media. The latter are practical to obtain but warrant further clinical evaluation. Photographic motility may be applied as a bulk test, i.e. the overall motility or else particular types of motion can be identified. If the photographic motility test is organised to detect the percentage of sperm displaying progressive corkscrew motility as opposed to other sorts of progressive motility the findings appear to correlate with fertility.

Mucus Penetration Test

It is our practice to evaluate all couples where routine semen analysis is normal and tests of ovulation and tubal patency are normal with sperm–cervical mucus contact testing or sperm–cervical mucus penetration testing. The main logistical difficulty is to obtain pre-ovulatory cervical mucus. We therefore hold a clinic twice a week and couples to be tested are given written instructions that they may attend the clinic without prior appointment on the clinic day that falls nearest to days 11, 12 or 13 of the menstrual cycle, assuming a 28-day cycle. Despite these arrangements the commonest cause of inadequate mucus is because the sample is collected at the wrong time in the menstrual cycle. Unfortunately, deep-frozen cervical mucus is not always reliable and therefore cannot be recommended.

Gynaecological Examination. The mucus samples are obtained during vaginal examination by using an insulin syringe to draw the sample from the cervical canal An Insler cervical score (Insler et al. 1972) is made at the time and the results are recorded on special forms (Appendix 4.2).

Sperm–Cervical Mucus Interaction Test For sperm–cervical mucus contact testing (Kremer and Jager 1976) a drop of mucus is placed on a slide and stretched along its strands. A drop of fresh semen is placed nearby and the two specimens brought into contact by placing a cover slip. Readings are taken of the distance sperm penetrate in a known period of time (usually 15 min) and also the type of motility seen, e.g. progressive or non-progressive (shaking) motility. In our laboratory this test is usually set up as a crossed hostility test, i.e. donor semen and husband's semen are cross-tested with donor cervical mucus and wife's cervical mucus. In this manner the test may be used to confirm that antisperm antibodies found by other tests are significant (Hendry et al. 1978). The advantage of the sperm–cervical mucus contact test is that less mucus is required than with the Kremer test and the type of sperm motility can be detected.

Capillary Tube Test (Kremer 1965). This test gives more accurate information than the sperm–cervical mucus contact test but is dependent on adequate cervical mucus. The mucus is drawn into a capillary tube (Fig. 4.8); one end is sealed with plasticine which causes the mucus to bulge from the other end and guarantees full contact with the semen. This open end is placed in a glass reservoir of semen. The

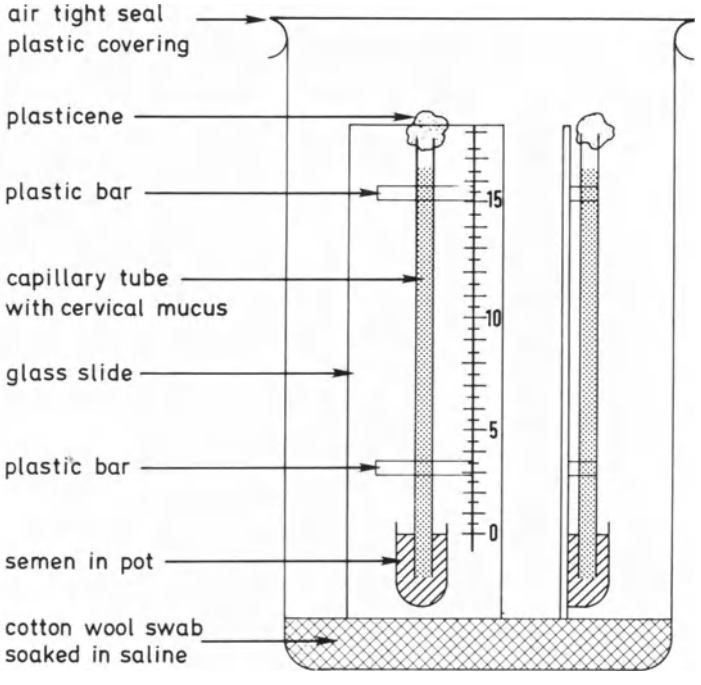


Fig. 4.8. Apparatus necessary for Kremer sperm penetration test

tube is incubated at 37°C in a moist environment and readings are made at 30, 60 or 180 min. Capillary tubes 70 mm in length and with a volume of 10 μl have provided the best results. We read the samples at 60 min. Readings are made of (a) the distance penetrated by the furthest sperm and (b) the distance penetrated by the main wave of spermatozoa; the latter reading shows a good correlation with conception rate. Conception rate is found to be reduced when the penetration distance is less than 3.9 μm/s, and no conception has occurred in cases we have studied when the penetration was less than 1.7 μm/s.

Substitutes for Cervical Mucus in Sperm Migration Tests. Cervical mucus from cows has been used as a test medium for sperm migration in vitro and for the evaluation of human infertility (Alexander 1981). Spermatozoa from ejaculates of humans and cattle migrate different distances depending upon the mucus donor and the length of time mucus is stored before testing. This variation in mucus makes it difficult or impossible to qualitatively compare the characteristics of semen samples obtained at different times from the same or different donors.

To overcome this problem an easily obtained and stable material is needed, with sperm migration characteristics similar to those of cervical mucus. Various mucus substitutes have been tried. A certain fraction of the white of hens' eggs has shown properties very similar to human cervical mucus and has the virtue of being easily obtainable. A fresh hen's egg is obtained then the layer of white situated immediately outside the highly viscous inner layer (Fig. 4.9) is transferred by

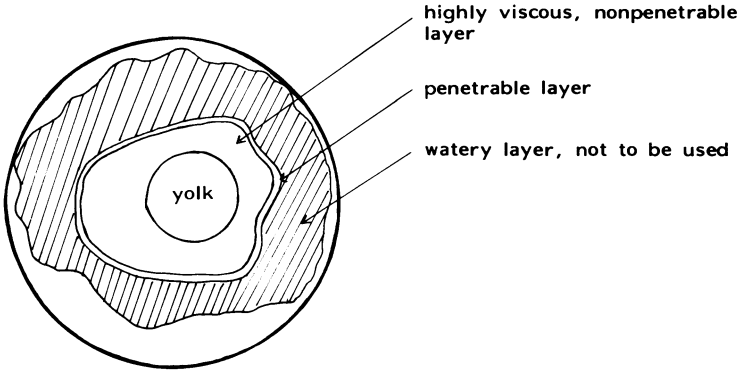


Fig. 4.9. Fresh egg, showing fraction of egg white which can be used as a cervical mucus substitute for sperm penetration tests

means of a glass pipette to a watch glass. Several glass capillary tubes as used for the Kremer penetration test are allowed to fill by bringing one end of the tube into contact with the egg white. One end of the tube is then sealed with plasticine and two tubes are placed in a reservoir of sperm in a Kremer meter. Readings are taken as for the capillary tube test described above and the average for the two tubes is read. This cheap test has been shown to give good correlation with reproductive ability and, despite the differences between cervical mucus and egg white, may be detecting progressive corkscrew motility which can otherwise only be accurately identified using more expensive photographic techniques.

Synthetic media for quantitative studies on sperm migration have been sought and have been prepared with reagents commonly used for polyacrylamide gel electrophoresis. Even more promising is a medium prepared with buffered 12.5% Healon, a hyaluronic acid preparation (Pharmacia Pharmaceuticals, Sweden). This synthetic medium has physical characteristics similar to that of oestrous human cervical mucus, and spermatozoa migrate in it in large numbers and parallel to each other. A full clinical evaluation is awaited but preliminary results suggest that this synthetic medium will, like human mucus, inhibit progress of antibody-coated sperm.

Conclusion

Measurement of semen volume, motility, density and morphology gives little prognostic information about a man's fertility except in cases of azoospermia or aspermia or when the density is less than 10 million/ml. Motility and morphology estimations may give more information but in most laboratories the techniques used allow considerable observer error. Because of the limitations of conventional techniques new tests are currently being evaluated. These include the detection of progressive corkscrew motility by photographic techniques, the assessment of the ability of spermatozoa to progress through columns of human or substitute cervical mucus and the assessment of the percentage of spermatozoa able to penetrate mammalian eggs from which the zona pellucida has been removed. Preliminary evidence suggests that these tests may give better prediction about the fertilising ability of a man's spermatozoa; the advent of human in vitro fertilisation as treatment for couples where the husband has poor semen quality may allow precise evaluation of the prognostic value of all methods of semen analysis. As yet there is little evidence that currently used semen measurements yield information on basic defects in sperm but if in the future it is possible to discover which tests have correlate well with a lack of fertilising ability it should then be possible to separate out and study populations of fertile and infertile sperm. An understanding of the basic defects causing lack of fertilising ability may in the future allow development of specific treatments.

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Appendix 4.1. Papanicolaou staining procedure

Fixed smears are stained according to the following schedule:

Hydration	Ethanol	80%	10 × 1 s dips
		70%	10 × 1 s dips
		50%	10 × 1 s dips
	Distilled Water		10 × 1 s dips
Harris' haematoxylin (filter before use)			3 min.
Running water			3–5 min.
0.5% HCl to differentiate nuclei			2 × 1 s dips
Running water			3–5 min.
1% Lithium carbonate to blue nuclei			1 min.
Dehydrate	Ethanol	50%	10 × 1 s dips
		70%	10 × 1 s dips
		80%	10 × 1 s dips
		95%	10 × 1 s dips
Orange G6 (obtained commercially[a])			2 min.
Ethanol wash			20 × 1 s dips
EA–50 (obtained commercially[a])			5 min.
Ethanol 95%			15 × 1 s dips
Absolute alcohol			2 min.
Mount in DPX			

[a] These stains if frequently used may be made more cheaply in the laboratory

Appendix 4.2. Form used for recording the cervical score when patients attend the Infertility Clinic to give cervical mucus for sperm/cervical mucus contact testing or for cross-hostility testing

INFERTILITY CLINIC WESTERN GENERAL HOSPITAL, EDINBURGH

Patient's name *Report to*

Cervical mucus assessment
Date of last menstruation
(1st day of bleeding)
= _____

Case No. — | | | | | | |

Today's date — | | | | | | |

∴ Day of cycle sample taken _____ | |

Normal cycle length (days) _____ | |

Insler cervical score

Parameter	0	1	2	3
Amount of mucus	NONE	SCANT A small amount of mucus can be drawn from the cervial canal	DRIBBLE Glistening drop of mucus in external os, easily drawn from cervix canal	CASCADE Abundant mucus pouring from external os
Spinnbarkeli	NONE	SLIGHT Uninterrupted mucous thread can be drawn $\frac{1}{4}$ distance between external os & vulva	MODERATE Uninterrupted mucous thread can be drawn $\frac{1}{2}$ distance between external os & vulva	PRONOUNCED Uninterrupted mucous thread can be drawn the whole distance between external os & vulva
Ferning	NONE Amorphous mucus	LINEAR Fine linear ferning in few spots. No side branching	PARTIAL Good ferning. Side branches in part of slide. Amorphous mucus in other parts	COMPLETE Full ferning of whole preparation
Cervix	CLOSED Mucosa pale external as hardly admits thin applicator		PARTIALLY OPEN Mucosa pink, cervix canal easily penetrable by an applicator	GAPING Epithelium hyperaemic; external os patulous

Score out of 12 _____ | |

Mucus quality

pH of mucus _____ | |

1 = Clear 2 = Cloudy 3 = Bloodstained _____ |

Comments

Chapter 5

The Zona-Free Hamster Egg Penetration Test

J. Aitken

Introduction

Conventional methods of semen analysis rely heavily upon simple measurements of sperm quality which do not equate well with fertility. Of all the measurements routinely assessed (morphology, motility and sperm density) the greatest reliance is placed on sperm density since this shows a statistically significant correlation with infertility at concentrations below 60 million/ml (David et al. 1979) and densities of less than 10 million/ml are conventionally regarded as indicative of infertility. The predictive value of sperm density has been called into question however, for when the fertility of the female partner is taken into account about 50% of men with a sperm density of less than 10 million/ml can initiate a pregnancy (Smith et al. 1977) (see also Fig. 4.4). Thus direct tests of the fertilising capacity of an ejaculate are required in order to assess male infertility. The ideal assessment would be an in vitro fertilisation system incorporating mature human ova. However, because of the obvious logistical and ethical difficulties, such a direct test of fertilising capacity is only possible if some surrogate for the human ovum can be devised. The identification of such a surrogate has been achieved through the elegant studies of Yanagimachi at the University of Hawaii. The key to this discovery was the observation that guinea pig spermatozoa could be induced to penetrate hamster oocytes which had had the zona pellucida removed by trypsin treatment (Yanagimachi 1972). The normal fertilisation of an oocyte involves the sperm penetrating the outer zona pellucida and then fusing with the inner vitelline membrane, and it is this outer covering, the zona pellucida, which is the major barrier to interspecific (i.e. between different animals) fertilisation. Zona-free hamster oocytes were subsequently shown to be susceptible to penetration by spermatozoa of every species tested including the rat, mouse (Hanada & Chang, 1972, 1976) guinea pig (Yanagimachi 1972; Barros et al. 1973) boar (Imai et al. 1977) rabbit, deer mouse (Hanada and Chang 1978) and human (Yanagimachi et al. 1976). Why zona-free *hamster* eggs should (Hanada and Chang 1972, 1976) be so readily penetrated by heterologous spermatozoa is not at all clear, but it is not a property shared to the same extent by the ova of other species. Human spermatozoa, for example, will not penetrate zona-free rat (Quinn 1980) or rabbit ova (Aribarg and Bedford 1977) and show only a limited affinity for zona-free mouse ova (Aitken and Mortimer,

unpublished observations). The zona-free hamster oocyte therefore appears to be a unique vehicle for evaluating the fertilising capacity of human spermatozoa.

The Technique

The method for the zona-free hamster egg penetration assay was originally described by Yanagimachi et al. (1976). The medium normally used for the preparation of human spermatozoa and hamster ova is a modified Krebs-Ringer salt solution (BWW medium) developed by Biggers et al. (1971); the formula for the stock solution is given in Table 5.1. Immediately before use 100 ml of this solution is supplemented with 210 mg sodium bicarbonate, 100 mg glucose, 3 mg sodium pyruvate, 370 μl sodium lactate (60% syrup) 300 mg of human serum albumin (HSA) and 2 ml of 1 molar HEPES buffer (Flow Laboratories*).

The semen samples are obtained by masturbation after at least 48 h abstinence, allowed to liquefy in a sterile plastic jar, and then, within 2 h of collection, 1 ml of the sample is diluted with 9 ml of BWW. The spermatozoa are then pelleted by centrifugation at 500 g for 6 min. and subsequently washed twice more with 9 ml volumes of fresh medium before finally being suspended in BWW at a concentration of 10 million spermatozoa per ml. Capacitation times of from 1 to 20 h have been reported in the literature but since the optimum period of capacitation shows considerable variation between individuals (Perreault and Rogers 1982) no single time will be appropriate for all samples. A survey of the literature reveals a tendency to link shorter capacitation times with an increase in the concentration of HSA in the medium. Hence Barros et al. (1979) incorporated 35 mg HSA/ml for a 1 h capacitation period while 18 mg HSA/ml are routinely used for the 6–7 h capacitation period employed in the author's laboratory (Aitken et al. 1981, 1982 a,b,c). When longer capacitation periods of 18–20 h are used then the HSA concentration should be reduced to 3 mg/ml (Rogers et al. 1979). The rationale behind such variations in albumin concentration is unclear since we have not detected any significant differences in the results obtained with a 6 h capacitation period in media containing either 3 mg/ml or 18 mg/ml HSA (Aitken et al. 1983a). Similarly the gas phase in which capacitation is induced has little influence on the outcome of the test; air or 5% CO_2 in air are both satisfactory according to Yanagimachi et al. (1976).

Mature ova are collected from female golden hamsters (*Mesocricetus auratus*) induced to superovulate by an intraperitoneal injection of 30 iu pregnant mare's

Table 5.1. Formula for stock BWW medium

Salt	Quantity (gm/litre)
NaCl	5.540
KCl	0.356
$CaCl_2.2H_2O$	0.250
KH_2PO_4	0.162
$MgSO_4.7H_2O$	0.294
(Phenol red	1.0 ml.)

* Flow laboratories P.O. Box 17 Irvine Scotland

serum (PMS) on day 1 of their oestrous cycle followed by an equivalent dose of human chorionic gonadotrophin (hCG) on day 3. The animals are autopsied 17–18 h after hCG administration and the cumulus masses released from the tubal ampullae into BWW at 37°C. The cumulus cells are subsequently dispersed in 0.1% hyaluronidase in BWW and the naked ova washed twice in fresh medium before being transferred to 0.1% trypsin to remove the zonae pellucidae. After 2 or 3 min. the oocytes are removed, washed twice with BWW, and transferred to 20 μl droplets of the capacitated sperm suspension under paraffin oil. The insemination mixtures are then incubated for 2 h (Rogers et al. 1979) or 3 h (Barros et al. 1979; Aitken et al. 1981; Dor et al. 1981) at 37°C in an atmosphere of 5% CO_2 in air. At the end of this culture period the eggs are washed in BWW, transferred to a clean microscope slide and compressed to a depth of about 30 μm under a 22 × 22 mm coverslip mounted on four paraffin wax supports. The eggs may then be visualised by phase contrast microscopy for the presence of swollen sperm heads, with an attached or closely associated tail, within the vitellus. The percentage of eggs containing swollen sperm heads is taken as the penetration rate and 40–60 ova are usually scored per sample.

A variation on this procedure has been to isolate and store large numbers of zona-free hamster ova at −50°C in Dulbecco's phosphate buffered saline containing 1.25 M dimethyl sulphoxide and 10% foetal calf serum. These frozen ova may then be thawed out at any time and used to test semen samples in the zona-free hamster egg assay. Approximately 90% of frozen-stored hamster ova appear to be normal after thawing and the penetration rates observed are no different from those obtained with fresh ova on the same semen sample (Fleming and Yanagimachi 1979). As a consequence of these results it is feasible that large numbers of zona-free hamster ova might be preserved at specialised centres and distributed to those laboratories lacking the appropriate animal facilities.

It should also be pointed out that frozen-thawed human spermatozoa exhibit the capacity to fertilise zona-free hamster ova (Binor et al. 1980) although there appears to be disagreement as to whether the cryostorage procedure does (Binor et al. 1980) or does not (Cohen et al. 1981) influence the penetrating capacity of the spermatozoa.

Repeatability

It is of critical importance in setting up bioassays such as the zona-free hamster egg penetration test, the outcome of which may be influenced by small variations in culture conditions, to ensure that the results obtained are reproducible. Some examples of the results obtained in the author's laboratory on semen samples collected from the same donor on separate days are given in Table 5.2. Both this analysis and similar studies carried out by Rogers et al. (1979) and Cohen et al. (1982) indicate that good repeatability can be obtained with this system despite day to day variations in sperm density and motility.

A further indication of the repeatability of this system is the extent to which the outcome of the hamster egg test conforms to mathematical models, constructed according to Poisson distribution theory (Aitken and Elton, unpublished observations). This is particularly true when the relationship between the number of eggs

Table 5.2. Penetration rates, sperm densities and motilities for donor semen collected on separate days

Donor	Penetration rate (%)	Density ($\times 10^6$/ml)	Motility (%)
1	57.9	250	64
	65.9	110	59
	60.0	62	55
	62.0	90	67
2	58.6	126	58
	67	70	72.5
	50	192	51
3	93	174	65
	70	406	64
	70.5	412	69
4	7.0	147	62.9
	2.7	230	59

penetrated by a particular sample and the degree of polyspermy (multiple penetrations of individual ova) is considered. As the proportion of penetrated ova increases so the extent of polyspermic fertilisation shows a corresponding rise, as described by the formula $y = 1 - e^{-x}$ (where y = % penetration/100 and x = mean number of spermatozoa per egg). A theoretical line drawn according to this Poisson formulation fits the raw data extremely accurately (Aitken 1983) and, as a result, some important conclusions can be drawn concerning the reliability of the hamster egg test. Firstly, it is clear that the hamster oocyte is unable to mount any resistance to the multiple entry of human spermatozoa; hence the conventional scoring system, per cent oocytes penetrated, can be supplemented with measurements of 'mean number of penetrations per egg' to yield important additional information, particularly when penetration rates rise above 60%. Secondly, there can have been no significant variations in the quality of the ova, media or incubation conditions over the 6 months during which the relevant 75 assays were carried out; i.e. it is possible to so standardise the conditions of this test that the only significant variable measured is the quality of the spermatozoa.

What Does the Test Measure?

Relationship of the Test Results to the Number of Motile Sperm

The outcome of the hamster egg penetration test is highly dependent upon the number of motile spermatozoa in the incubation medium (Binor et al. 1980; Aitken et al. 1982a). Serial dilution curves indicate that there must be at least 0.4–0.6 $\times$ 10^6 motile spermatozoa per ml of medium in order to observe penetration of hamster oocytes by human spermatozoa under conventional conditions. This relationship between the concentration of motile spermatozoa in the incubation mixture and the proportion of penetrated zona-free hamster oocytes is described by Poisson distribution theory, according to the formula $y = 1 - e^{-x}$ (where y = % penetration/100 and x = concentration of motile spermatozoa). Significantly, the shape of the regression line describing the relationship between penetration rate

and the concentration of motile spermatozoa in the incubation medium is identical for all men. Differences between men are represented by changes in the position of the regression line along the x axis (Aitken 1983). These differences are significant; some normal fertile donors will fertilise 100% of hamster oocytes at a concentration of 1.0×10^6 motile spermatozoa per ml whereas, at the same concentration, others will only penetrate 10% of oocytes. Hence, although the number of motile spermatozoa in the incubation medium is an important element to standardise in the test, there are other, unrelated, major differences in fertilising capacity between men.

Relationship of the Test Results to Sperm Motility

If the number of motile sperm cells in the incubation medium does not account for the differences in fertilising potential between men perhaps it is the type or manner of movement exhibited by the spermatozoa which is critical? Using a time-exposure photographic technique (Overstreet et al. 1979) we have examined the relationship between the movement characteristics of human spermatozoa and their performance in the zona-free hamster egg penetration test (Aitken et al. 1982 a,b,c). The aspects of sperm movement which appear to bear the most significant relationship to the ability of spermatozoa to penetrate hamster oocytes are a progressive swimming speed (> 25 μm/s), a straight swimming mode of progression and a small amplitude of lateral head displacement. Using the data obtained from time-exposure photography it was found that the presence of subnormal fertilising capacity in patients exhibiting unexplained infertility could have been accurately predicted in 60% of cases. In the remaining 40%, the spermatozoa exhibited apparently normal swimming patterns despite their inadequate capacity for fertilisation (Aitken et al. 1983c).

This dissociation between the quality of sperm movement and their capacity for fertilisation was recently emphasised in an analysis of a patient suffering from the immotile cilia syndrome (Aitken and Ross, unpublished observations). In this condition spermatozoa are completely immotile because of the absence of dynein arms in the microtubules of the axonemal complex. Despite this immotility the sperm were able to undergo the sequence of changes that comprise the capacitation process, culminating in a perfectly normal acrosome reaction and by direct contact could to fuse with and penetrate zona-free hamster oocytes!

The motility of sperm may not, therefore, give a consistently reliable indication of their fertilising potential. Conversely, positive scores in the hamster egg assay may not necessarily indicate that the spermatozoa are capable of exhibiting a normal pattern of motility. Hence the ability of a sperm cell to engage in a process such as zona pellucida penetration, which is a major aspect of sperm function heavily dependent on an adequate degree of motility, might not be reflected in the outcome of the hamster egg test. This deficiency of the hamster egg assay has recently been underlined by Overstreet et al. (1980), who have detected cases of male infertility in which the spermatozoa possess specific lesions influencing their capacity for zona penetration, but not their ability to fertilise hamster ova. Ideally therefore, an accurate description of the fertilising capacity of human spermatozoa necessitates a 'mixed gamete assay' in which capacitated human spermatozoa are incubated with both zona-free hamster oocytes and human zonae pellucidae (Overstreet et al. 1980).

Relationship of the Test Results to the Ability of Sperm to Migrate Through the Female Genital Tract

Another aspect of sperm function which depends on motility is the migration of spermatozoa to the site of fertilisation. The first component of this transport process is the colonisation of the cervical mucus to form a cervical sperm reservoir. With the aid of a well defined cervical mucus penetration test (Katz et al. 1980) we have recently demonstrated that the penetration of cervical mucus in vitro is correlated, not only with motility ($p < 0.01$), as might have been expected, but also with the outcome of the hamster egg penetration test (Schats, Aitken, Best and Lees, unpublished observations). Intriguingly, the ability of human spermatozoa to ascend the female reproductive tract in vivo, as assessed by a laparoscopic sperm recovery technique (Templeton and Mortimer 1982), is also correlated with the outcome of the hamster egg assay (Templeton et al. 1982). The basis of these associations between sperm transport and the fertilising capacity of human spermatozoa in vitro is unknown. Undoubtedly sperm motility forms a common link between these processes, although this relationship will clearly break down in cases where there are specific defects in the locomotory apparatus (as in the immotile cilia syndrome) which do not influence the fertilising capacity of the spermatozoa. Whatever its basis, the correlation between the outcome of the hamster egg test and sperm transport encourages the belief that the results obtained with this in vitro fertilisation assay may reflect the capacity of a semen sample to achieve a pregnancy in vivo.

Relationship of the Test Results to the Ability of Sperm to Undergo the Acrosome Reaction and to the Ability of Sperm to Fuse with the Vitelline Membrane

Within the fertilisation process itself, the hamster egg assay is clearly capable of generating information on the ability of human spermatozoa to undergo the acrosome reaction, since only acrosome-reacted cells will fuse with the vitelline membrane of the hamster oocyte (Yanagimachi 1981). This association is a curious one however, since the purpose of the acrosome reaction is generally thought to be the release of proteolytic enzymes, such as acrosin, which serve to cleave a path through the zona pellucida. Since, by definition, the oocytes employed in the hamster egg assay have been divested of their zonae, such a change on the part of the spermatozoon would seem irrelevant. It has therefore been proposed that the acrosome reaction must be accompanied by a change in the conformation of the plasma membrane overlying the equatorial/post-acrosomal region of the sperm head, promoting the fusion of this region of the spermatozoon with the vitelline membrane of the oocyte.

The appearance of a fusogenic region on the equatorial segment of the sperm plasma membrane may possibly be the most important aspect of sperm function measured by the hamster egg test. This suggestion has arisen because although the acrosome reaction is necessary for penetration of zona-free hamster oocytes, the differences in fertilising capacity between samples are not a direct reflection of the size of acrosome-reacted population. This finding emerged from a study in which a fluorescein conjugated *Ricinus communis* lectin was used to determine the

size of the acrosome-reacted population (Talbot and Chacon 1980) in samples of spermatozoa which were simultaneously evaluated with the zona-free hamster egg penetration test (Aitken et al. 1983b). Although correlations between hamster egg penetration and per cent acrosome reaction were observed within each individual sample, there were large differences in penetrating capacity between samples which were not correlated with this factor. As an example of this discrepancy, two individuals with 46% and 45% of their spermatozoa acrosome-reacted, showed 2.7% and 92.0% penetration of hamster ova respectively (Aitken et al. 1983b). One possible explanation for these differences is that in some samples loss of the acrosomal cap is not associated with the appropriate changes on the plasma membrane of the equatorial segment, permitting fusion with the vitelline membrane. In such cases a high proportion of acrosome-reacted spermatozoa in the incubation medium would not equate with a high hamster egg penetration score. As an assay of the competence of human spermatozoa to fuse with the vitelline membrane, interspecific fertilisation appears to be a valid procedure, since ultrastructural studies indicate that the fine details of the fusion process are essentially similar whether zona-free hamster oocytes or homologous ova are used (Barros and Herrera 1977; Koehler et al. 1982).

Clinical Applications of the Test

The clinical evaluation of this test is dependent upon the establishment of a relationship between the results obtained with the penetration assay and the fertility status of the semen donor. Rogers et al. (1979) established a mean penetration rate for men of proven fertility of 56.3% with a range of 14%–100%, while Hall et al. (1980) obtained a mean of 64% and a range of 20%–100%. In the author's laboratory, means (and ranges) of 44.0% (14%–90%) (Aitken et al. 1982a) and 63.8% (20%–100%) have been recorded for normal fertile donors using conventional and hyperosmotic media respectively (Aitken et al. 1983b). As a result of these and similar studies (Karp et al. 1981; Cohen et al. 1982; Stenchever et al. 1982) there appears to be a general concensus of opinion that the lower limit of normal fertility is indicated by a penetration score of around 11%.

In cases of unexplained infertility the distribution of hamster egg penetration scores is quite different (Aitken et al. 1982b). A group of 85 such patients exhibited a mean ($\pm$ SE) penetration rate of 30.8 $\pm$ 3.4%, which was significantly lower than that observed in the normal fertile controls. Examination of the frequency distribution of penetration scores within the unexplained infertility group revealed the presence of a sub-population of males exhibiting $<10\%$ penetration which was not present in the normal fertile population. Intriguingly, the identity of this subnormal group could not have been predicted from a knowledge of the conventional criteria of semen quality, and only 60% of these men would have been selected on the basis of the movement characteristics of their spermatozoa (Aitken et al. 1982b).

The clinical significance of the hamster egg penetration test in such cases is that on follow up, fewer patients who had exhibited a penetration rate of 0–10% fathered children compared with those who had scored within the normal fertile range of 11%–100% ($P < 0.05$). In this study, in which 68 patients with unexplained infertility were followed up for 2.3 years, none of the four patients

exhibiting zero penetration were found to have initiated a pregnancy, although five out of 21 patients exhibiting scores of 1%–10% were successful in this respect. Clearly, penetration scores below the normal fertile range (<10%) are not incompatible with pregnancy although the fertility of such patients is significantly reduced. That this cohort of subfertile patients could not have been identified by any other means emphasises the diagnostic value of the hamster egg penetration test in cases of unexplained infertility.

The test may also be of value in diagnosing the fertility status of oligozoospermic males. In a study of 27 such males (Aitken et al. 1982c) a mean penetration rate of $2.8 \pm 1.5\%$ was observed, lower ($P < 0.001$) than the value recorded for the normal fertile population (Aitken et al. 1982a). Only 2 of these oligozoospermic males scored more than 10% in the hamster egg test while 19 of them scored zero. The fact that only 8 (30%) of these patients exhibited any fertilising capacity in this study, is in reasonable agreement with the reported outcome of AIH therapy in cases of oligozoospermia, which indicates that around 20% of men initiate pregnancies as a result of this form of treatment (Nachtigall et al. 1979). Furthermore, the results of this study are in good agreement with the cumulative pregnancy rates observed in our own hospital for men exhibiting oligo-asthenozoospermia, which after 6 years reaches a plateau at which 32% of patients' wives are pregnant (West et al. 1982). The zero penetration scores observed in the hamster egg assay may therefore be of significant clinical importance in identifying those oligozoospermic patients for whom there is little chance of ever achieving a pregnancy. It will be fascinating to see whether this promise is fulfilled when the results of long term follow-up studies on these patients are available.

The hamster egg assay may also be of value in screening patients with varicoceles, in order to identify those cases in which there is an accompanying defect of sperm function. Studies in this area indicate that a majority of men attending infertility clinics with a detectable varicocele do, in fact, have severely defective spermatozoa. An Edinburgh-based study (Aitken, Hargreave, Shaw and Tolley, unpublished observations) has revealed that 10 out of 14 varicocele patients scored below the normal fertile range in the hamster egg assay, while half of these men exhibited a complete failure of ovum penetration. Similarly, Rogers et al. (1980) detected subnormal fertilising capacity in 83.6% of varicocele patients. In the light of these results it is possible that the hamster egg test may be of value in selecting patients for whom treatment is appropriate.

The ability of the zona-free hamster egg penetration test to measure certain aspects of sperm function suggests another potential application for this system, in the selection of patients for in vitro fertilisation therapy. Although the in vitro fertilisation/embryo transfer procedure was initially conceived as a means of treating bilateral tubal occlusion, it may also be of profound importance in the treatment of male infertility. Clinical conditions, associated with symptoms of oligozoospermia, teratozoospermia or asthenozoospermia, in which there may only be a small population of spermatozoa in the ejaculate capable of fertilisation, might benefit from such therapy. Similarly in cases of unexplained infertility, patients with defective semen specimens identified by the hamster egg test, but in whom the conventional semen profile is perfectly normal, may be ideal candidates for this form of treatment. The major advantage of in vitro fertilisation therapy in such cases is that it circumvents the need for sperm transport to the site of fertilisation. This is a significant factor because there is good evidence, particularly in cases of unexplained infertility, that a major cause of infertility is a failure on the part of the

spermatozoa to ascend the female reproductive tract (Templeton and Mortimer 1982). Furthermore, failure of the spermatozoa to reach the site of fertilisation is significantly correlated with the outcome of the hamster egg penetration test, but *with no other aspect of the semen profile* (Templeton et al. 1982). It therefore seems appropriate to use the hamster egg test to select patients for whom in vitro fertilisation therapy might be employed in order to remedy situations in which the size of the fertile sperm population is small and likely to be further diminished during the ascent of the female reproductive tract in vivo.

The use of in vitro fertilisation in such situations carries with it a secondary advantage in that it provides an opportunity to supplement the incubation media with compounds that could artificially stimulate the fertilising capacity of the spermatozoa. The hamster egg test has already proved useful in helping to identify appropriate stimulants, such as caffeine (Aitken et al. 1983a) and exposure to hyperosmotic media (Aitken et al. 1983b).

An important consideration in employing the hamster egg test to select patients for in vitro fertilisation therapy is the significance of a zero penetration score. If such a result meant that the spermatozoa were completely unable to fertilise human ova, then zero scores would assume considerable importance as an exclusion criterion for in vitro fertilisation therapy. In a study involving 17 cases of simultaneous interspecific and intraspecific fertilisation of hamster and human oocytes respectively, we have observed subsequent cleavage of the human ovum with sperm samples scoring from 0 to 88.5% in the hamster egg test (Aitken, Templeton, Van Look, Evans and Baird, unpublished observations). An inherent defect of such studies is that cleavage may not invariably mean that fertilisation has taken place, parthenogenic activation of the ovum always being a possibility. Nevertheless, these results indicate that a failure to fertilise hamster oocytes may not necessarily mean that the spermatozoa are incapable of fertilising the human ovum. The fact that there must be at least $0.4–0.6 \times 10^6$/ml motile spermatozoa in the incubation medium to achieve fertilisation of zona-free hamster oocytes (Aitken et al. 1982a; Binor et al. 1980), whereas human ova can be routinely fertilised with 0.2×10^6 cells/ml, also suggests that the hamster oocyte is the more formidable obstacle for human spermatozoa.

Conclusions and the Future

The aspects of sperm function assessed by the zona-free hamster egg penetration test include the ability of spermatozoa to capacitate, acrosome-react, fuse with the vitelline membrane, become incorporated into the ooplasm and undergo nuclear decondensation. Of all the functions measured by this interspecific system, perhaps the most important is the acquisition of the ability to fuse with the vitelline membrane. At the ultrastructural level, the fusion of human spermatozoa with the vitelline membrane of the hamster oocyte appears to be an exact reflection of sperm–egg fusion in the homologous situation. However, the fact that human ova can be fertilised with spermatozoa which fail to penetrate hamster oocytes suggests that at the molecular level, there are differences in the composition of human and hamster vitelline membranes. Another obvious difference between the human ovum and the hamster oocyte is the presence of zona pellucida around the former. Lesions in the spermatozoa which impair their ability to bind to or penetrate the

zona will not, therefore, be detected in the hamster egg system. As a means of screening human sperm samples for their ability to fertilise human ova, the zona-free hamster egg must be regarded as an imperfect surrogate.

Nevertheless, the information generated with this procedure is unique and clinically valuable. The test promises to give better information about a man's fertility than any of the standard methods of semen analysis. Men can be identified who have defects of sperm function which cannot be found using standard semen measurements. Although it is not possible to exclude patients from in vitro fertilisation therapy because their spermatozoa fail to penetrate hamster oocytes, this system could still be of value in identifying patients appropriate for such treatment.

The laboratory study of normal and abnormal sperm will also be facilitated by the zona-free test. It may be possible in the future to identify populations of infertile sperm associated with a specific defect, e.g. the failure of fusion with the vitelline membrane. Once this has been done it should be possible to understand the molecular basis of the defect and new specific methods of treatment may emerge.

The techniques used for the zona-free test are similar to those employed for in vitro fertilisation. In the light of results of laboratory research it should be possible to identify those men who will benefit from in vitro techniques being used to enable a fertilisation to take place that would otherwise not have occurred. One can predict that in vitro fertilisation techniques may become one of the mainstays of treatment for proven male infertility and that the zona-free penetration test may become the most widely used technique in the assessment of male fertility.

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Chapter 6

Endocrinology of Male Infertility and Fertility

F. Wu

Introduction

The gonads are unique amongst endocrine glands in that they have two functions: steroid hormone synthesis and gamete production. Spermatogenesis takes place within the seminiferous tubules while the sex steroids are synthesised in the Leydig cells in the interstitial spaces. As well as anatomical proximity (Fig. 6.1) there is close functional interrelationship. It has recently become clear that high intratesticular androgen concentrations are necessary for normal spermatogenesis and this concept has influenced thinking about patient management and also determined the direction of recent research.

Normal Testicular Function and Control

Steroidogenesis

Leydig cells synthesise sex steroids via biosynthetic pathways common to other steroid-secreting tissues, from acetate via cholesterol and pregnenolone (Hall 1970). The major testicular secretory product is testosterone but weaker androgens such as dehydroepiandrosterone, androstenedione and also oestradiol are produced. Although a small amount is secreted by the testis, dihydrotestosterone is mainly derived from testosterone at the target tissues where it serves as an effector hormone by binding to specific cytoplasmic receptors (Bruchowsky and Wilson 1968).

Testicular steroidogenesis is principally regulated by the anterior pituitary hormone luteinising hormone (LH) but follicle-stimulating hormone (FSH) and prolactin may also have minor synergistic roles (Bartke et al. 1978). The mechanism of LH stimulation of testosterone production typifies the actions of peptide hormones (Catt and Dufau 1976). It involves the specific binding of LH to surface membrane receptors on Leydig cells resulting in the sequential activation of adenyl

cyclase and cyclic-AMP-dependent protein kinases leading to messenger RNA and protein synthesis. Specific rate-limiting steps in the testosterone synthetic pathway, such as side-chain cleavage of cholesterol to form pregnenolone, are influenced by this series of membrane-associated intracelluar events (Moyle et al. 1973). In addition, local regulatory mechanisms involving the loss of spare receptor sites on Leydig cells and local oestrogen production have also been demonstrated (Dufau et al. 1978).

Testosterone is secreted in a pulsatile manner (Baker et al. 1975). Maximum levels occur at 0600 h and minimum levels at 2200 h–2400 h (Sjöberg et al. 1979). Only 2% of circulating testosterone is biologically active as the remaining 98% is bound to the carrier proteins sex-hormone-binding globulin (SHBG) and albumen (Anderson 1974). Testicular steroidogenesis declines in some but not all men after the fifth decade with decreased total and free testosterone and increased oestradiol, SHBG and plasma LH levels (Baker et al. 1976a, b; Vermeulen 1979).

Androgens in the systemic circulation are necessary for the intrauterine development of internal and external genitalia, the development and maintenance of secondary sexual characteristics and normal male sexual activity. The extremely high intratesticular concentration of testosterone (Steinberger et al. 1974a) is thought to be important in promoting normal germ cell maturation (Steinberger 1971).

Spermatogenesis

Germ Cells

The spermatogonia undergo a strictly coordinated series of mitotic and meiotic divisions followed by metamorphosis of cell structures (spermiogenesis) to yield spermatozoa with their haploid chromosome complement and inherent capacity for motility and fertilisation (Clermont 1963) (see p. 124).

Sertoli Cells

These tall columnar somatic cells possess extensive cytoplasm enveloping the developing germ cells as they migrate towards the lumen (Fig. 6.1). Tight junctions between neighbouring Sertoli cells form the anatomical basis of a blood–testis barrier dividing the seminiferous tubules into basal and adluminal compartments (Setchell and Waites 1975) (Fig. 6.2). Within the secluded and avascular environment of the latter, the leptotene primary spermatocytes, secondary spermatocytes and spermatids develop. The Sertoli cells secrete large amounts of hyperosmolar tubular fluid rich in potassium, inositol, aspartate, glutamate and androgens (Setchell 1980a). This highly specialised microenvironment is essential for the completion of germ cell meiosis and spermiogenesis (Frizt 1978). It is generally believed that most factors influencing germ cell development are mediated via the Sertoli cells and it is therefore of interest to note that FSH and testosterone bind specifically to Sertoli cells (Mulder et al. 1976; Orth and Christensen 1977).

Hormonal Requirements for Spermatogenesis

Gonadotrophins are required to maintain the specific intratesticular milieu conducive to germ cell development. Hypophysectomy (Clermont and Morgentaler 1955)

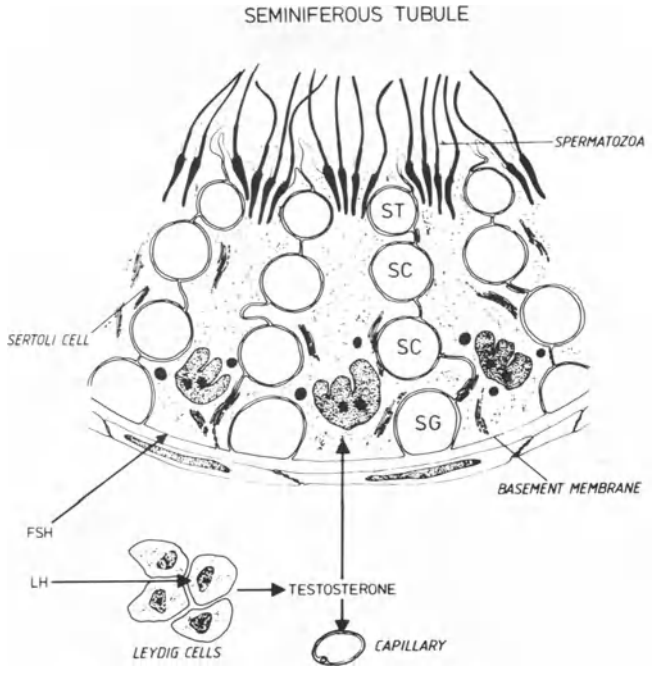

Fig. 6.1. Diagrammatic representation of the cellular composition and structural relationship between different cell types in the testis. *S*, Sertoli cells; *SC*, spermatocytes; *SG*, spermatogonia; *ST*, spermatids (Dorrington 1980)

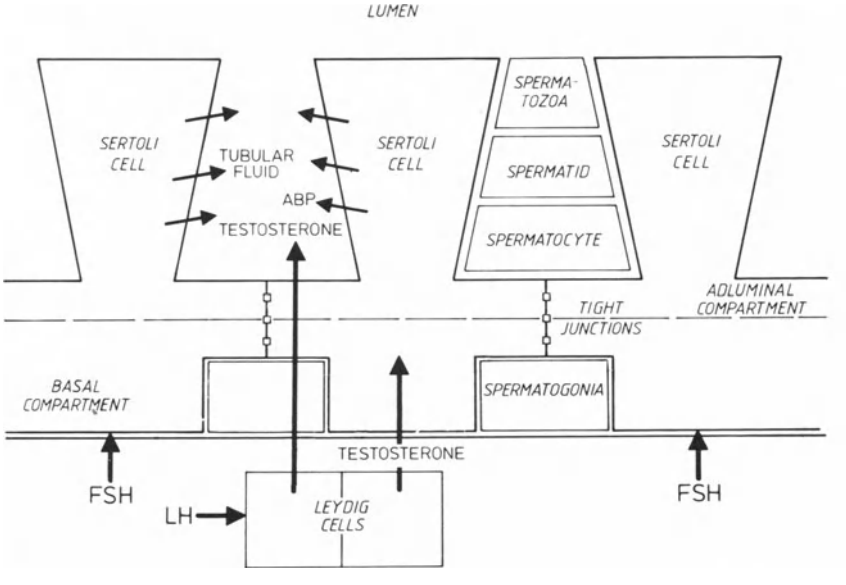

Fig. 6.2. Schematic view of the cellular arrangement in the seminiferous tubules. The tight junctions between the Sertoli cells separate the tubule into basal and adluminal compartments and the various possibilities of hormonal transport and action are indicated. (Adapted from Dorrington 1980)

and reduction of gonadotrophin by specific antisera in rats (Raj and Dym 1976) and in monkeys (Wickings et al. 1980) result in failure of spermatogenesis and Leydig cell function. Replacement studies after hypophysectomy demonstrate that androgens as well as FSH are required for the restoration of spermatogenesis (Steinberger 1971). However, in some species (rats and rabbits) but not others (man, monkey and ram) very high doses of exogenous testosterone alone were able to maintain spermatogenesis after hypophysectomy if treatment was started before testicular regression occurred (Ahmad et al. 1973). The experimental findings in the rat fostered the concept that androgens alone can maintain spermatogenesis, but both FSH and androgens are required to initiate or restore it (Steinberger 1971). However in man, monkeys and rams, FSH is also required for the maintenance of normal spermatogenesis in the adult (Mancini et al. 1971; Wickings et al. 1980). Nevertheless, the differential hormone requirement for initiation and maintenance of spermatogenesis in rodents has provided a basis for the study of molecular mechanisms of hormonal control.

Androgen-Binding Protein

The action of FSH in rat seminiferous tubules has been intensively studied in recent years using in vitro cell culture techniques (Means et al. 1976, 1978). Sertoli cells are probably the sole target cells for FSH in the seminiferous tubules (Orth and Christensen 1977) but they also possess cytoplasmic receptors for androgens (Mulder et al. 1976). Sertoli cells synthesise an androphilic macromolecule—androgen-binding protein (ABP)—in response to FSH and also to stimulation by testosterone (Fritz et al. 1975; Elkington et al. 1975). Testicular levels of ABP fall following hypophysectomy (Sanborn et al. 1975). Androgens can maintain ABP synthesis if their administration is started immediately after surgery but FSH and androgens are required to reinitiate ABP production after testicular regression (Weddington et al. 1975). Thus the Sertoli cell requirement for ABP production closely parallels the hormonal requirement for spermatogenesis in the rat. This suggests that ABP may have a crucial role in spermatogenesis although the exact nature of its function in the seminiferous tubules remains uncertain.

Hormonal Control of Spermatogenesis—A Hypothetical Model

Based on the above findings in rats it is possible to conceive a model for the molecular mechanism of spermatogenesis (Fig. 6.2). FSH and androgens stimulate the synthesis of ABP by Sertoli cells; it is secreted into the adluminal compartment of the seminiferous tubules. ABP has a high affinity for testosterone diffusing into the tubules from the interstitium and the binding of the incoming androgen may prevent its rapid metabolism to oestradiol and androstanediols thus maintaining a high concentration of androgens in the tubule (Dorrington 1980). At present, it is not clear whether androgens act directly on germ cells or indirectly via Sertoli cells.

There are, however, deficiencies in the model. In man, it has not been possible to differentiate between testicular ABP and circulating SHBG (Vigersky et al. 1976). Furthermore, Cunningham and Huckins (1979) have challenged the concept that high intratesticular testosterone is essential for spermatogenesis. Clearly, much more basic information is needed before the mechanism of hormonal control of spermatogenesis can be fully understood.

Control of Gonadotrophin Secretion

Hypothalamus—Gonadotrophin Releasing Hormone

A single hypothalamic hormone, gonadotrophin-releasing-hormone (GnRH) is responsible for stimulating the release of both LH and FSH (Schally et al. 1971). LH and FSH are always produced together as a functional unit. It is generally accepted that episodic secretion of GnRH into the hypophyseal portal vessels is responsible for the pulsatile fluctuation of plasma LH (Carmel et al. 1976). FSH secretion is also episodic, but due to its lower plasma concentration and longer circulating half-life, pulsatile changes are less obvious. In man, episodic LH secretion first becomes detectable during sleep just before the onset of puberty (Wu et al. 1980). The increased LH pulse frequency and amplitude is important in the development of pituitary and testicular functions during puberty (Foster et al. 1978). In adult men, LH pulsatile secretion is no longer entrained to sleep and approximately 10–12 secretory pulses occur every 24 h (Santen and Bradin 1973) with no diurnal variation (Krieger et al. 1972). In seasonally reproducing animals frequency and amplitude of GnRH secretion constitute an important mechanism for the control of pituitary–gonadal function (Lincoln and Short 1980).

Testes

Feedback by Testicular Steroids

It is now well established that testicular steroids exert negative feedback over gonadotrophin secretion (Brown-Grant 1977). Testosterone is the most important quantitatively but its metabolites 5α-dihydrotestosterone and oestradiol are also independently capable of suppressing LH and to a smaller extent FSH (Santen 1977). All three steroids act on the hypothalamus as well as the pituitary but their effects on these two sites are distinct (Kingsley and Bogdanove 1973; Santen and Ruby 1979).

Inhibin

Failure of testicular steroids to suppress FSH to normal levels after castration and the rise of FSH without LH after selective damage to the seminiferous epithelium indicate the presence of an additional feedback mechanism (Setchel et al. 1977). The existence of this mechanism was first proposed by McCullagh in 1932. It was not until the last few years however that inhibin was isolated and it has still not been completely characterised (Davies et al. 1978). Inhibin is said to be a peptide molecule with molecular weight between 1200 and 16 000 and its site of production has been traced to the Sertoli cell (Steinberger 1979). On the evidence available at present, its exact physiological role in the negative feedback control of gonadotrophins is yet to be defined.

Prolactin

The physiological role of prolactin in the human male is unclear. Prolactin

receptors occur in Leydig cells (Charreau et al. 1977) and the hormone is present in the human seminal plasma in concentrations 4–7-fold higher than in blood (Sheth et al. 1975). Prolactin is said to influence oxidative metabolism and motility of sperm (Shah et al. 1976). Animal studies have suggested that prolactin may have a synergistic effect with LH on Leydig cell function and spermatogenesis (Bartke 1971). Rubin et al. (1975, 1976) found a correlation between nocturnal elevation of prolactin and testosterone in man. Hyperprolactinaemia is associated with hypogonadism and impotence in man (Thorner et al. 1977) but the effect of hyperprolactinaemia on seminiferous tubular function has not been systematically studied.

Endocrine Causes of Male Infertility

The majority of patients presenting with infertility do not have any primary endocrine abnormalities, but hormonal changes secondary to seminiferous tubule damage are common.

The incidence of primary endocrine defects in subfertile males has been reported to be 0.5%–3% (Van Zyl et al. 1975; de Kretser 1979; Vermeulen 1979), but this small minority is significant since specific hormonal treatment is often successful. Moreover, the few that have underlying pituitary tumours will require definitive treatment. There is no specific endocrine condition that will damage seminiferous tubules without also affecting Leydig cells because LH and FSH are always produced together as a functional unit.

Hypogonadotrophic Hypogonadism

Acquired lesions of the pituitary such as adenomata, infarction or granulomata may give rise to hypogonadotrophic hypogonadism. This may be associated with other pituitary hormone deficiency or excess which usually presents more urgent clinical problems. Isolated gonadotrophin deficiency is a congenital disorder due to a selective deficiency of hypothalamic GnRH (Hashimoto et al. 1975). This is commonly associated with agenesis of the olfactory bulb leading to anosmia or hyposmia. This clinical association was first described by Kallmann et al. in 1944; he has since given his name to this syndrome, in which other somatic abnormalities such as colour blindness, nerve deafness, short fourth metacarpal, syndactyly, mental subnormality, midline cranio-facial defects and renal abnormalities are also encountered (Santen and Paulsen 1973). A familial incidence may be found. Recognition of these clinical features, especially anosmia, in hypogonadal patients will facilitate the clinical diagnosis of hypogonadotrophism. LH and FSH are deficient in both the congenital and the acquired form and the predominant clinical picture in these patients is of androgen deficiency presenting as a lack of sexual development, impotence and loss of sex drive.

Fertile Eunuch

In hypogonadotrophic hypogonadism, the degree of GnRH deficiency is variable. In states of partial deficiency subnormal amounts of GnRH may stimulate FSH

secretion preferentially (Valk et al. 1980). The normal FSH and subnormal LH may produce sufficient intratesticular testosterone to establish spermatogenesis even though circulating testosterone is inadequate for full development of secondary sexual characteristics. These patients were originally described as 'fertile eunuchs' (McCullagh et al. 1953), a misnomer based on the erroneous concept that the primary defect was isolated LH deficiency and that fertility was normal if spermatogenesis was present. A recent report of two patients who fulfilled the diagnostic criteria for both Kallmann's and 'fertile eunuch' syndrome confirmed that these are but phenotypic variants in the spectrum of hypogonadotrophic hypogonadism associated with variable degrees of GnRH deficiency (Rogol et al. 1980).

Isolated FSH Deficiency (see also p. 219)

There are two reported cases of this in the English literature. The first (Rabinowitz et al. 1974) was a patient with X0/XXY karyotype, germ cell aplasia and 'undetectable' plasma FSH, who nevertheless responded to GnRH stimulation with increases in LH and FSH. The other case (Stewart-Bentley and Wallack 1975) was an oligozoospermic patient with basal plasma FSH below the detection limit of assay. There was no response of FSH to either clomiphene or GnRH stimulation. These reports need further confirmation before isolated FSH deficiency can be accepted as a true clinical entity. However they serve as a useful reminder that the lower end of the physiological range of plasma gonadotrophins, especially in the case of FSH, may be close to the detection limits of radioimmunoassay (Ross 1970).

Hyperprolactinaemia

In man, persistent hyperprolactinaemia is usually associated with pituitary chromophobe adenoma or acromegaly. Impotence is the characteristic feature (Thorner et al. 1977). There is no general agreement on the incidence, nature, severity or reversibility of abnormal spermatogenesis in these cases. In patients who primarily present with infertility, the incidence of elevated basal plasma prolactin has been reported to be 11%–40% (Segal et al. 1976; Roulier et al. 1976; Saidi et al. 1977; Hermabessiere et al. 1977; Blacker et al. 1977). In a large series of men presenting with infertility no case of significant hyperprolactinaemia was found when results were compared with those from a fertile control population (Hargreave et al. 1981). Another study however found lower mean prolactin levels in infertile men (Pierrepoint et al. 1978). The significance of prolactin in men presenting with infertility is doubtful on present evidence.

Miscellaneous

It has been postulated that in some cases of 'idiopathic' infertility, specific enzyme defects in the androgen synthetic pathway may give rise to inadequate intratesticular concentrations of testosterone while the circulating levels remain within the normal range. Several reports claim to have demonstrated defects in androgen synthesis in in vitro studies of testicular biopsy material from infertile men

(Rodriguez-Rigau et al. 1978), while others are unable to confirm this (Nieschlag et al. 1979).

Recently two infertile patients with normal secondary sexual characteristics were found to have partial androgen insensitivity with elevated gonadotrophins and elevated testosterone production rates (Aiman et al. 1979a). It was suggested that the abnormal spermatogenesis was a consequence of a biochemical defect in androgen action.

Endocrine Sequelae of Defective Spermatogenesis

Follicle-Stimulating Hormone

Since FSH is believed to be under the negative feedback control of the seminiferous tubular hormone inhibin, conditions where the seminiferous epithelium is severely damaged will result in loss or reduction of inhibin production and elevated FSH (Scott and Burger 1980). Qualitative assessment of the severity of germ cell damage in testicular biopsies from infertile men showed an inverse relationship with plasma FSH concentration (Wu et al. 1981). Quantitative cytological studies confirmed a similar inverse relationship between FSH and the number of spermatogonia (de Kretser et al. 1974) (Fig. 6.3) or more mature gem cells (Franchimont et al. 1972). Furthermore, when sperm density was used as the index for seminiferous tubular function, the same inverse relationship with FSH was demonstrated (Fig. 6.4). However, the finding of occasional patients with high FSH in spite of a normal sperm density has led to current research on interaction between germ cells and Sertoli cells and the possibility that this interaction may occasionally break down (Baker et al. 1976a).

LH and Androgens

Elevated LH levels are commonly found in infertile men with more severe degrees of germ cell depletion. There is good correlation between plasma LH and FSH concentrations in infertile men (Wu et al. 1981). This implies either that inhibin has a negative feedback effect on LH via GnRH (Franchimont et al. 1979) or that Leydig cell dysfunction may be associated with defects of spermatogenesis. This latter possibility is compatible with the findings of lower mean plasma testosterone (Nieschlag et at. 1978), diminished response to hCG stimulation (de Kretser et al. 1975), and impaired testicular steroidogenesis in vitro in infertile men (Rodriguez-Rigau et al. 1978). Ultrastructural studies in rats with experimentally induced spermatogenic damage revealed changes in the Leydig cell compatible with abnormal steroid production (Kerr et al. 1979a). Lower mean concentrations of dihydrotestosterone in blood and seminal plasma were found in infertile men compared with normal men (Purvis et al. 1975a,b); but this was not confirmed by Nieschlag et al. (1978).

In a few cases the combination of elevated LH and elevated testosterone levels may suggest the presence of androgen resistance (Aiman et al. 1979b).

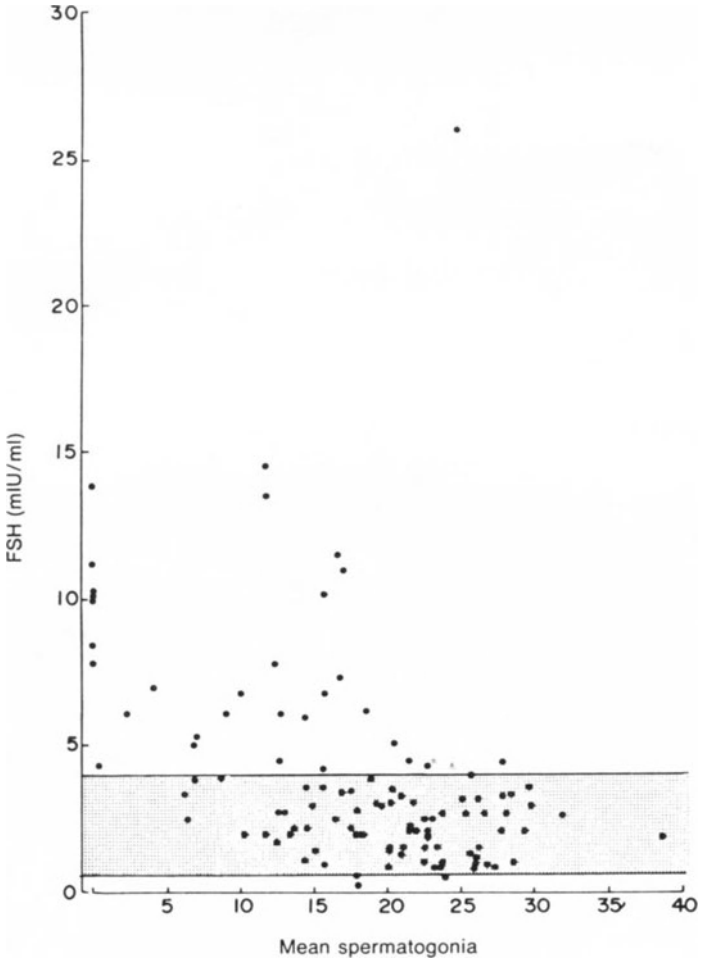

Fig. 6.3. Relationship between spermatogonial numbers and FSH levels in 111 infertile patients (de Kretser and Holstein 1976)

Oestrogens

Oestrogens in infertile males have not been extensively studied. Recently, the plasma concentrations of oestradiol, oestrone and oestrone sulphate in a group of infertile men with elevated FSH were found to be significantly higher than in matched fertile controls (Wu et al. 1982a). This was accompanied by an increase in SHBG concentration. Although the source of excessive oestrogens is uncertain in these infertile men, animal studies have demonstrated increased conversion of testosterone to oestradiol by Sertoli cells in response to FSH stimulation (Dorrington and Armstrong 1975). Human spermatic vein concentration of oestradiol was also elevated in patients with raised FSH (Wu et al. 1982b). There is considerable evidence that oestradiol can directly inhibit Leydig cell function without LH

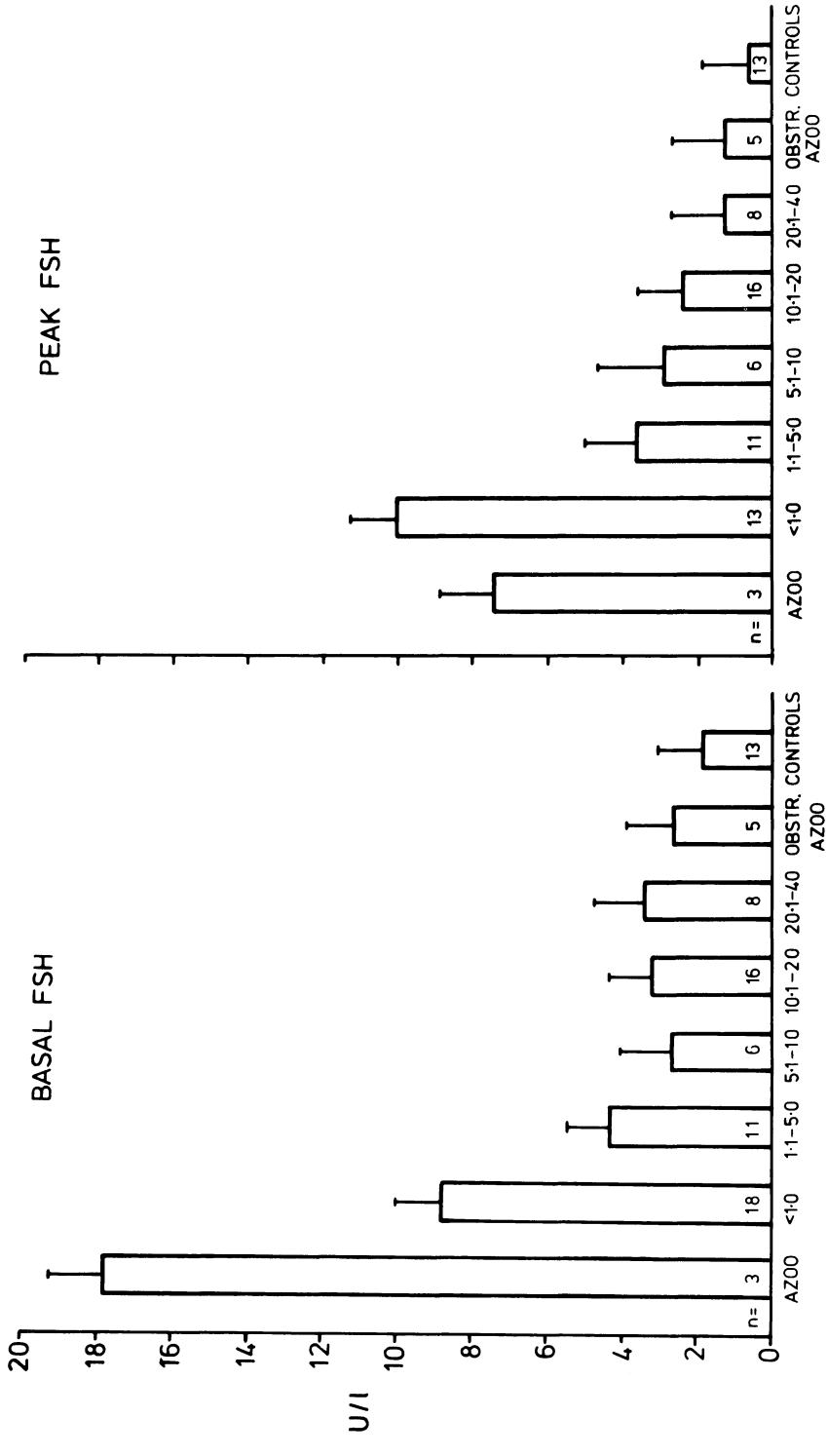

Fig. 6.4. Mean (±SEM) levels of basal and peak FSH (response to 50 ug GNRH IV) at different sperm densities. Patients with obstructive azoospermia had grade 1 or 2 histologies and major abnormalities of the efferent ducts (Wu et al. 1981)

suppression (Dufau et al. 1978). Thus, primary lesions of the seminiferous tubules can give rise to increased oestrogen synthesis in the Sertoli cells from excessive stimulation by the pathologically elevated FSH. Increased testicular oestrogen can inhibit Leydig cell functions and suppress androgen production. This series of deranged hormonal reactions may aggravate or perpetuate the initial lesions in the seminiferous tubules (de Kretser 1979).

Varicocele

Lower mean plasma testosterone concentrations have been reported in men with varicocele especially when they are over the age of 35 (Comhaire and Vermeulen 1975). In vitro studies in human testicular biopsy tissue showed reduced conversion of pregnenolone to testosterone in 16 patients with varicocele compared with one normal control (Weiss et al. 1978). However, normal concentrations of gonadotrophins, testosterone and oestradiol in the peripheral and spermatic veins of patients with varicocele have also been reported (Swerdloff and Walsh 1975).

Cryptorchidism

Elevated FSH levels are commonly found in adults with bilateral or unilateral cryptorchidism even after successful orchiopexy. The level of LH may also be raised, especially in those with bilateral cryptorchid testes in association with grossly elevated FSH levels, but testosterone is seldom abnormal (Bramble et al. 1974). In cryptorchid prepubertal boys, there is no agreement as to whether FSH or LH is raised, normal or low. These observations have several clinical implications. Cryptorchidism is a common clinical feature of patients with hypogonadotrophic hypogonadism (Santen and Paulsen 1973). In adults, the elevated gonadotrophin level associated with undescended testes serves to exclude the diagnosis of gonadotrophin deficiency but in prepubertal children the rise in gonadotrophin level is unpredictable. This emphasizes the importance of adequate follow-up of prepubertal patients after surgical correction of cryptorchidism to ensure that normal sexual development occurs. Lastly, in those with unilateral cryptorchidism, elevated FSH levels may be compatible with relatively adequate sperm production from the eutopic testis.

Klinefelter's Syndrome

In this condition, the presence of supernumerary X chromosome(s) results in the premature degeneration of testicular germ cells so that with very rare exceptions effective spermatogenesis is absent (Foss and Lewis 1971). FSH secretion will consequently be increased. Associated with this are variable degrees of Leydig cell hypofunction evidenced by low to low normal levels of circulating testosterone, impaired response to hCG stimulation and elevated LH (Wang et al. 1975). The nature of the Leydig cell abnormality is not clear. Specific enzymatic defects in the testosterone synthetic pathway have been proposed (Stewart-Bentley and Horton 1973). However, a heightened testosterone synthetic capacity of the testis in vitro

was also found in spite of low circulating testosterone levels (Steinberger et al. 1974a). Another consistent finding in Klinefelter's syndrome is high or normal plasma oestradiol concentration and blood production rate (Sitterii and MacDonald 1973). This may be explained by the increased conversion of testosterone to oestradiol in testicular (Sitterii and MacDonald 1973) and non-testicular tissues (Wang et al. 1975).

Cancer Chemotherapy (see also p. 13)

Antitumour drugs, including alkylating agents, procarbazine, cytosine arabinoside, daunorubicin and vinblastine, are known to produce testicular germ cell atrophy in a dose-related fashion. In recent years the dramatic improvement in remission and survival rates, especially in Hodgkin's disease and lymphoblastic leukaemia, has generated more interest in the long-term effect of treatment on reproductive function of these patients. This subject has been recently reviewed by Schilsky et al. (1980).

In nearly all patients (>80%) treated by combination chemotherapy, germ cell atrophy results in reduction in testicular size, azoospermia and elevation of plasma FSH. The adult, pubertal and prepubertal testes are all susceptible although there is some evidence that in the prepubertal testes the extent of germ cell atrophy may not be as complete as in adults (Lendon et al. 1978). Recovery of the germinal epithelium with normalization of FSH levels (Roesen et al. 1978) and even fertility (Holmes and Holmes 1978) does occur, although only in a small minority (Chapman et al. 1979). Recovery is dependent on the total dose of drugs and the time since cessation of treatment; but no definite prognostic conclusions on spermatogenetic recovery can be offered at present due to the lack of prospective data.

In both treated adults and children, evidence of mild or compensated Leydig cell failure are detectable in the form of elevated basal plasma LH, exaggerated response to LHRH and lower testosterone concentrations (Mecklenburg and Sherins 1974; Chapman et al. 1979). Clinical evidence of androgen deficiency includes gynaecomastia and diminished sexual function (Sherins et al. 1978).

Endocrine Diagnosis of Male Infertility

The major objective of endocrine investigations is to assess the severity of abnormalities in the seminiferous epithelium in azoospermic and oligozoospermic patients. The measurement of FSH, by virtue of its inverse relationship to sperm density and germ cell population in the seminiferous tubules, has to a large extent replaced testicular biopsy as the adjunct to semen analysis in the investigation of male infertility. LH and testosterone measurements may also contribute to the complete assessment of hypothalamic–pituitary–testicular functions and identify the few patients with gonadotrophin deficiency and androgen insensitivity.

Follicle-Stimulating Hormone

The general availability of radioimmunoassay has enabled the measurement of FSH to be used routinely in the assessment of the infertile male. Results of a survey of 100 consecutive oligozoospermic patients (<40 million/ml) attending the Male subfertility clinic, Royal Infirmary, Edinburgh are summarised in Fig. 6.5. Testicular histology was assessed qualitatively (McIlree et al. 1966) from: grade 1—normal, grade 2—moderate hypospermatogenesis with germ cells present in all tubules, grade 3—germ cell aplasia in some but not all tubules, to grade 4—germ cell aplasia in all tubules. In the azoospermic and severely oligozoospermic (<5 million/ml) groups, elevated basal FSH denotes significant germ cell destruction—grade 3 or grade 4. There is general agreement that this is irreversible due to the depletion of stem cells. In azoospermic patients, normal FSH is compatible with obstruction or malformation of the efferent duct system since testicular histology was more or less normal (grade 1 or grade 2). In patients with sperm density above 5 million/ml, all except one patient had grade 1 or grade 2 histology and 40 out of 43 had normal FSH levels. Thus FSH estimation in this group is unhelpful. In the remaining patients, those with normal FSH and sperm density under 5 million/ml, the testicular histological grading cannot be reliably predicted. This may theoretically be explained by partial obstructed lesions of the efferent ducts, pituitary/hypothalamic abnormalities or unrepresentative unilateral testicular biopsy specimens. This sizeable group may therefore require further investigations.

Bearing in mind the limitations of measurement of basal FSH, it was of interest to see whether the diagnostic accuracy in terms of prediction of testicular histology could be improved with the use of GnRH stimulation as well as LH and testosterone measurements. It was found that basal FSH was the best single discriminator between histological groups while the additional measurement of FSH response to GnRH, LH and testosterone gave no further information in this respect (Table 6.1). It was also shown that basal FSH and GnRH response were highly correlated (Fig. 6.6). Based on these results, the most informative hormonal measurement would seem to be basal FSH alone. The episodic fluctuation of FSH was small—the coefficient of variation of four half-hourly samples was under 10% so that single estimations are representative.

Certain pitfalls in the interpretation of FSH results are worth emphasising. As a general rule, elevated FSH and azoospermia or oligozoospermia are synonymous with irreversible germ cell depletion and severely impaired fertility. One notable exception is that arising from cytotoxic chemotherapy where recovery of spermatogenesis with normalisation of FSH has been reported in a small proportion of patients (Roesen et al. 1978). In patients with unilateral testicular atrophy from whatever aetiology, e.g. cryptorchidism, varicocele, orchidectomy, high FSH levels are compatible with reasonable sperm production, presumably from the contralateral testis, and even fertility (Wu et al. 1981). In some patients with azoospermia and normal FSH, the underlying abnormality may be a late maturation arrest rather than destruction in the outflow tract. It is wise therefore to examine fluid from the caput epididymis for sperm at the time of attempting epididymovasostomy. In most cases sperm are seen and the operation can proceed. If no sperm are seen then a testicular biopsy should be performed.

The application of FSH measurement to male infertility can now be rationalised. In most instances, this procedure has obviated the need for testicular biopsy. Abnormally high levels associated with azoospermia or oligozoospermia and small

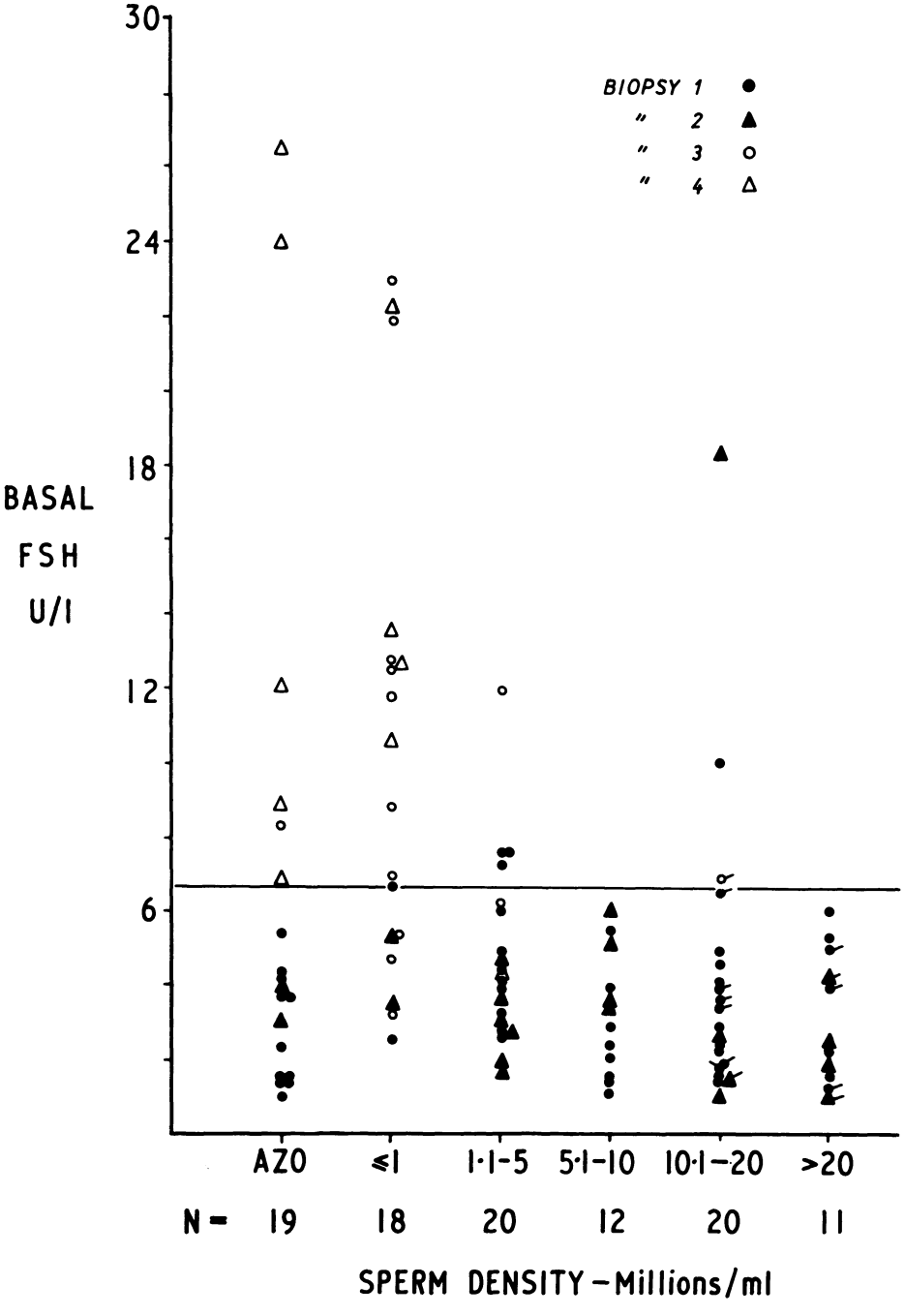

Fig. 6.5. Basal FSH, sperm density and testicular histology (grades 1–4—see text) in 100 subfertile patients. Horizontal line represents the upper limit of the normal range (6.7 U/l) for basal FSH. Markers indicate patients who subsequently impregnated their wives with or without treatment. (Wu et al. 1981)

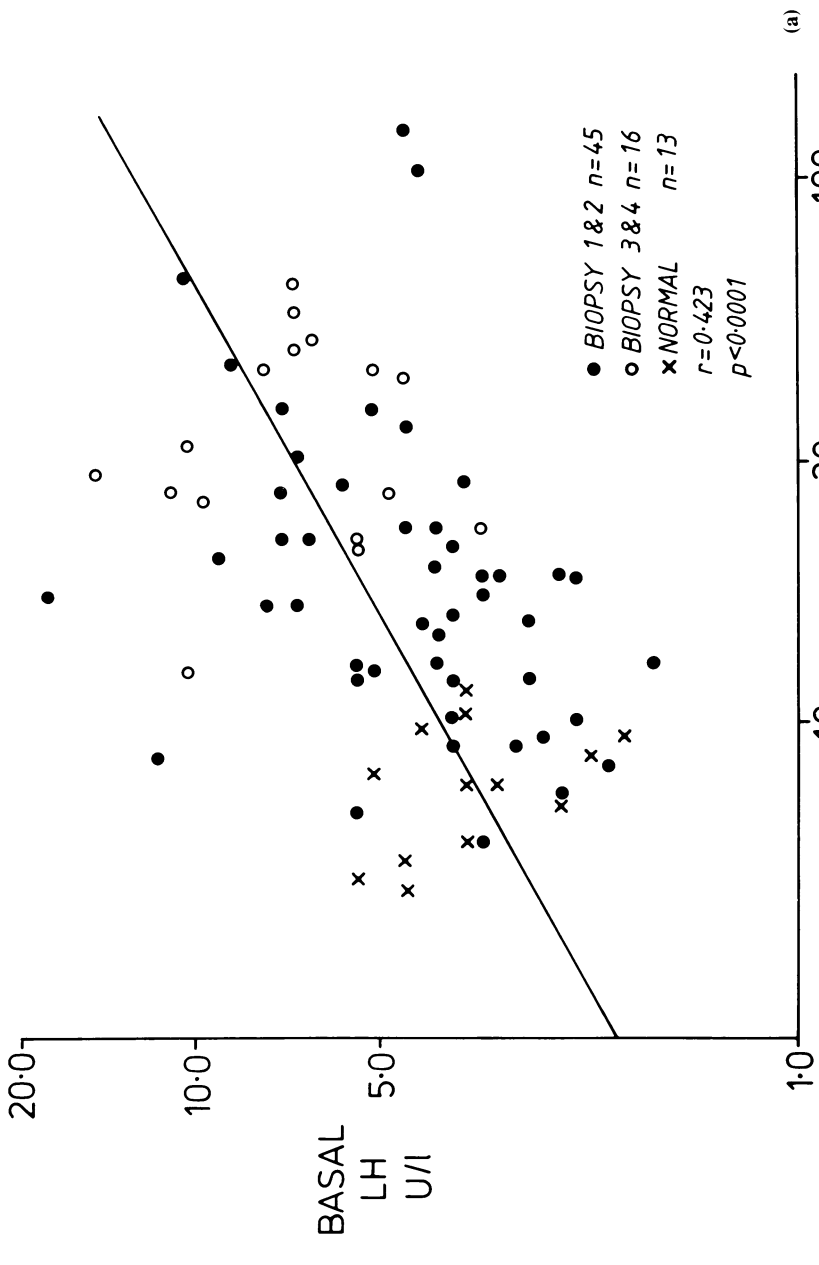

Fig. 6.6. Basal and peak levels (response to 50 ug GnRH i.v.) of LH (**a**) and FSH (**b**) in 61 patients and 13 controls (Wu et al. 1981)

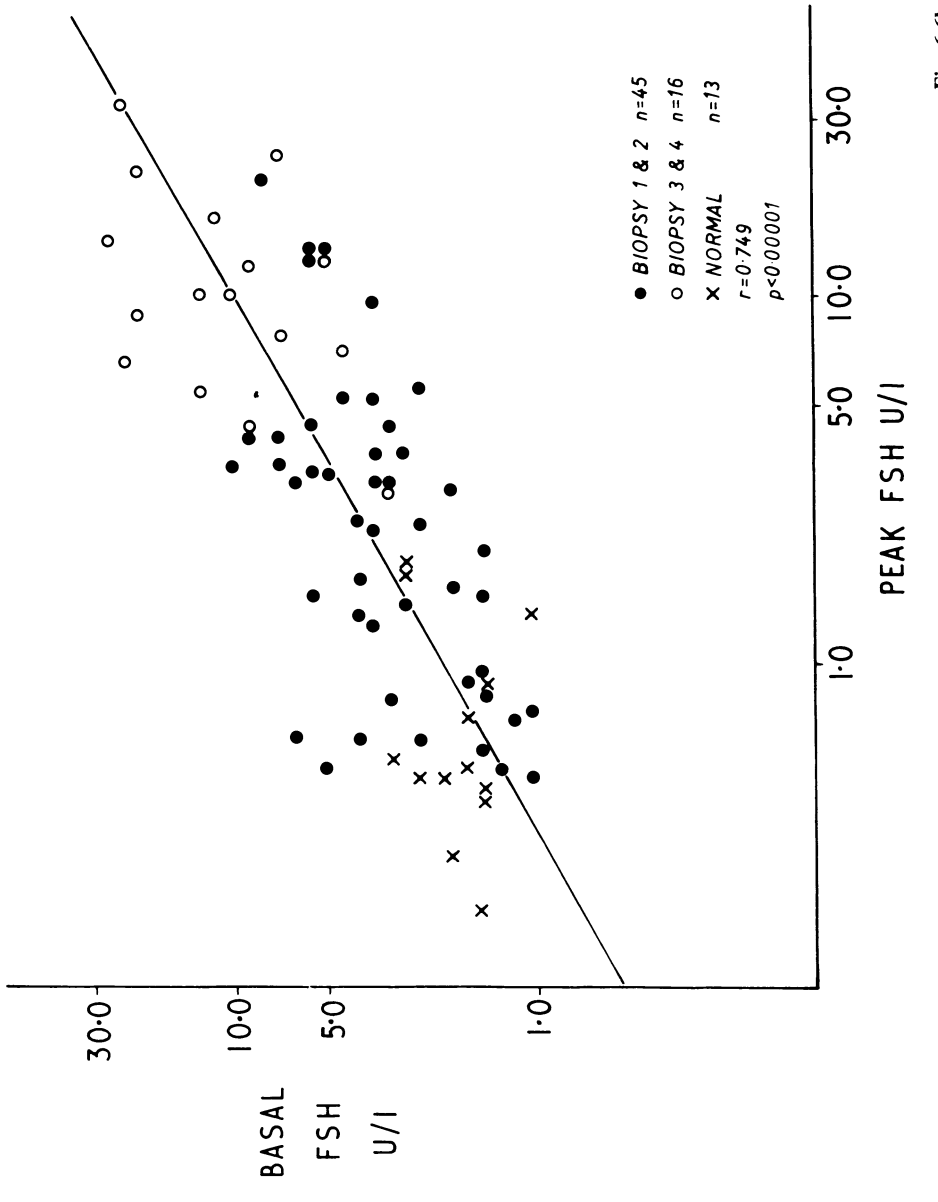
BASAL FSH U/l
30·0
10·0
5·0
1·0
1·0
5·0
10·0
30·0
PEAK FSH U/l
● BIOPSY 1 & 2 n=45
○ BIOPSY 3 & 4 n=16
× NORMAL n=13
r=0·749
p<0·00001

Fig. 6.6b

Table 6.1. Discriminant functions of the hormone measures (multivariate discriminant analysis), basal LH (LB), basal FSH (FB), peak LH (LP) and peak FSH (FP) in differentiating testicular histological groups as represented by the generalised distance (D) between them.

Hormone Measure		D Testicular histology grades 1 & 2 vs 3 & 4
LB alone		1.07
FB alone		2.00
LP alone		0.97
FP alone	FB	1.71
Best pair	+	2.18
All 4 together	FP	2.22

testes indicate severe germ cell atrophy which is usually but not always irreversible. However, in oligozoospermic patients with normal FSH, primary spermatogenic defects cannot be distinguished from obstructive lesions by hormonal investigations alone. In this group scrotal exploration, bilateral testicular biopsy and vasography could be considered.

It is clear that FSH measurement has come to occupy a crucial role in the initial assessment of infertile patients and guidance as to their further management in terms of investigations, treatment and prognosis. If is therefore essential that each laboratory or infertility clinic should establish its own valid normal range with great care.

LH and Testosterone

Up to one-third of the infertile male population may have some biochemical evidence of Leydig Cell dysfunction. Patients with Klinefelter's Syndrome are an extreme example of this. However, in infertile men with normal karyotype clinical symptoms and signs of androgen deficiency are rarely present. Furthermore, marginally low levels of circulating testosterone are compatible with apparently normal sexual activity (Wu et al. 1982c). Thus a clear case for the immediate institution of androgen replacement in these patients cannot be made, although there is the likelihood that Leydig cell function may eventually decline further. The possibility of partial androgen resistance as a cause of infertility has been raised recently (Aimen et al. 1979b). Although the exact incidence of androgen receptor deficiency is unknown, elevated LH found in association with elevated testosterone in azoospermic and oligozoospermic patients should suggest such an abnormality and further study of skin fibroblast culture may be indicated.

Since LH and FSH are secreted together as a functional unit and isolated FSH deficiency has not been confirmed, the measurement of LH and testosterone is useful when FSH levels are inappropriately low for the degree of oligoozoospermia or when there is clinical evidence of hypogonadism. If LH and testosterone are within the normal range the diagnosis of gonadotrophin deficiency is untenable. With recent improvements in sensitivity of gonadotrophin radioimmunoassay (Hunter and Bennie 1979), a good correlation between basal LH level and LHRH response over a wide range of concentrations can be achieved with the result that LHRH testing is no longer required (Fig. 6.6).

Further difficulties in the interpretation of LH and testosterone levels are due to variations introduced by episodic secretion of these hormones and the diurnal variation in testosterone levels. Consequently, borderline results from single samples should be confirmed by repeat estimations preferably of three samples taken at 20 min intervals.

Prolactin

The routine assessment of prolactin in infertile men cannot at present be justified (see p. 93). However when specific indications such as erectile impotence, diminished sex drive or suspected pituitary tumour are present, prolactin estimation is important. Sampling for prolactin should be carried out under standardised conditions since a number of factors can acutely elevate the circulating levels, for example psychological stress from venepuncture or clinic attendance, physical exercise and sleep.

A Suggested Scheme for Hormonal Investigation of Infertile Men

A scheme for the routine endocrine assessment of the infertile male is proposed in Fig. 6.7. Patients with clinical abnormalities such as varicoceles, congenital malformations, undescended testes, sexual problems, ejaculatory disturbances and systemic diseases are selected out at the initial consultation and are excluded from the diagnostic scheme. The flow chart starts with patients with azoospermia or oligozoospermia (under 5 million/ml). They are further categorised with respect to plasma FSH concentration and testicular size. The latter is considered to be reduced when the volume compared to Prader's orchidometer is under 12 ml. In patients with elevated FSH no further investigations of spermatogenic function is required. All that remains is to diagnose chromosomal abnormalities such as Klinefelter's syndrome and its variants—these patients usually have atrophic or small testes. Estimation of the level of circulating androgen allows assessment of the need for replacement therapy.

All patients with FSH levels below the lower limit of the laboratory normal range should have a preliminary pituitary screen with measurements of LH, testosterone and prolactin as well as radiology of the pituitary fossa. If any of these results are abnormal comprehensive pituitary assessment and visual field charting should be performed.

Patients with normal FSH but reduced testicular size should also have preliminary pituitary screening. In this group, the search for reversible aetiological factors may yield opportunities for appropriate treatment.

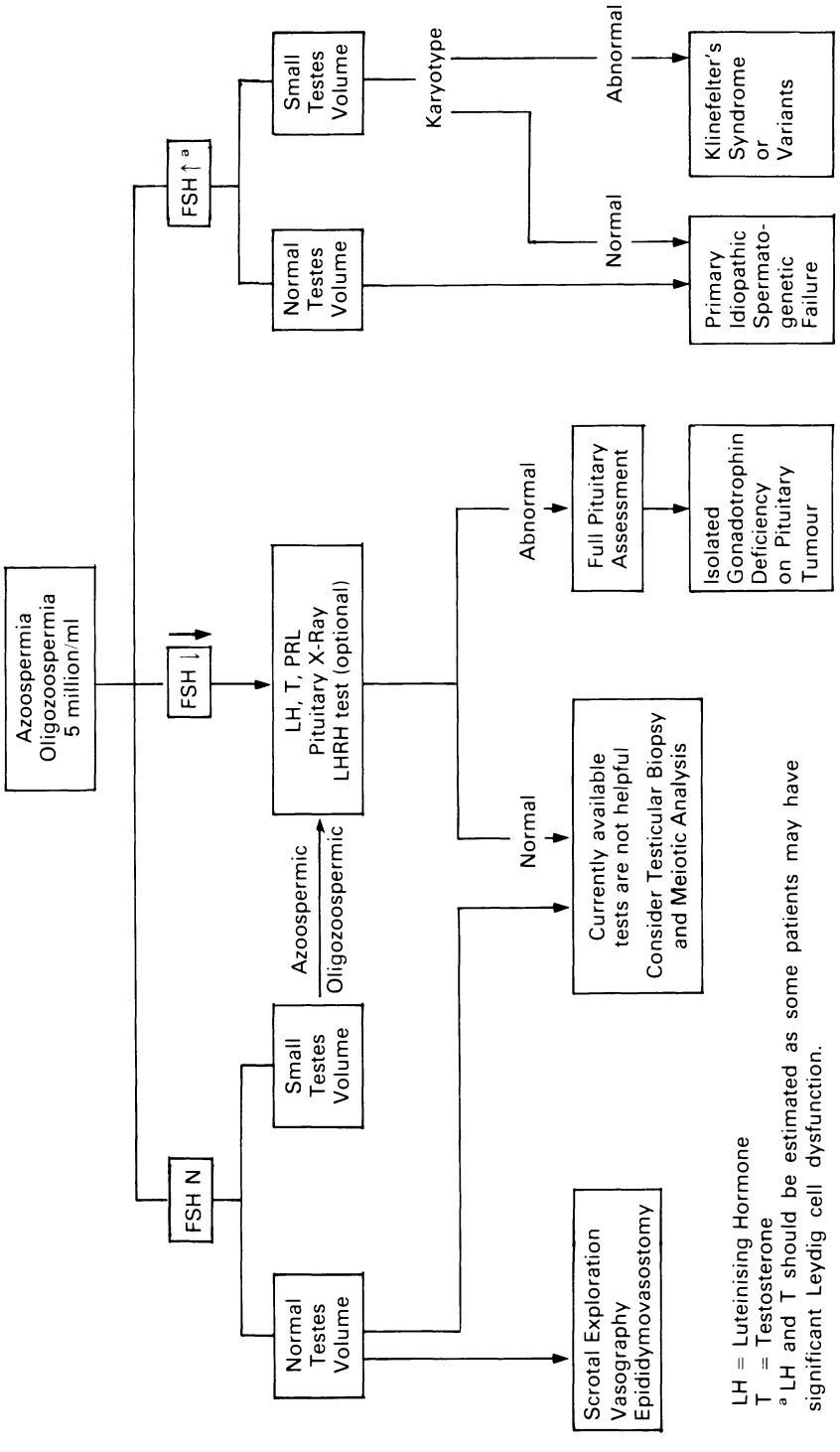

Fig. 6.7. A scheme for the endocrine and related investigations of the infertile male

Specific Endocrine Treatment of Male Infertility

GnRH and Gonadotrophins

In certain patients the basic defect is deficiency of hypothalamic GnRH production. The logical treatment would be to administer GnRH to induce the secretion of pituitary gonadotrophin which in turn stimulates testicular spermatogenesis and androgen production. This has been successful in two patients with isolated gonadotrophin deficiency after LHRH treatment for 41–50 weeks (Mortimer et al. 1974). A more promising approach is the pulsatile intravenous administration of natural sequence GnRH at near-physiological doses using portable automatically-timed syringe pumps (Valk et al. 1980).

Until the use of GnRH becomes established, purified gonadotrophin preparations for induction of spermatogenesis can be used, and are often successful. The regime is based on the recommendations of Paulsen (1974). Human chorionic gonadotrophin (hCG, Pregnyl) which has identical biological properties to LH is used on its own for the initial 6 months at a dose of 1500–3000 IU three times weekly by intramuscular injection. This is relatively inexpensive and initiates or potentiates androgenisation with production of ejaculates. At the end of this period, adequate spermatogenesis may have been induced in those patients with 'fertile eunuch' variant syndrome. If azoospermia persists, hCG is supplemented by human menopausal gonadotrophin (HMG, containing 75 IU FSH and 75 IU LH per ampoule) at a dose of 1–2 ampoules three times weekly. If a pregnancy is achieved during this time, semen can be stored and gonadotrophin treatment replaced by androgen supplements. This type of treatment is extremely expensive and demands a high degree of motivation and cooperation in both the patient and the doctor. Careful selection of patients for gonadotrophin treatment is essential. Ideally, a testicular biopsy should have been performed before treatment to establish the presence of primitive germ cells in the testis. In practice this may not be advisable since the trauma to the immature testis during biopsy may actually jeopardise the subsequent response to treatment. It is also essential to confirm that there is no barrier to fertility in the wife before this treatment is undertaken.

Future Trends

Confirmation of the key role of Sertoli cells in spermatogenesis by the studies of molecular mechanisms of FSH action has increased the urgency of the search for a marker of Sertoli cell function. Androgen-binding protein in rodents seems to fulfil this role, but in man ABP is as yet indistinguishable from SHBG in plasma. The establishment of a reliable bioassay and radioimmunoassay for inhibin is becoming important in clinical practice. A recent report has correlated inhibin levels in seminal plasma with seminiferous tubular function (Scott and Burger 1980). These newer approaches will certainly overtake the measurement of FSH as an index of the functional integrity of the germinal epithelium and Sertoli cells. The increasing use of hormone measurements in seminal plasma may also be informative about testicular and accessory glandular functions (Niechlag et al. 1978).

In vitro studies on Leydig cell function have revealed the existence of local intratesticular regulatory mechanisms in which oestrogen from Sertoli cells may play a role. Further studies especially using testicular biopsy samples, should reveal whether these mechanisms are relevant to male infertility. However, until much more is known about the mechanism of hormonal control of germ cell development, the exact role of intratesticular androgens and the interaction between androgens and FSH, the clinical management of male infertility will remain in its present unsatisfactory state.

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Chapter 7

Histopathology

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Indications for Testicular Biopsy

Historical

Testicular aspiration was first advocated by Huhner (1928) as a diagnostic measure in cases of azoospermia. In 1940 Charny suggested testicular biopsy to distinguish between obstructive and non-obstructive azoospermia. The value and role of testicular biopsy has remained the subject of considerable controversy for many years. Several workers have felt it to be of minimal value, except in patients with azoospermia (Scott 1968). Hanley (1955) felt that it was of no value as a regular procedure. More recently, however, testicular biopsy as a routine procedure has been advocated by Ellis (1968) and Meinhard et al. (1973).

In recent years newer methods of drug treatment and microsurgical techniques in obstructive azoospermia have meant that an accurate diagnosis of the cause of subfertility is highly desirable. Testicular biopsy allows precise information on the state of spermatogenesis to be obtained and also allows a quantitative scoring technique to be adopted (Johnsen 1970). Although many histological patterns are recognised by the pathologist, their interpretation in relation to treatment and prognosis is still debatable.

Advances in hormone analysis have decreased the number of indications for testicular biopsy. The advent of routine FSH estimation has allowed adequate prediction of testicular damage without recourse to testicular biopsy. Thus, in patients with grossly elevated FSH levels combined with azoospermia and soft, small testes, no treatment is possible and testicular biopsy can be avoided (Pryor et al. 1976).

Veterinary Practice

It is of interest that testicular biopsy was similarly advocated in veterinary practice for many years, but fell into disrepute because of technical complications due to the different vascular supply of the testes in domestic animals and particularly in the ram, where damage can easily occur. Biopsy of farm animals was carried out

routinely to assess fertility (Galina 1971). This was easily done using a Silvermann needle and fixing the sample in Cleland's fluid. Damage to the seminiferous epithelium was reported (Gassner and Hill 1955) following biopsy in animals and, in recent years, this effect has led to the abandonment of routine biopsy. Interestingly, histological features similar to those seen in man, such as spermatogenic arrest, occur in bulls, although obstruction of the epididymis is seldom seen.

Specific Indications

Indications for testicular biopsy have decreased significantly with the advent of hormone estimations and where chromosomal analysis is possible. The prospect of inhibin estimation in the near future may further reduce the indications for biopsy. It will continue to be a yardstick against which newer techniques can be measured. Some indications are:

1. Azoospermia with normal sized testes in association with mild elevation of FSH to between normal and twice normal levels;
2. In cases of obstructive azoospermia bilateral testicular biopsy is indicated to determine on which side microsurgical anastomosis is best attempted (Silber 1978);
3. Infertility of long duration where semen analysis gives poor results and other tests on both husband and wife are non-contributory; certain rare disorders of meiosis may only be detected by meiotic analysis;
4. Severe oligozoospermia ($\leq$10 million/ml) with equivocal reduction in testicular size and/or FSH within normal or nearly normal limits;
5. Cases of undescended testis associated with infertility. The presence of carcinoma in situ might contraindicate stimulatory hormonal treatment;
6. Research applications: (a) assessment of long-term effects of male contraception (Heller et al. 1963); (b) assessment of environmental hazards, e.g. irradiation, industrial chemicals, etc.; and (c) assessment of damage caused by cytotoxic agents.

Technique

Needle Biopsy

There is no indication for a needle biopsy because it induces unacceptable traumatic artefacts, and the volume of tissue obtained is small. A biopsy should contain 30–150 cross-sections of seminiferous tubules as this amount of tissue has been shown mathematically to be adequately representative of the testicular parenchyma (Roosen-Runge 1956).

Stab Biopsy

The traditional method is a stab biopsy carried out under general anaesthesia. The testis is held by the surgeon so that the overlying scrotal skin is tight; a stab incision is made with a sharp, narrow scalpel (e.g. 23 blade). The incision is made anteriorly to avoid any blood vessels and the blade inserted vertically through the skin, tunica vaginalis and tunica albuginea of the testis. A small quantity of serous fluid is often obtained and with gentle pressure on the testis a small portion of yellowish tubular tissue is made to protrude. This is cut off with scissors and immediately placed in Bouin's fluid. The skin is sutured with one or two interrupted absorbable sutures and sprayed with a plastic spray dressing, and the patient is given a suspensory bandage. In view of the decrease in the need for testicular biopsy as the technique for establishing diagnosis, and also because supplementary investigations, e.g. vasography, are often indicated, there is now a case to be made for abandoning the stab biopsy technique in favour of a full exploration. The testis and epididymis can be delivered out through the wound, allowing identification of any abnormality of the testis, epididymis or terminal portion of the vas (see Chap. 12).

Complications are minimal and rare. There may be some postoperative pain radiating into the groin or lower abdomen and, rarely, a small haematoma or haematocele may occur. Infection is uncommon. The most controversial complication of testicular biopsy is that of depression of the sperm count. Gassner and Hill (1955) first noticed germinal epithelial damage following testicular biopsy in the bull. More recently Rowley and Heller (1966) have shown significant depression of the sperm count occurring 3–4 weeks following biopsy and returning to normal after a period of 10 weeks. Rowley et al. (1969) have therefore suggested that any treatment should be delayed until 10–18 weeks have elapsed after testicular biopsy.

Frozen Section

A rapid haematoxylin and eosin stain of a cryostat section gives little useful information. A better alternative is to boil the tissue in modified Carnoy's solution for 2 min and, after cutting the frozen section at 10 μm thickness, stain with carbol fuchsin and toluidine blue (Levin 1979).

Suspected testicular tumours should never be biopsied.

The cytological features of premalignancy and carcinoma in situ are subtle and difficult to visualise except in well prepared, thin, stained sections after paraffin embedding. Immunofluorescent staining for antigenic determinants, which also requires cryostat sections, is still a research procedure only.

Fixatives

For permanent embedding and staining procedures, the choice of fixative is vital. Buffered 10% formol saline often results in unacceptable shrinkage and distortion. Testicular tissue should be fixed in either Bouin's solution, Zenker's formol solution (Helly's solution) or one of the zinc-substituted Zenker's fixatives (Lillie and Fulmer 1976). Cleland's (Rowley and Heller 1966) and Stieve's

(Johnsen 1969) fixatives also ensure minimal shrinkage artefact and give good cellular and nuclear definition in various staining procedures. A section 4–6 μm in thickness is necessary for diagnostic purposes.

Stains

A well-differentiated haematoxylin and eosin stain shows most of the details needed for a histopathological assessment. A Masson trichrome stain shows up connective tissue in the septa and around the tubules and also the finer details of Leydig cells. To demonstrate the basement membrane as well as the collagen fibres of the tunica propria, a combination of the Masson trichrome and the Jones' periodic acid–methenamine silver stain is useful (Lillie and Fulmer 1976). The value of elastic stains is controversial and their use usually adds little to the diagnostic or prognostic data. Periodic acid–Schiff (PAS) staining demonstrates intracellular glycogen particularly within germ cells and Sertoli cells.

Immunoperoxidase staining of paraffin-embedded tissue for detecting immunoglobulins, the demonstration of antigens of micro-organisms and the localisation of steroid hormones and their receptors (Kurman et al. 1978) are currently in use for research and may be of value in the future investigation of infertility. The fixative used for these studies is critical and perhaps a mercuric fixative may be required.

Scoring Methods

All quantitative assessments of testicular tissue must take into account that pathological testicular tissue is heterogeneous with the testes being affected in a patchy, random fashion. Testicular tubules also develop at unequal rates, so that counting the cells in a limited number of tubules may not be representative. In progressive testicular degeneration, cells are invariably lost from the germinal epithelium in a fixed order; loss of spermatozoa and spermatids precedes that of the spermatocytes and spermatogonia; Sertoli cells are the last to disappear (Johnsen 1967). The earliest attempts at quantitation used a *point counting technique* which allows the calculation of the relative volume of the various constituent elements of the testis; (Roosen-Runge et al. 1957; Federling et al. 1965). This time consuming method showed little correlation with sperm count and clinical diagnosis and took little account of the lack of homogeneity of the testicular parenchyma. In the method of Mancini et al (1965), 50 transverse sections of testicular tubules were selected in each biopsy, all the cells within them were counted and *a mean cell count* was computed *for each cell type per tubule*. This method is useful in infantile testes where good tubular cross-sections can be seen but is of limited value in adults. Clermont (1966) studied *cell cycles* by meticulously examining serial sections from testicular biopsies. This method presupposes excellent biopsy material and large numbers of serial sections and is therefore not useful as a routine service bench technique. Steinberger and Tjioe (1968) suggested projection of the histological sections, numbering of the tubules and measurement of their circumference by detailed *planimetry*. The cells were then individually and differentially counted in 25 tubules and the mean count to a standard length obtained.

Another easy but relatively crude method still in use is that of Mack et al. (1961) in which 100 tubules are counted and the number of tubules containing germ cells in any stage of maturation is expressed as a percentage, the value being called the *tubular fertility index*. This method is free from observer error and practical in terms of fertility assessment both before and after puberty. Its exact prognostic value has been well documented in cryptorchidism (Mancini et al. 1965; Hecker and Hienz 1967).

Another grading method in use in Edinburgh for a few years has enjoyed good clinical acceptance with helpful correlation. It was described by Chandley et al. (1976) and is based on general morphology:

Grade I: *Active* spermatogenesis with production of spermatozoa. Only *minor abnormalities* present, e.g. a slight excess of Sertoli cells in a minority of tubules or some slight thickening of the tunica propria

Grade II: *Arrest or depression* of spermatogenesis at various stages but no tubules show complete atrophy of the germinal cells

Grade III: *Some* tubules show *complete absence* of germinal cells and only contain Sertoli cells

Grade IV: *All tubules* show absence of germinal cells, their only cellular content, if any, being Sertoli cells

The *score count method* of Johnsen (1970) is a conveniently rapid and more accurate method which is gaining wider acceptance. The biopsy is scanned with a 10× objective lens; the crushed tubules at the edges of the biopsy are discounted. Each tubule is given a score ranging from 10 for complete spermatogenesis (i.e. numerous spermatozoa lying within the open lumen and an organised germinal epithelium arranged in a regular thickness all around the basement membrane) to 1, where no cells are seen in the section of the tubules (Table 7.1). To obtain a mean score, each score, x, is multiplied by its frequency, f, and the sum, $\Sigma(fx)$ is divided by the total number, N, of tubules scored. The Leydig cells may also be counted on a sliding scale of 1 to 6 where a score of 6 equates with maximal hyperplasia, 3 is normal and 1 indicates the absence of Leydig cells. This method shows a high correlation with sperm counts and other parameters used in the study

Table 7.1. Johnsen's method for scoring testicular biopsy (after Johnsen 1970)

Score	*Criteria*
10	*Complete* spermatogenesis with many spermatozoa. (Cell achieving the small head form of the spermatozoon) and a germinal epithelium organised in a *regular thickness* leaving an open tubular lumen
9	Many spermatozoa present but germinal epithelium *disorganised* with marked sloughing or obliteration of lumen
8	Only few spermatozoa (5–10) present
7	*No* spermatozoa but *many* spermatids present
6	*No* spermatozoa and only a *few* spermatids (5–10) present
5	*No* spermatozoa, *no* spermatids but *several* spermatocytes present
4	Only a *few* spermatocytes (<5) and no spermatids or spermatozoa present
3	Spermatogonia the *only* germ cells present
2	*No* germ cells; Sertoli cells present
1	*No* cells in tubular section (complete hyalinisation)

of male fertility and is well suited for mathematical treatment of data for scientific purposes and for computer analyses.

A mathematical model for calculating *mean seminiferous tubule length* was devised by Lennox et al. (1970). The values required for this calculation are the total testicular volume obtained by calipers and the volume of a number of tubular cross-sections in a unit area determined by a point-counting method. Variations in tubule length have been demonstrated in abnormal testes but the practical clinical value of this method is limited.

Electron Microscopy

Electron microscopy has little to offer in diagnosis except perhaps in the 'immotile cilia syndrome' where ultrastructural studies of spermatozoa are also of diagnostic value. If electron microscopy is contemplated 1 mm^3 blocks of testicular tissue should be fixed immediately after biopsy in 1% buffered glutaraldehyde.

Chromosome analysis

If chromosome analysis of germ cells is envisaged a portion of the biopsy should also be dispensed into 1% sodium citrate and taken to the laboratory immediately (see also p. 152)

Testicular Development

John Hunter (1762) described the mechanism of testicular descent with some accuracy but this was later misinterpreted leading to the long-held and fallacious view that the testicle was pulled into the scrotum by the cremaster muscle. The modern view described below is based on the work of Backhouse (1964).

During the 5th week of fetal development cells of the coelomic lining and underlying mesenchyme proliferate to form urogenital folds on either side of the midline of the posterior abdominal wall. The testis arises from the medial aspect of the middle of this fold and is recognisable during the 7th fetal week. A strand of mesenchyme connects the developing testis to the inguinal region and later swells to form the gubernaculum. The mesonephric duct, the future vas deferens, runs in this strand and then passes medially. There is a gap in the developing abdominal musculature at the site of the future inguinal canal. The scrotum is formed by expansion of the gubernaculum and also by growth of the developing epididymis as the mesonephric duct grows to join the testis. The prostate arises in the distal part of the primitive urethra, the corresponding cells in the female becoming para-urethral glands of Skene. The seminal vesicles arise as an outgrowth near the termination of the mesonephric duct. As the embryo grows, tethering by the gubernaculum causes the testis to migrate caudally; the testicular vessels grow in length to compensate for this changing anatomical relationship. Testicular descent usually takes place at the end of the 7th month of fetal life and is preceded by rapid downward growth of the processus vaginalis and cremaster into the gubernacular mesenchyme.

This differentiation towards the male state results from the influence of fetal androgens. These androgens are secreted by the fetal testis in response to placental gonadotrophin. There is no evidence of corresponding ovarian activity causing differentiation towards the female state. Radiation castration of male fetal mice results in rapid regression towards the female state (Raynaud 1958). Inappropriate androgens in cases of adrenocortical hyperplasia cause scrotal development in female babies (Maxted et al. 1965).

Testicular Descent

Normally, the testes will descend through the inguinal canal during the 9th fetal month and it has been shown that if they do not lie in the scrotum by the age of 1 year, spontaneous descent is unlikely (Scorer and Farrington 1971). These authors argue that spontaneous late descent is very rare. The development of the gubernaculum and much of the process of testicular descent are under the influence of fetal androgens. These androgens and the interstitial cells secreting them have disappeared by the 4th week after birth; thus most testes will lie in the scrotum by 3 months of age. In premature babies full descent may occur up to 6 months after birth. Scorer (1964) found the incidence of undescent at birth to be 2.7% in full term infants. At 1 year, however, this had fallen to 0.77% in 3162 boys. Campbell (1959), using a much larger series, found an incidence of undescent of 0.28% in 12.5 million military recruits. Campbell's figure may be falsely low because some cases could have had previous orchiopexy. It is probable that the true incidence of undescent lies somewhere between these two estimates.

The Onset of Spermatogenesis

The testes start producing spermatozoa in early puberty in response to increased levels of FSH and LH (see p. 91). The trigger for this increased gonadotrophic secretion is not understood. FSH levels increase two-fold, whereas LH secretion is characterised by nocturnal pulses (Finkelstein et al. 1973). The exact stage of puberty when spermatogenesis starts is difficult to define. Charny et al. (1952) showed by histological examination that normal testicular development falls into three phases:

1. A resting phase (from birth to the end of the 4th year) during which the seminiferous tubules remain small in diameter with no evidence of cell differentiation;
2. A growth phase (5th to 9th year) in which the seminiferous tubules elongate and become tortuous; the tubular diameter steadily increases but there is no differentiation of the lining cells;
3. A maturation phase (9th to 15th year) during which the tortuosity and diameter of the seminiferous tubules increases and there is cell differentiation into the various spermatogenic layers;

The clinical stages of puberty have been well described by Marshall and Tanner (1970). Testicular enlargement occurs before the other changes:

Stage 1: Pre-adolescent stage—persists from birth until the pubertal development of the testes has begun. General appearance of testes, scrotum and penis changes very little during this period although there is some overall increase in size.

Stage 2: Shown by enlargement of testes and scrotum with some reddening and change in texture of scrotal skin. Attainment of this stage is usually the first external evidence that puberty has begun.

Stage 3: Penis increases in length and to a lesser extent in breadth. Further growth of testes and scrotum.

Stage 4: Length and breadth of penis have increased further and glans has developed. Testes and scrotum further enlarged with darkening of scrotal skin.

Stage 5: Genitalia are adult in size and shape.

There is clinical evidence that the main bulk of the testis is made up of seminiferous tubules; thus infertile men with azoospermia who have the Sertoli-cell-only syndrome (del Castillo et al. 1947) have small soft testes. Sniffen et al. (1951) described spermatogenesis occurring in a testicular biopsy from a boy aged 12. Charny et al. (1952) described complete spermatogenesis at the age of $11\frac{1}{2}$ years in two negro boys. Unfortunately the clinical stage of puberty was not always accurately, recorded and another approach has been to examine the urine for sperm. Baldwin (1928) reported sperm in the urine from a boy aged 11 and Richardson and Short (1978) found the mean age at which spermaturia first developed was 13.3 years in 134 boys studied. In this latter study some specimens were negative after previous positive specimens and this makes it likely that 13.3 years is in fact an overestimate. It seems probable that the first stage of puberty when the testes enlarge (mean age 11.64 years) occurs when the seminiferous tubules are growing and sperm production starts. Thus boys appear to go through a fertile eunuch stage (Fig. 7.1).

Testicular Histology

Each testis is covered by a flattened mesothelial lining, the visceral layer of the *tunica vaginalis*, and enclosed in a thick fibrous capsule consisting of collagenous bundles admixed with a few smooth muscle cells (the *tunica albuginea*). On its internal aspect is an areolar, well-vascularised connective tissue mantle (*tunica vasculosa*) which also extends into the interstices of the testicular parenchyma to provide a delicate interstitial connective tissue padding (Kormano and Suoranta 1971). From the tunica albuginea arise fine, vascular, often incomplete, fibrous septa (*septula*) which subdivide the testicular parenchyma into 200–300 pyramidal *lobules* and which converge superomedially into a dense collagenous nodular thickening of the capsule, the *mediastinum testis*. Each testicular lobule comprises 1–4 straw-coloured highly convoluted serpentine *seminiferous tubules*, 30–80 cm long and 130–300 μm in diameter. The total length of each tubule in a normal testis is approximately 540 cm (Dykes 1969; Lennox et al. 1970). As the tubules converge towards the mediastinum testis they form 20–30 straighter tubules (*tubuli recti*) each about 0.5 cm in diameter. These in turn communicate with a plexiform system of epithelial lined spaces, the *rete testis*, which is applied to the postero-superior

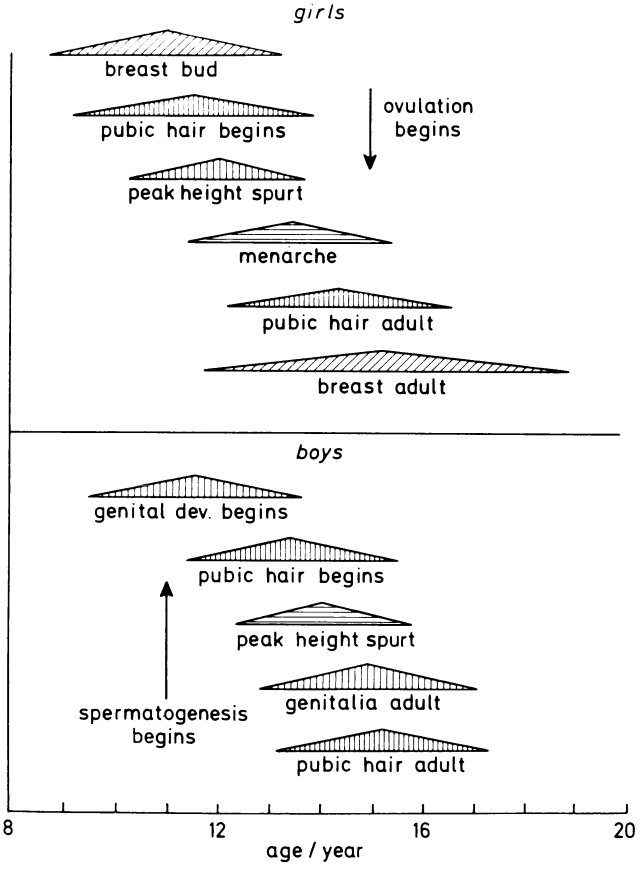

Fig. 7.1. Sequence of pubertal events in boys and girls (adapted from Marshall and Tanner 1970 by Short 1976)

aspect of the testis; from the latter *efferent ductules* penetrate the tunica albuginea and proceed into the epididymis (Sniffen 1950). Each seminiferous tubule is surrounded by a *basement lamina (membrane)* around which is arranged a concentrically laminated sheet composed of several layers of flattened spindle shaped cells admixed with abundant elastic fibres. Its outermost layers retain some ultrastructural features of smooth muscle cells but in man, in contrast to other mammals, no contractile power has been demonstrated. These layers increase with age and in testicular disorders (Davis and Langford 1969).

Internal to the basement membrane lies the stratified *seminiferous (germinal) epithelium* which consists of a complex population of cell types comprising the *supporting* (*sustentacular*) *cells* (*Sertoli cells*, *sustenocytes*) and the *spermatogonic cells* (*germ cells*, *spermatogonia*). There are several morphologically distinct germ cell types representing the successive differentiation stages which lead to the formation of spermatozoa, the holocrine cellular secretion of the testis. The Sertoli cells are a relatively fixed non-proliferating population whereas the spermatogonia

proliferate continuously in the basal aspect of the germinal epithelium and differentiate, causing displacement of the more mature and differentiated cells towards the tubular lumen.

Admixed with these cells are spherical structures composed of islands of cytoplasm in which there are membranous and mitochondrial residues and abundant free ribosomes (*residual bodies*) (Vaughn 1966). These are derived from meiotic activity and undergo spontaneous autolysis, or phagocytosis by Sertoli cells (Carr et al. 1968); their exact role in spermatogenesis has yet to be determined.

Sertoli Cells

The cytoplasmic outline of the Sertoli cell is remarkably polymorphic and its contour is difficult to make out on light microscopy. The morphology needs to be considered three-dimensionally (Flickinger and Fawcett 1967). These cells appear columnar in tubular transverse sections and polygonal in tangential sections. They rest directly on the basement membrane occupying almost its entire surface (except for an occasional basal spermatogonium) and extend through the width of the entire epithelium as far as its free luminal aspect. Sertoli cells have several folds and recesses in their apices and herein are held spermatids and spermatozoa prior to their liberation into the lumen. Complex, slender, irregularly branched, cytoplasmic processes from their lateral borders ramify through the entire germinal epithelium between the germinal cells and maintain the structural cohesion of the epithelium by occupying the intercellular spaces. The rapidly dividing germ cells mould the cell borders. Ultrastructural studies prove that Sertoli cells do not form a syncytium and that each cell retains its individuality. At points of contact between cell processes above the spermatogonia, specialised junctions (similar to the 'tight junctions' of epithelial cells) of at least three different varieties can be identified (Fawcett et al. 1959; Fawcett 1961; Nicander 1967). The multiple parallel lines of fusion of opposed cell membranes at these sites act effectively as a blood/testicle barrier separating the differentiating germ cells from potentially noxious chemicals and immunoglobulins in the blood stream.

Their 9–12 μm nucleus is irregular with one or more deep surface indentations, and one or two large, pyroninophilic, tripartite, polymorphic nucleoli consisting of a nucleololemma and two associated dense chromatic bodies. The nucleoplasm is otherwise homogeneous and evenly distributed.

Abundant, slender, elongated mitochondria with lamellar cristae are present within the cytoplasm and are often aligned parallel to the long axis of the cell. There are also a few ribosomes, a diversity of primary and secondary lysosomes (some containing lipofuscin), smooth endoplasmic reticulum (SER), an extensive simple Golgi complex (with little evidence of secretory activity), and intracytoplasmic myofilaments and microtubules each 70–90 Å which act as a cytoskeleton and account for the cohesive properties of these cells. The well-developed SER is usually localised basally and consists of a mesh of smooth-surfaced tubules. In certain stages of the spermatogenic cycle the SER becomes closely aggregated and is deployed close to the developing acrosomal caps of spermatids.

Sertoli cells act as 'nurse cells' to the spermatogonia by aiding their metabolism and nutrition, by providing mechanical support and by phagocytosing residual bodies and other meiotic debris. They also appear to have a local endocrinal function on the germ cells and they are capable of converting testosterone to

dihydrotestosterone through their 5α-reductase activity (Payne et al. 1973). The adenohypophyseal hormones FSH and LH (ICSH) affect these cells directly and they show considerable modifications during the seminiferous cycle. Sertoli cells also play an active role in the release of mature spermatozoa and in the mechanical escalation towards the lumen of the differentiating germinal cells. During fetal development Sertoli cells are thought to be responsible for the secretion of Müllerian/inhibitory factor, a substance which inhibits the muellerian duct from developing into female internal organs.

Within the cytoplasm of Sertoli cells are crystalloids (of Charcot-Böttcher) 10–25 μm in length consisting of, parallel, slender, fusiform subunits each about 150 Å in diameter. Their physiological and clinical significance is uncertain (Johnsen 1969).

The number of Sertoli cells per transverse section of the seminiferous tubule diminishes significantly from birth (40 ± 3) to adult life (10 ± 1) (Hadziselimovic and Seguchi 1974). This ratio may be reversed in pathological conditions.

Leydig Cells

Interstitial (*Leydig*) *cells*, which account for about 12% of the testicular volume (Ahmad et al. 1969), occupy the angular vascularised spaces between the convoluted tubules; gross interspecies differences in their contribution have been observed (Bouin and Angel 1903). They arise mostly from mesenchyme with some contribution from the mesonephric blastema (Witschi 1951). Their undifferentiated precursors are found at the same site and they differentiate and mature locally under the influence of gonadotrophins. The cells are clustered close to blood vessels which tend to have a polyhedral outline at the centre of a cluster and an elongated, spindle shape in the periphery. Each cell measures 14–20 μm across and they are often binucleate. The large, spherical, excentric nuclei contain peripherally arranged heterochromatin and one or two nucleoli; a clear perinuclear area, seen on light microscopy, houses the Golgi apparatus. The latter enlarges with gonadotrophic stimulation but its exact functional role in the cell is ill-understood.

The scanty, eosinophilic cytoplasm contains lipid droplets (triglycerides, phospholipids and cholesterol) similar to those in other endocrine cells, and abundant polymorphous mitochondria, some with tubular cristae (Christensen 1975). The extensive, ascorbic-acid-rich SER is arranged as a complex branching and anastomosing tubular network with some distended cisternae; the enzymes of androgenic steroid biosynthesis are deployed along it. Lysosomes are abundant and contain golden-brown lipofuscin pigment which increases in volume with age. Specific to human interstitial cells are proteinaceous, highly variable crystalloids (Reinke crystals) about 3 μm thick and 15–20 μm long; their ends are tapering or rounded (Nagano and Ohtsuki 1971). They show no specific staining affinity, often appear colourless and are stainable nonspecifically by azocarmine. They are isotropic in polarised light and are ultrastructurally composed of filamentous subunits each 50 Å thick resembling catalase (Yamada 1965).

Leydig cells constitute the endocrine portion of the testis and produce androgens; these act locally to stimulate germ cell proliferation and differentiation and systemically, at puberty, to influence the development of accessory reproductive glands and secondary sexual characteristics. In later years they maintain the

function of the testis and the activity of the accessory sex glands. Leydig cell function is depressed by oestrogens but is resistant to heat, radiation, and chemicals toxic to spermatogonia (Lipsett 1980). Leydig cells multiply rapidly in early fetal life (probably under the influence of placental gonadotrophins), atrophy at about the time of birth and the immediate postnatal period, and proliferate once more prepubertally (Sohval 1954; Hayashi and Harrison 1971) under the influence of pituitary gonadotrophins.

Spermatogenesis

The cells of the germinal epithelium are dynamically interrelated and a complex lineage is established as cells move from the basement membrane towards the lumen (Johnsen 1970). Various quantitative aspects of this sequence of change from spermatogonia to spermatozoa have been investigated and calculated and the existence of a *cycle* firmly established (Clermont 1963; Heller and Clermont 1964; Clermont 1966). There is wide interspecies variation and in man the cycle is estimated to take 22.5 to 23 days (Leblond and Clermont 1952; Clermont 1966; Clermont 1969). The stages of this cycle were elucidated by studying the acrosomic system of spermatids and the changing morphology of germ cell nuclei. In humans, three stages were identified (Leblond and Clermont 1952). Successive phases of the cycle occur at consecutive intervals along the length of the tubule with an undulatory progression (*spermatogenic wave*). This 'wavelength' is difficult to measure in men as it varies irregularly along individual tubules.

Spermatocytogenesis

In this first phase the primitive germ cells derived from the germinal primordium of the developing testis replicate by mitotic division to replace themselves and to produce several successive generations of spermatogonia. These cells are intimately related to Sertoli cells, which insinuate cytoplasmic processes among them (Leeson 1966). The spermatogonia are large rounded cells of three main types: type A light, type A dark and type B (Table 7.2) in Zenker's fixed material (Roosen-Runge and Giesel 1950: Clermont 1969). These cell types are interrelated (as shown in

Table 7.2. Classification of spermatogonia

	Nucleus	
Spermatogonia	Nucleoli	Nucleoplasm
Type A light (A^2)	1–3, eccentric, irregular—adjacent to nuclear membrane	pale, finely granular
Type A dark (A^1)	1–3, eccentric, irregular—adjacent to nuclear membrane	dark, with a pale-staining nuclear vacuole
Type B	central	coarsely granular, peripherally arranged chromatin

Fig. 7.2). The number of mitotic divisions at this stage is the basis for the large numbers of spermatozoa formed during the active reproductive life of mammals.

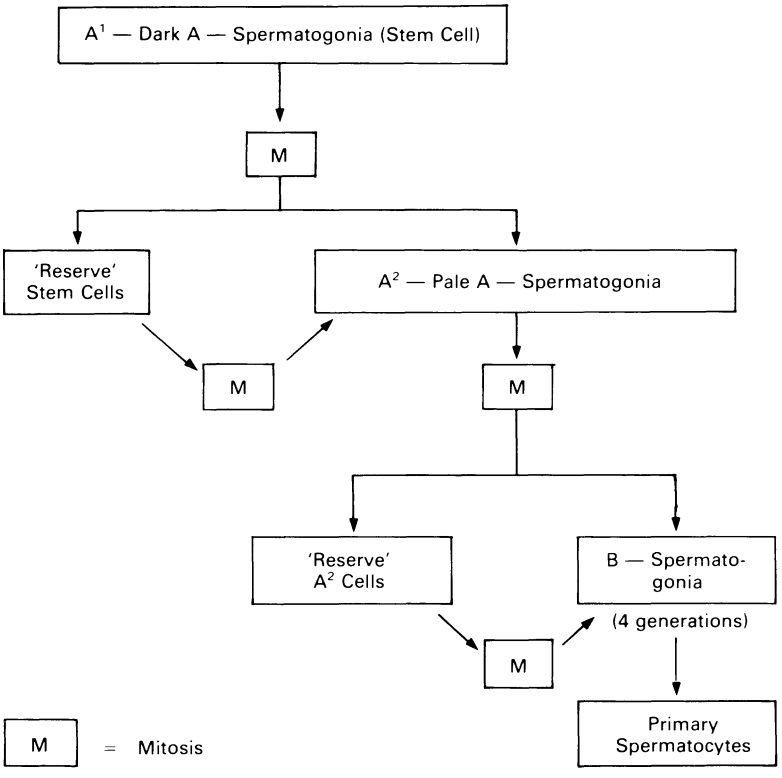

Fig. 7.2. Interrelationship of the three main types of spermatogonia

Meiosis (Maturation or Reduction Division)

Each type B spermatogonium divides into two diploid resting *primary spermatocytes* and these are essentially morphologically similar to their precursors. They are considered to be in the *preleptone* phase of the first maturation division (*Meiosis 1*) and after an increase in their cytoplasmic volume and reorganisation of nuclear chromatin into chromosomes they enter *prophase* of this division which lasts, through the stages of *leptotene*, *zygotene*, *pachytene*, *diplotene* and *diakenesis*, for an average of 22 days. The nuclear membrane disappears at this stage and *metaphase 1*, *anaphase 1* and *telophase 1* are completed with the formation of *two haploid, secondary spermatocytes* per primary spermatocyte. In this maturation division corresponding regions of chromatids, of paired chromosomes and their genetic contents, are exchanged ('crossing over'). Ultrastructural studies of germ cell divisions show that although each nuclear division is complete, somatic

subdivision is incomplete due to the persistence of the cytoplasmic spindles. There is therefore protoplasmic communication between germ cell progeny throughout differentiation as the daughter cells are still partly conjoined as part of a large syncytium. This is responsible for the synchrony of development of germ cells in any one area of a tubule. Spermatozoa leave the syncytium at their time of release into the lumen of the tubule (de Kretser 1969). The second maturation division (*Meiosis 2*) then quickly begins and after a second prophase, metaphase, anaphase and telophase, four haploid *spermatids* are produced for every primary spermatocyte. Spermatids may further divide mitotically to produce similar haploid cells.

Spermiogenesis (Spermateleosis)

This involves a process of nuclear and cytoplasmic metamorphosis whereby spermatids become *spermatozoa.* It can be subdivided into four phases. The spermatids are now small spherical or polygonal cells with a central, finely granular, nucleus, 5–6μm in diameter, and scanty cytoplasm with a small juxtanuclear Golgi complex consisting of flattened, parallel, membrane-enclosed vesicles and smaller rounded vesicles (Brokelmann 1963).

Golgi Phase

Several small carbohydrate-rich granules (*proacrosomal granules*) appear within vesicles of the Golgi complex and eventually coalesce into a large, PAS-positive, hemispherical granule (*the acrosomal granule*) still located within a dilated membrane-bound vacuole (*the acrosomal vesicle*). The acrosomal granule adheres to the outer nuclear membrane at the site destined to become the anterior tip of the spermatozoon. The Golgi complex continues to contribute to the acrosomal vesicle by producing small vesicles which coalesce with it causing it to enlarge gradually.

Cap Phase

The acrosomal granule becomes flattened and its central portion bulges into an indentation of the now ovoid nucleus. The acrosomal vesicle further increases its volume and its content of carbohydrate and proteolytic enzymes. Its area of contact with the nuclear envelope is also increased and it covers the anterior two-thirds of the nucleus in the shape of a bilaminar cap (*head cap*). The remainder of the cytoplasmic contents, including the two centrioles, migrate to the lower pole of the cell; the distal centriole becomes orientated perpendicular to the cell's surface and gives rise to a slender *flagellum* projecting into an narrow cleft between the spermatid and the adjacent Sertoli cells. The granular nucleoplasm is transformed into strands and filaments which shorten and thicken into coarse granules.

Acrosomal Phase

The acrosomal granule remains at the anterior nuclear pole but spreads laterally as a thin layer to form an *acrosomal cap.* The nucleus becomes flattened, elongated and more densely and coarsely granular. The pair of centrioles and flagellum form a structure consisting of a central bifibrillary axial filament (*axoneme*) and nine concentrically arranged peripheral doublets.

Maturation Phase

The nuclear chromatin granules coalesce to produce a uniformly dense nucleus (apart from an occasional variable vacuole) and the nucleus assumes a flattended and pyriform shape. Cytoplasmic microtubules arise and become aggregated to form a cylindrical structure (*manchette*) which extends into the cytoplasm in the elongated portion of the cell caudal to the nucleus.

Sperm Morphology

The mature sperm (spermatozoon) is an actively motile, free swimming, complete cell and consists of two principal portions (Fawcett 1958, 1975). The *head* lies anteriorly and contains the dense, usually homogeneous, nucleus which gives the sperm its ovoid or pyriform configuration. Some nuclei appear variegated and contain clear spaces (nuclear 'vacuoles') within them. Condensation of nuclear chromation ensures compactness, aiding sperm mobility and helping to protect it during its trail along the female passages and through the lining membranes of the ovum. The sperm head measures 4–5 μm in length and 2.5 μm in width. The acrosomal cap covers its anterior two-thirds and this cap contains a large membrane-bound vacuole in which proteolytic, mucolytic and hydrolytic enzymes are concentrated: these enzymes disperse the interadherent cells of the corona radiata and cause focal degeneration of the zona pellucida of the ovum (Stambaugh and Buckley 1968, 1972; Allison and Hartree 1970; Gaddum-Rosse and Blandau 1972; Zamboni and Stefanini 1968).

The most anterior part of the acrosome is termed the *apical segment* and its shape is species-specific. The main portion of the acrosome is the *principal segment.* Its lower end is concentrically narrowed to form the *equatorial segment.* (Franklin et al. 1970; Fawcett 1975).

Caudal to the acrosome a specialised *dense layer* is formed between the cell and the nuclear membranes: it is at this site that outer membranes of ovum and sperm fuse initially (Barros and Austin 1967). Behind the nucleus, the rest of the head forms into the shallow *implantation fossa.*

The other portion of the sperm, the *tail*, provides mobility and allows for an appropriate orientation at the time of fertilisation. It measures 55 μm in length and tapers from a width of 1 μm to 0.1 μm. Four major subdivisions are ultrastructurally identifiable in it: the calibre of the tail and its covering sheath varies in these subdivisions (Ånberg 1957; Gaddum 1968; Pedersen 1969).

Immediately adjacent to the head is the *neck* which in human spermatozoa is often surrounded by a droplet of redundant cytoplasm from the germinal epithelium. Within the neck are nine segmented, symmetrically deployed columns, each 1–1.5 μm thick, attached anteriorly to a dense ring (*capitulum*) situated behind the implantation fossa (Fawcett and Phillips 1969; Zamboni et al. 1971; Zamboni and Stefanini 1971). A few longitudinally orientated mitochondria and the juxtanuclear (proximal) transversely orientated centriole are also present.

The next segment (*middle piece*) is 5–7 μm long and 1 μm wide. Surrounding it are circumferentially arranged mitochondria. The latter are placed end to end and encircle the tail in the thick helix: they provide energy for motility. In the middle piece the nine segmented columns are continuous below with the outer dense fibres of the flagellum. Its core is occupied by the *axoneme* which comprises two central singlet and nine outer evenly spaced doublet microtubules. In its distal end is a dense shelf-like ring (*annulus*) which prevents changes in the orientation and position of the mitochondria during the active movement of the sperm.

The *principal part* is 45 μm long and about 0.5 μm wide and is surrounded by a dense fibrous sheath consisting of ventral and dorsal longitudinal columns connected by regularly spaced circumferential ribs which extend half-way around this sheath and are continuous at their ends with the longitudinal columns. Two of the outer dense filaments (in the 3 and 8 o'clock positions) terminate below the annulus. This results in relative asymmetry between the two sides of the tail with a 'minor' compartment containing three outer dense filaments and a 'major' compartment with four filaments.

The caudal *tailpiece* is not covered by a fibrous sheath and consists of the structures present in an ordinary flagellum, i.e. two central and nine peripheral doublets (Telkka et al, 1961).

Ejaculated spermatozoa undergo changes (*capacitation*) in the female genital tract prior to fertilisation of the ovum. The duration of this reaction varies between species and is the order of 7 h in man (Austin 1974). The process may be changed by female hormones and results in the increase of oxygen uptake by the sperm and a changed motility pattern. Other changes occur within the epididymis during sperm storage (Fawcett and Hollenberg 1963; Blandau and Rumery 1964).

Pretesticular Causes of Infertility

The pretesticular causes of infertility are largely hormonal and therefore often amenable to hormonal manipulation (Wong et al. 1974). The endocrinopathies associated with infertility are discussed in Chap. 6. The pubertal maturation of the testes is under the direct influence of the pituitary hormones and their hypothalamic releasing factors. Pathological lesions of the brain, hypothalamus and adenohypophysis result in depleted anterior pituitary hormone production. Congenital conditions, e.g. the Laurence–Moon–Biedl Syndrome; traumatic damage; cysts, including craniopharyngioma; infections, including tuberculous meningitis; and tumours, e.g. histiocytosis X, can all produce hypopituitarism, in which the testes will comprise small miniature tubules and Leydig cells.

Hypopituitarism occurring after puberty results in maturation arrest with progressive loss of germ cells, a diminished diameter of seminiferous tubules, and progressive thickening and hyalinisation of the tunica propria. The Leydig cells are small and shrivelled.

Any type of oestrogenic excess may lead to subfertility. Endogenous excess occurs in hepatic cirrhosis due to excessive oestradiol production (Galvao-Teles et al. 1973), a higher rate of aromatisation of peripheral testosterone (Olivo et al. 1978), impaired testosterone production (Baker et al. 1976), and a decreased level

of free and protein-bound testosterone in spite of higher levels of sex steroid-binding globulin (Chopra et al. 1973; Galvao-Teles et al. 1973). Testicular histopathology shows features of hypospermatogenesis to maturation arrest with eventual depletion of the germinal epithelium and a diminished tubular diameter with thickening of the tunica propria and eventual hyalinisation (Bennett et al. 1950). Eventually there is complete tubular sclerosis and loss of interstitial cells with an endstage similar to post-pubertal hypopituitarism (Federling et al. 1965). Treatment with exogenous oestrogens from any source results in identical testicular changes. Some of the testicular changes of ageing may be caused in a similar manner.

Klinefelter's syndrome patients also show an increased synthesis of oestradiol. Stimulation with human gonadotrophin results in a diminished testosterone response from the testes and this increased synthesis is not sustained: this suggests an intrinsic Leydig cell metabolic defect which needs further elucidation (Wong et al. 1973b; Smals et al. 1974).

Similar histological changes in the testes may result from excessive glucocorticoids due either to endogenous excess as in Cushing's syndrome or from therapeutic administration. Diabetes mellitus (Fairburn 1981) and hypothyroidism result in similar changes. Giesthovel et al. (1975) also observed diminished levels of free testosterone in diabetic men.

Endorgan failure in the shape of defective interstitial cell function or a congenital depletion of Leydig cells is another rare cause of infertility. Leydig cell agenesis has been described in only two patients, who were shown to have a male phenotype, high levels of LH and low plasma testosterone levels. Their testes contained only tubules; a vas deferens and epididymis were present. Stimulation with human gonadotrophin releasing factor had no effect (Berthezene et al. 1976; Brown et al. 1978).

Congenital enzymatic defects of steroid biosynthesis affect both the adrenal cortex and testes. Inadequate fetal secretion of testosterone results in the formation of ambiguous external genitals: in such cases, the fetal secretion of muellerian inhibitory factor is intact and therefore there are no female internal organs (Saez et al. 1972; Givens et al. 1974).

All states of gross metabolic abnormality lead to diminished Leydig cell function probably due to failure of the hypothalmic–pituitary axis; hepatic failure, chronic azotaemia and gross calorie–protein malnutrition from any cause lead to hypogonadism with low secretion rates and plasma concentrations of testosterone with increased blood levels of luteinising hormone and impaired stimulation by human gonadotrophin (Holdsworth et al. 1977; van Kammen et al. 1978). Dystrophia myotonica (Febres et al. 1975) and leprosy (Morley et al. 1977) cause both interstitial cell damage and tubular dysfunction. Features resembling hypopituitarism are seen on biopsy.

Toxins in the systemic circulation impair Leydig cell function. Ethanol excess in the absence of chronic liver disease affects Leydig cell function (Lipsett 1980). Testosterone biosynthesis is suppressed by ethanol in vitro (Ellinboe and Varanelli 1979), in experimental animals (Gordon et al. 1976) and in humans whose calorific intake is half composed of alcohol (Gordon et al. 1979). Alcohol augments hepatic degradation of testosterone (Rubin et al. 1976) and aromatisation of testosterone to oestradiol (Gordon et al. 1979). Lead compounds may have a similar effect (Braunstein et al. 1978). Neither chemotherapeutic agents for malignancy nor irradiation appear to have a deleterious effect on interstitial cells (Lipsett 1980).

Testicular Causes of Infertility

Germ Cell Aplasia (Sertoli-cell-only syndrome)

This condition, illustrated in Fig. 7.3, is often congenital (*del Castillo syndrome*); the postulated cause is a failure in the migration of the germ cells from the yolk sac endoderm to populate the immature testicle (del Castillo et al. 1947). In acquired cases, cytotoxic drugs (e.g. chlorambucil and azothioprine) and perhaps deep x-ray therapy, have been incriminated (Richter et al. 1970; Sherins and de Vita. 1973). All the seminiferous tubules are affected; but on rare occasions focal spermatogenesis is retained and in these tubules various stages of maturation are present, in which case the histological picture of hypospermatogenesis prevails. The diameter of the tubules is decreased and they are devoid of germ cells, the germinal epithelium being replaced exclusively by mature Sertoli cells; the latter are aligned vertically on the basement membrane with their apical aspects displaced to one side giving them a resemblance to 'windswept treetops' (Sniffen et al. 1951). Vacuoles often appear within their cytoplasm and other characteristic ultrastructural alterations are observed (Chemes et al. 1977). The tunica propria is not thickened; the Leydig cells are present in normal numbers. This is an endstage condition as germ cells do not regenerate. These patients are potent but infertile, with testes slightly smaller than normal and with well developed sexual characteristics. Azoospermia is present but the urinary 17-ketosteroid levels are normal: the levels of FSH and LH are elevated.

After exposure of the testes to irradiation, although the germ cells degenerate, there is an associated thickening of the tunica propria which progresses to hyalinisation and sclerosis of the seminiferous tubules.

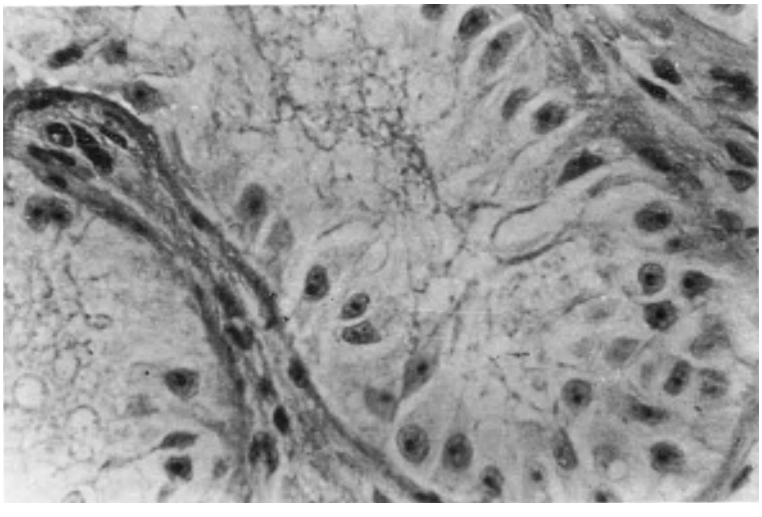

Fig. 7.3. Germ cell aplasia. The testicular tubular diameter is decreased and the germinal epithelium consists solely of mature Sertoli cells. The tunica propria is of normal thickness and there is no hyperplasia of the Leydig cells. H & E, × 200

Maturation Arrest

This condition, illustrated in Fig 7.4, was found, in a number of studies (Wong et al. 1973a; Levin 1979), to be one of the more important causes of male infertility. The condition may be complete or partial, and is characterised by an abrupt halt at some stage of spermatogenesis with no further differentiation beyond this given level. The arrest is usually at about the primary spermatocyte stage in which case no secondary spermatocytes, spermatids or spermatozoa are present. On occasions the halt is at the later spermatid stage and in such patients some spermatozoa may be formed. All tubules in an individual patient show the arrest at the same level. The Sertoli cells are normal, the tunica propria is not thickened and the tubular diameter is unaltered. The Leydig cells are present in normal proportions. The patient shows oligozoospermia or azoospermia. Gonadotrophin and urinary 17-ketosteroid level are normal. The condition is often bilateral, and bilateral biopsy may be required to assess its completeness. It may be difficult to distinguish this condition from hypospermatogenesis.

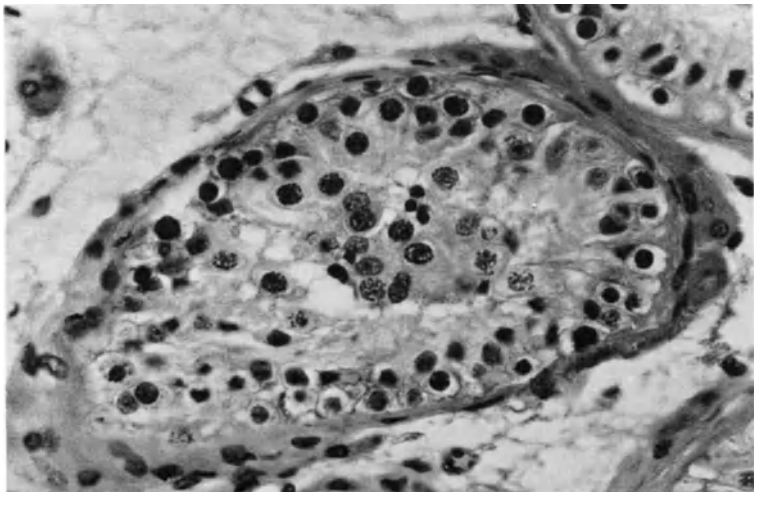

Fig. 7.4. Maturation arrest. Although meiotic activity is occurring within the tubules, no mature spermatozoa are present within the lumen. The Sertoli and Leydig cells are normal and there is no thickening of the tubular tunica propria.

Hypospermatogenesis

This is caused by diminished activity of spermatogonia and a proportional hypoplasia of all germ cells resulting in thinning of the germinal epithelium (Fig. 7.5). Quantitation of this condition is particularly useful.

Severity varies from a slight generalised reduction in numbers of spermatogonia in a few foci within tubules to a severe form where there is such an extensive reduction in germ cells as to resemble germ cell aplasia. Three subcategories are specified by Levin (1979). The tubular diameter is normal, but there is hyalinisation

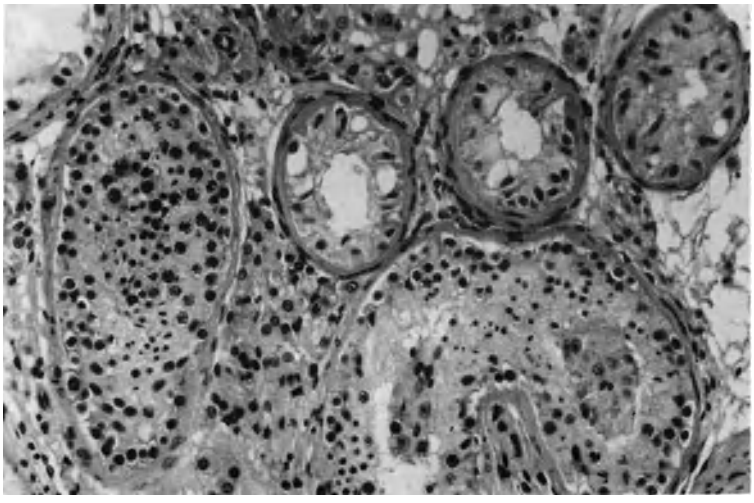

Fig. 7.5. Hypospermatogenesis. Reduced spermatogenic activity is present; some tubules show marked germ cell losses and others relatively normal meiotic activity. H&E, × 120

and peritubular connective tissue thickening with no reduplication of the tunica propria. Disorganisation and luminal sloughing of the germinal epithelium, although it may be artefactual, has in some cases been shown to be a genuine feature in optimally fixed material. There may be an excessive interstitial fibrosis; the Sertoli cells and Leydig cells are unremarkable. Oligozoospermia or azoospermia are present but levels of gonadotrophins, 17-ketosteroids and semen fructose are normal.

Hypospermatogenesis and maturation arrest are often considered as phases of the same spectrum (Wong et al. 1973a); they are also among the least understood causes of male infertility. There is evidence that both physical and chemical environmental agents and agents used in chemotherapy may be responsible. In such cases, infertility is potentially reversible. Removal of the noxious environmental agent, withdrawal of the chemotherapeutic agent (Buchanan et al. 1975; Roeser et al. 1978) or surgical treatment of a varicocele (Dubin and Amelar 1970; Johnsen and Agger 1978) may be effective.

Irradiation Damage

Direct exposure to deep x-rays, (Lushbaugh and Casarett 1976) or to radioactive agents, either therapeutically or accidentally, may induce hypospermatogenesis of differing degrees of severity (Platt 1947; Rowley et al. 1974) depending on time-related dosage. These changes were studied experimentally in laboratory animals exposed to graded doses of irradiation (Oakberg and Diminno 1960; Wyrobek 1979). Prolonged treatment or high dosage resulted in germ cell loss. The Leydig cells are not adversely affected; the Sertoli cells showed pseudohyperplasia. There was a consequent decrease in the diameter of the tubules with thickening of the tunica propria eventually resulting in tubular hyalinisation and sclerosis.

Klinefelter's Syndrome (Sclerosing Tubular Degeneration)

First described in 1942 (Klinefelter et al. 1942) and illustrated in Fig. 7.6, this is a progressive condition characterised by patchy spermatogonic failure leading to a complete and universal loss of germ cells, thickening of the tunica propria, gradual replacement of the seminiferous tubules by hyalinised fibrous collagenous tissue, nodular 'hyperplasia' of the Leydig cells and atrophy of the Sertoli cells (Ferguson-Smith et al. 1957). The end result is a small, shrunken, firm testis of which the

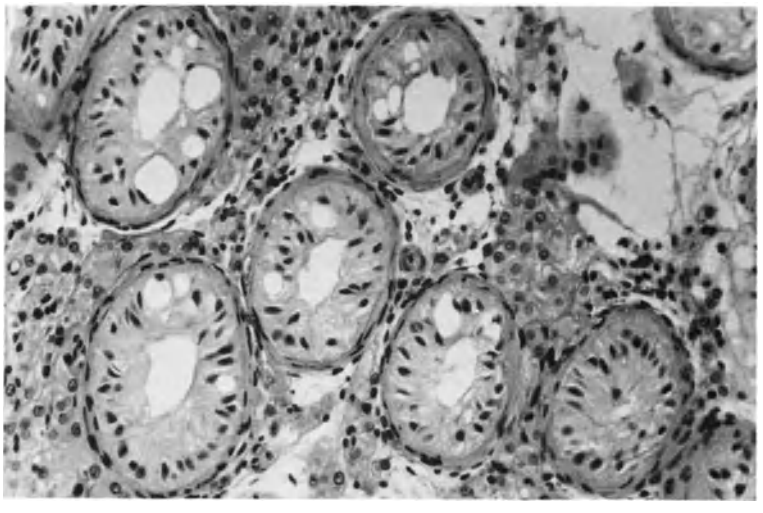

Fig. 7.6. Klinefelter's syndrome. Complete loss of germ cells, increased thickness of the tunica propria, 'hyperplasia' of the Leydig cells and Sertoli cell atrophy are the predominant features. H&E, × 120

tubules have become totally obliterated and replaced by thick collagenous cords appearing as 'ghost' tubules on histology. Controversy still exists as to whether this 'adenomatous clumping' of interstitial cells is due to proliferation or whether it results from depletion of intervening testicular parenchyma. Failure of condensation of the testicular reticulin fibre meshwork suggests that this is a true proliferation (Wong et al. 1973a) yet mathematical calculations of the volume of Leydig cells suggests a pseudohyperplasia (Ahmad et al. 1969; Ahmad et al. 1971). The local pathogenesis of this condition is still debatable. It has been demonstrated on occasions that the elastic fibre content of the peritubular connective tissue is decreased, suggesting the existence of a congenital defect which prevents the normal appearance of elastic tissue at about the time of puberty (de la Balze et al. 1954). Loss of elastic tissue is, however, at most patchy and therefore cannot be the full explanation.

The clinical features of Klinefelter's syndrome are discussed in Chap. 8.

Cryptorchidism

The changes in the germinal epithelium in testicular maldescent are severe but variable (Fig. 7.7). On the basis of animal experiments the higher body temperatures that prevail other than in the scrotum were advocated as their cause (Atkinson 1973; Jones et al. 1977). It has also been postulated that such testes possess intrinsic congenital defects, i.e. they are dysgenetic (Sohval 1974). Defects in Sertoli cell maturation may also be present. There is often a combination of all these abnormalities in the individual maldescended gonad.

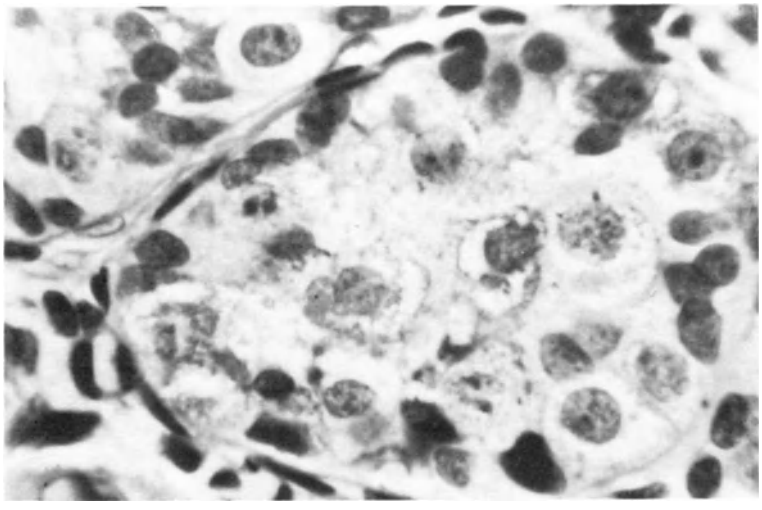

Fig. 7.7a,b. Cryptorchidism. **a** Immature testicular testicular tubules with markedly reduced calibre and contain inactive spermatogonia and immature Sertoli cells. H & E, × 80 **b** Immature germ cells and Sertoli cells in tubules. H & E, × 200

One method of classifying these testicular changes is that of Nistal et al. (1980) who studied 246 biopsies (220 prepubertal and 26 postpubertal) of undescended testes (Table 7.6) and subdivided them in Four types which differ histologically and in their specific postpubertal evolution. The lesions, more commonly seen in ectopic (obstructed) testes, appear to constitute the counterpart of the temperature-induced changes seen in experimental cryptorchidism, namely a slight hypoplasia of the germ cells, slowing of the growth of the seminiferous tubule and the absence of a wave of growth. The other subtypes represent testicular tubular dysgenesis with the effects of raised temperature superimposed: the dysgenetic changes are manifested as germinal cell hypoplasia, Sertoli cell hypoplasia or hyperplasia and ultrastructural modifications in both cell types. Focal lesions in the shape of ring-shaped tubules and intratubular calcospherules are encountered in the severest degrees of dysgenesis.

In unilateral cryptorchidism the development of the contralateral testis varies between compensatory hypertrophy (Laron and Zilka 1969) and variations on the dysgenetic theme; changes similar to, but perhaps less marked than, those of the maldescended testis occur in about a quarter of such patients. Precocious prepubertal maturation may also occur (Holsi 1971).

Table 7.3. Histological findings in 220 prepubertal testicular biopsies (after Nistal et al. 1980)

Type	Description	Germ Cells	Tubular fertility index[a]	Mean tubular diameter[a]	Sertoli cell index[a]	%	No. of Cases	High Scrotal
I	*Minimal Lesion*	N or slight hypoplasia	N or slightly ↓	N or slightly ↓	N	26%	58	9
II	*Marked Germinal Hypoplasia*	Marked hypoplasia	Markedly ↓	Slightly or moderately ↓	N	24%	53	16
III	*Diffuse Tubular Hypoplasia*	Severe hypoplasia	Severely ↓	Severely ↓	↓	33%	78	24
IV	*Diffuse Sertoli cell hyperplasia*	variable	variable (N ⇄ ↓)	variable (N ⇄ ↓)	↑	17%	35	4

Table 7.3. *(continued)*

Type	Canalicular	Abdominal	Ectopic (Obstructed)	Prognosis
I	1	11	37	after puberty normal spermatogenesis
II	31	2	4	marked hypospermatogenesis or maturation arrest
III	43	3	8	Sertoli cell only syndrome
IV	8	24	0	failure of Sertoli cell maturation with germ cells not reaching adult development

[a] N = normal, ↓ = diminished, ↑ = elevated.

Germ cell tumours occur more frequently in atrophic (Twonbly 1947) and maldescended (Krabbe et al. 1979; Pugh 1976) testes. Tubular atrophy with atypical features in the residual germinal epithelium is often observed in close proximity to malignant tumours (Azzopardi et al. 1961); but this may only represent the direct reactive or regenerative effect of the tumour rather than its precursor. Skakkebaek's (1972) studies on infertile males showed atypical germ cell changes which he labelled as *carcinoma-in-situ* (CIS) or *atypical germ cell syndrome*. These changes occur in only 0.6% of such patients: in his series CIS was seen in nine patients; in seven of these the testes were normally descended and in two there had been unilateral childhood orchiopexy; four patients developed invasive tumours. Sigg and Hedinger (1980, 1981) in a 30-year survey of testicular biopsy material found a 0.2% incidence; four of these ten patients developed invasive tumours.

These morphological changes occur in totally atrophic tubules with no residual meiotic activity. The atypical germ cells are lined singly over the unusually thickened basement lamina and displace the Sertoli cells towards the luminal aspect of the tubule (Mark and Hedinger 1965).Their large round nuclei contain coarsely dispersed chromatin and increased numbers of nucleoli; the cytoplasm contains abundant glycogen. Histologically abnormal spermatogonia may be seen in normal testicular biopsies and are increased in frequency if spermatogenesis is impaired in any manner (Heller and Heller 1970). Electro microscopy has shown a close association between the morphology of CIS cells, seminoma cells (Nielsen et al. 1974; Schulze and Holstein 1977; Schulze et al. 1978) and immature germ cells (Holstein and Körner 1974). Similar changes have been shown in two of eight patients with 46XY chromosome complement and testicular feminisation (Skakkebaek 1978) but no CIS changes have been observed in other testicular causes of infertility. The possibility of CIS should be considered before prescribing treatment to patients with a history of cryptorchidism.

The Timing of Orchiopexy

The development of the seminiferous tubule starts with tubule elongation at the end of the fourth year and spermatogenesis may already be established by the age of $10\frac{1}{2}$ years (see p. 118). In order to preserve this full potential for fertility, orchiopexy should be done as early as possible to minimise damage to the developing tubule and to allow any post-operative ischaemia to resolve. It is probable that the testis is ischaemic for some months after orchiopexy (Yeates 1976). Thus to conserve full fertility the operation should be done before the age of 6 years. Unforunately many cases are not discovered until school medical examinations at a later age. In these circumstances orchiopexy should be done as early as possible and certainly before puberty starts; in such cases some fertility may be preserved.

The adult who presents with undescended testes should also be explored. If in the adult the undescent is unilateral and the testis cannot be placed in the scrotum, then orchiopexy with placement of a testicular prosthesis is indicated. If there is bilateral undescent then orchiopexy should be attempted for psychological reasons and also to place the testicles in a position where they can be examined. In bilateral adult cases where orchiopexy is impossible, bilateral orchidectomy and hormonal replacement can be considered. It should be remembered, however, that the testosterone production will often be normal despite undescent. Each case must be judged individually.

Mumps Orchitis

Infection with the mumps virus at puberty and in the post-pubertal period leads to progressive degenerative changes resulting in permanent damage to the seminiferous tubules; prepubertal infections resolve completely (Danielsson 1978). In the acute infective phase there is intercellular oedema, a mixed lymphocytic and polymorphonuclear neutrophil infiltration, and degeneration and sloughing of the germinal epithelium (Gall 1947; Charney and Meranze 1947). In the healing phase, which may take years, there is progressive loss of spermatogonia followed by tubular sclerosis and hyalinisation (Barlew and Masters 1954). Tubular involvement is not uniform and individual testicular lobules are damaged to varying degrees. The Leydig and Sertoli cells are preserved. The testis becomes smaller and oligozoospermia or azoospermia results. The FSH level is raised, the LH and 17-ketosteroids are normal.

Post-Testicular Causes of Infertility

The post-testicular causes of infertility comprise primary obstruction of efferent testicular ducts and defects in the morphology and motility of spermatozoa. Trauma and diminished blood supply due to prolonged partial torsion to the testes may also be causes of infertility. Such factors must be bilateral to produce sterility.

Obstruction of the ducts that lead spermatozoa from the testis may be congenital or acquired. The percentage of cases of azoospermia secondary to congenital failure of an anatomical union between the vas and the epididymis has not yet been established. Acquired blocks are the result of stricture formation and scarring following non-specific pyogenic infection as associated with chronic active prostatitis or as an end result of gonococcal and tuberculous infection. The tail of the epididymis at its junction with the vas deferens is the usual site of this blockage. The seminal vesicles, prostate and ejaculatory ducts are usually also involved. Voluntary vasectomy is now the commonest extratesticular cause of sterility; but it can also occur as a complication of other inguinal or scrotal surgery.

This type of obstruction has no direct effect on the testicular germinal epithelium or on the Leydig cells (Phadke and Phadke 1967; Wong et al. 1973b) as shown by successful reconstruction operations (Silber 1978). The anatomical, endocrinological and immunological sequelae of vasectomy are discussed in Chap. 17.

Mucoviscidosis (fibrocystic disease of the pancreas) is another inherited condition which causes sterility through testicular outflow obstruction. This condition occurs approximately once every 2000 to 2600 live births; now that such patients may survive into early manhood, resulting azoospermia has been demonstrated. There is also a diminished volume of seminal plasma with a diminished fructose concentration, and increased citric acid and acid phosphatase concentrations. The vas deferens may be normal at autopsy but the Wolffian ducts fail to mature or are secondarily obliterated at an intrauterine phase (Landing et al. 1969; Taussig et al. 1972).

In many cases of oligozoospermia diminished motility is also found. This may be partly related to faulty spermatogenesis but there may also be faulty maturation and storage during the slow progress of sperm through the epididymis. Biological

abnormalities of the seminal plasma, which is largely made up of secretions from the seminal vesicles and prostate, have also been described and may lead to defective motility (Moon and Burge 1968; Eliasson 1968).

The flagella of spermatozoal tails (Afzelius 1959; Allen 1968) and the cilia found on respiratory, ependymal, and genital epithelia have been shown to be ultrastructurally identical and also essentially similar to cilia of other species. On transverse sectioning normal cilia consist of nine identical pairs of microtubules deployed at regular intervals concentrically around another centrally located doublet of minitubules. Each pair consists of an 'A' and a 'B' tubule; the 'A' tubules are linked to adjacent 'B' tubules and by a radial spoke to the central pair. In addition, there is an extra area on the 'A' tubules—these inner and outer 'dynein arms' form a transient connection enabling the control of the sliding movements of adjacent microtubules (Satir 1965). ATPase is situated on the inner dynein areas, this being the enzyme which catalyses the breakdown of ATP to produce the energy required for ciliary motion (Satir 1974).

In a small number of infertile men a syndrome known as the *immotile cilia syndrome* was described (Afzelius et al. 1975; Afzelius 1976). In this condition the sperm count is normal and the spermatozoa appear morphologically unremarkable yet no motility can be demonstrated in fresh ejaculates. Electron microscopy of such sperm has shown an absence of dynein areas (Eliasson et al. 1977). Such ciliary abnormalities have also been demonstrated in Kartagener's syndrome (Kartagener and Stucki 1962), in which diminished mucociliary clearance in the respiratory tree in associated with chronic sinusitis, bronchiectasis and situs inversus.

Another version of this syndrome is associated with normal dynein arms but absence of the radial spokes so that the outer microtubules are displaced and the central doublet is excentric (Sturgess et al. 1979). The theoretical proposition of treating this condition by in vitro addition of ATP to stimulate motility has been considered (Forrest et al. 1979).

Recently it has been reported (Averbach and Wight 1979a) that testicular biopsies in the infertile male which appear histologically normal may contain tubules which, as measured stereologically, are hypercurved. There is some dispute as to the interpretation of these findings (Averbach and Wight 1979b); such hypercurvature is said to result in diminished outflow of spermatozoa.

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Chapter 8

Chromosomes

Anne Chandley

Introduction

In the United Kingdom, about 10% of all marriages are childless through infertility (HMSO Report 1960). In a proportion of cases, a genetic or chromosomal factor exerting its effect on gamete formation or function is responsible. To the clinician involved in the treatment and counselling of the infertile couple, a sound knowledge of the likely consequences of gene mutation or chromosomal aberration acting specifically on the reproductive system is essential if the right advice is to be proffered and the correct course followed.

The purpose of this chapter is to consider some of the ways in which genetic factors can operate to impair human fertility, to indicate just how common such factors are within the subfertile or infertile population, and to stress the importance of early diagnosis and correct counselling.

The Detection of Chromosome Abnormalities

The practical problems involved in analysing the somatic karyotypes of patients who present at an infertility clinic are considerable (Chandley 1977). The blood lymphocyte culture technique is straightforward enough, but analysis of the human chromosome complement requires the skills and experience of highly trained staff and the judgement of a knowledgeable cytogeneticist who can realise the implications of the findings. Moreover, chromosomal analysis is time consuming and many urologists, faced with the variety of tests and treatments that have to be administered to their patients, may find that resources cannot be spared for a full-scale operation of this kind. In this event, valuable information in a few cases will undoubtedly be lost, but the clinician may be consoled by the fact that the overall frequency of chromosomal abnormalities within the subfertile population as a whole is low. However, a frequency of ever-increasing significance is likely to be found if attention is confined to men with low sperm density.

One practical alternative to a full-scale chromosomal investigation is to carry out a simple buccal smear test to determine the nuclear sex of each individual. This, combined with a clinical examination, should be sufficient to reveal those sex chromatin-positive cases with Klinefelter's syndrome and a 47,XXY karyotype. One of the very earliest cytogenetic investigations carried out on a subfertile male population in Glasgow, relied entirely on nuclear sexing (Ferguson-Smith et al. 1957). The number of chromatin-positive individuals identified was entirely consistent with the frequency of XXY types which have subsequently been shown, by chromosome analysis, to occur within the infertile male population (Chandley et al. 1975).

However, the buccal smear test can give no useful information on the *autosomal* complement of an individual; for this, a full chromosome investigation is still required. Only in the last few years has it been realised to what extent the internal control of spermatogenesis in mammals is dependent on the normal arrangement of the autosomal component of the genome and therefore detection of an anomaly in an autosome is just as important as in a sex chromosome (see below). The frequency with which chromosome abnormalities are to be found within the male and female human subfertile populations and the ways in which chromosome abnormalities can exert their effects on the reproductive system, are considered here.

Chromosome Surveys Among Patients Attending Infertility Clinics

Surveys of Males

A number of surveys of male patients attending infertility clinics have now been carried out. Tiepolo et al. (1981) summarised the findings from several of the largest surveys where karyotyping has been carried out on more than 1000 men (Table 8.1). They include data from Pavia, Uppsala, Brussels and Edinburgh. Ascertainment procedures have varied from one survey to another so that the frequency of abnormalities has also varied from 2.1% in an unselected group studied in Edinburgh (Chandley 1979), to 9% in a selected group studied in Pavia (Tiepolo et al. 1981). In the latter survey, males were referred from infertility

Table 8.1. Total number and percentage of chromosome abnormalities in four different surveys of subfertile males

Survey	Number	Sex chromosomes	Autosomes	Total	%
Pavia[a]	2247	163	37	200	8.9
Uppsala[b]	1363	70	20	90	6.6
Brussels[c]	1000	27	6	33	3.3
Edinburgh[d]	2372	33	18	51	2.1

References
[a] Tiepolo et al. (1981)
[b] Kjessler (1974)
[c] Koulischer and Schoysman (1975)
[d] Chandley (1979)

centres all over Northern Italy. Since such differences in ascertainment invalidate the pooling of data, only the results of the Edinburgh survey on unselected men will be considered in the following discussion.

This survey was carried out over a 10-year period between September 1968 and July 1978. During this time, a total of 2372 men, (97% of all males who attended the clinic) were karyotyped. After 5 years (1968–1973), the overall frequency of chromosome abnormalities was 2.2% (Chandley et al. 1975), a frequency which did not change when the data obtained over a further five years (1973–1978) were included (Chandley 1979).

This figure, for the incidence of major chromosomal changes such as aneuploidies, translocations and other structural rearrangements (Table 8.2), but excluding minor chromosome 'variants', was three times that reported for the newborn male population of Edinburgh at that time (Jacobs et al. 1974) (Table 8.3). (Minor variants such as larger-than-normal or smaller-than-normal Y chromosomes or autosomes were not included because they are known to be common among normal fertile individuals in the population (Court-Brown et al. 1965) and their frequencies within the subfertile male population were approximately the same as in the population at large).

Men with a 47,XXY karyotype occurred nearly nine times more often among the subfertile males and accounted for nearly half of all the chromosomally abnormal

Table 8.2. Chromosome abnormalities found in the somatic karyotype of 2372 unselected men who attended a subfertility clinic in Edinburgh between September 1968 and July 1978

Karyotype	n
47,XXY	24
47,XYY	5
46,XY/47,XYY	1
45,X/48,XYYY	1
45,X/46,X,r(Y)	1
46,X,inv(Y)(p11;q11)	1
47,XY,mar+	4
Robertsonian translocation	4
Reciprocal autosomal translocation	10
Total	51

Table 8.3. Frequency of occurrence of chromosome abnormalities among 2372 subfertile males and among 7849 newborn male babies in Edinburgh

	Subfertile males		Newborn males	
	n	Frequency/1000	n	Frequency/1000
All abnormalities	51	21.50	57	7.00
Sex chromosome abnormalities	33	13.91	24	3.05
47,XXY	24	10.11	9	1.15
47,XYY	5	2.10	10	1.27
Robertsonian translocation	4	1.69	6	0.76
Reciprocal translocation	10	4.22	6	0.76
Marker chromosome	4	1.69	1	0.13

men within this group (Table 8.3). Male carriers of reciprocal autosomal translocations were five times more frequent and men carrying a supernumerary or 'marker' chromosome in their karyotype 12 times more frequent than within the newborn male population.

The incidence of 47,XYY males and carriers of Robertsonian translocations was not significantly different for the two groups, but all other comparisons were highly significant.

A study of chromosomal abnormalities in relation to sperm count showed that among azoospermic men, 15.38% were chromosomally abnormal, 12.85% being 47,XXY chromatin-positive males, the other 2.53% being chromatin-negative (Table 8.4). As mean sperm count increased, so the numbers of chromosomally abnormal individuals declined, an observation which has been made by other authors who have analysed their subfertile male data in this way (Kjessler 1972). This point will be raised again later in the chapter when consideration is given to the general question of selection of males for karyotyping purposes.

Table 8.4. Frequency of chromosomal abnormalities among 2275 men attending the Edinburgh subfertility clinic classified according to sperm count

Sperm count ($\times 10^6$ per ml)	% chromosome abnormalities
0	15.38
< 1–20	1.76
21–60	0.94
61–100	0.70
>100	0.20

Surveys of Females

Apparently, the only survey that has been conducted among women attending an infertility clinic is that of P. A. Jacobs (unpublished work) over the period 1970–1972. During this time, the somatic karyotypes of 850 women who presented at a subfertility clinic in Edinburgh were examined, and five found to be abnormal. One patient was heterozygous for a 13q 14q Robertsonian translocation, three carried a reciprocal autosomal translocation and the fifth had a small extra marker chromosome in an otherwise normal karyotype.

The overall frequency of abnormalities within this subfertile female group was 0.6%, a figure not significantly different from the control frequency of 0.4% for women in the Edinburgh general population. This survey of subfertile females was thus discontinued.

Chromosomal Effects On Fertility

The 47,XXY Individual

The testes of adult XXY males with Klinefelter's syndrome are small, hyalinised and devoid of germ cells. The seminal analysis usually reveals azoospermia. At birth

however, the testes of XXY babies are generally of normal size and consistency and show a normal histological appearance (Ratcliffe et al. 1979). By the age of 6 months, they have usually decreased in size and become unusually soft. The histological changes which take place in the XXY testis are mainly postpubertal, although some may be seen earlier (Mikamo et al. 1968).

The generally observed sterility of XXY males, both in man and other investigated mammalian species, has led to the postulate that the presence of two X chromosomes in a testicular germ cell results in its perinatal death (Burgoyne 1978).

Chromosomally-Derived Sterility

As previously noted, the incidence of chromosomal abnormalities among males attending infertility clinics rises as sperm count declines. This is because not only is the XXY karyotype associated with hypogonadism, but several other abnormal chromosomal situations, both sex chromosomal and autosomal, impair germ cell maturation in males. This type of effect has come to be known as 'chromosomally-derived' sterility (Searle et al. 1978). In other species in which it has been investigated, the effect seems to stem from a defect in spermatogenesis which leads to the production of few or no spermatozoa. Such effects are also observed in man and through studies in man and the mouse, a clearer picture is now emerging of the particular kinds of chromosome abnormality involved. Searle et al. (1978) have listed the chromosomal conditions in the mouse which are likely to generate male sterility through spermatogenic impairment. These include the XYY condition, failures of association between X and Y chromosomes at meiosis, heterozygosity for X–autosome or Y–autosome translocations and some conditions involving autosomes only. In all of these, the fertility of female carriers for the same abnormality appears unaffected.

A priori, one might expect that some or all of these types of anomaly having effects on spermatogenesis might be found among men attending infertility clinics and this is indeed the case.

In their Dundee survey, Faed et al. (1982) found an X–autosome and a Y–autosome translocation heterozygote, both showing spermatogenic disturbance. The former patient was azoospermic, with a complete maturation arrest at the pachytene stage of meiosis; the latter was severely oligozoospermic with a partial arrest in the spermatid stage. Other human sex–autosome translocations are known to generate male sterility through spermatogenic disturbance (Dutrillaux et al. 1972; Smith et al. 1979; Laurent et al. 1982).

The XYY condition in man can have a deleterious effect on spermatogenic development in some individuals, but the testes of others appear to display a normal histological picture (Skakkebaek et al. 1973). The frequency of the XYY karyotype among males attending infertility clinics is not, apparently, significantly greater than among newborn males (Table 8.3). Failure of X and Y pairing at meiosis in man, as in the mouse (Beechey 1973), is associated with maturation breakdown, the arrest in germ-cell development occurring at the first meiotic division (Chandley et al. 1976a). In some cases, pairing failure in one pair of chromosomes occurs because of the presence of a structural rearrangement in one homologue, and this too can lead to arrested spermatogenic development. Either the sex chromosomes

or an autosomal pair may be involved (McIlree et al. 1966; Chandley and Edmond 1971). In other rare cases, the pairing defect may be of a more general nature, affecting a high proportion of the chromosomes, and again, spermatogenic breakdown is an associated phenomenon (see Thomson et al. 1979 for review).

Perhaps the most fascinating and informative type of rearrangement associated with spermatogenic breakdown is the purely autosomal reciprocal translocation. Here, the data from the mouse are particularly useful for studies of the mechanisms involved because different individuals exhibit an almost complete range of effects, from those with normal sperm production to those in which spermatogenic arrest occurs right at the onset of meiosis (Searle 1974; Searle et al. 1978).

The effects on spermatogenesis are associated in these cases of purely autosomal exchange with special characteristics of the translocations themselves. There is a tendency for one breakpoint to be close to the centromere and the other fairly distal on the chromosome involved. This often leads to the formation of long and short marker chromosomes in the somatic karyotype and at meiosis, because of failure of chiasma formation in one arm or in adjacent arms of the translocation configuration, to the preponderant formation of a type of meiotic configuration known as an 'open chain' at metaphase I (Fig. 8.1). In the mouse, a positive correlation has been noted between severity of effect on the sperm count and percentage of spermatocytes showing such chain configurations at meiosis (Searle 1974). Thus, as the proportion of cells with chains rises, so the sperm count declines.

In man, a few reciprocal autosomal translocations which seem to follow the same rules as those described for the mouse have now been reported (Chandley et al. 1976b; Laurent et al. 1977; Léonard et al. 1979; San Roman et al. 1979; Blattner et al. 1980). Where meiotic investigations have been performed in male heterozygotes, again a preponderance of chain configurations has been noted (Fig. 8.1b) and, as in the mouse, their frequency appears to increase as sperm count declines (Table 8.5). Also, the sterilising effects of these purely autosomal reciprocal translocations appear to be limited to male heterozygotes. In studies where the translocation has been shown to be familial (Chandley et al. 1976b; Léonard et al. 1979; Blattner et al. 1980), sterility and azoospermia have been reported in more than one male heterozygote while no obvious effects on fertility in females carrying the same translocation have been observed. The defect would thus appear to be one of disturbed meiotic function that can be accommodated in oocyte but not spermatocyte development.

The mechanism by which these autosomal translocations produce the disruption of spermatogenesis is not yet understood although it is believed that they act autonomously on the germ cells carrying them (Searle et al. 1978). This could be true of all other situations of 'chromosomally derived sterility' and, at least in man, the detection in the karyotype of any one of the kinds of abnormality associated with this kind of phenomenon would appear to offer a poor prognosis for normal male fertility.

Recurrent Abortion

Translocations and certain other structural rearrangements are a well-known source of heritable chromosomal imbalance, both in man and other species. The factors which affect segregation in human translocation heterozygotes have been reviewed

Table 8.5. Correlation between break point positions, meiotic configurations and effects on sperm production in human reciprocal autosomal translocations. C, chain; R, ring

Case	Translocation	CIV or CIII + I (%)	RIV (%)	No. of cells analysed	Sperm count ($\times 10^6$ per ml)
1	t(9;22)(q12;p11)	100	0	22	Azoospermic
2	t(9;11)(q34;q13)	100	0	68	<0.1
3	t(13;18)(p13;q12)	meiotic studies not performed			Azoospermic
4	t(8;15)(q22;p11)	meiotic studies not performed			<0.1
5	t(10;13)(q25;q11)	60	40	30	8.5
6	t(9;22)(q21;q11)	34	66	38	Not known but normal spermatogenic activity seen in only a few seminiferous tubules of the testis.
7	t(9;14)(q22;q32)	1	99	103	7.5
8	t(2;10)(q33;q24)	0	100	58	20.0
9	t(5;6)(q22;q13)	0	100	110	23.0
10	t(1;18)(q32;q21)	0	100	79	31.0
11	t(2;11)(p21;q25)	0	100	64	Not known but normal spermatogenic activity reported in all seminiferous tubules.

References:
Cases 1, 6, 7, 9 and 10 Chandley et al. (1976b).
Cases 2 and 11 San Roman et al. (1979).
Case 3 Blattner et al. (1980)
Case 4 Léonard et al. (1979)
Case 5 Laurent et al. (1977)
Case 8 Faed et al. (1982)

in detail by a number of authors (Ford and Clegg 1969; Lindenbaum and Bobrow 1975). It is important to emphasise that each translocation is likely to be unique in respect of the relative frequencies of different types of gamete it produces.

A significant reduction in reproductive fitness has been shown for both male and female carriers of a human reciprocal translocation (Jacobs et al. 1975). In both sexes, there is a significant reduction in numbers of live births and a significant increase in both numbers of fetal deaths and in the generation time between live births by comparison with controls. Such carriers can therefore be rendered subfertile in instances where the translocation is of the type to segregate a high proportion of chromosomally-abnormal gametes, leading to abortion of the conceptus. In cytogenetic surveys, approximately 10% of couples who are normal except for recurrent pregnancy wastage have been found to carry balanced translocations (Mennuti et al. 1978).

Perhaps the most extreme example of a type of human translocation giving a high proportion of abnormal segregants is the homologous Robertsonian centric fusion type of rearrangement (Palmer et al. 1980). Here, abortion levels can reach 100%, owing to the continual segregation of either nullisomic or disomic gametes. These in turn produce monosomic or trisomic offspring, the majority of which abort; thus carriers of these rare translocations are rendered almost completely sterile, not by their inability to produce gametes but by their inability to produce chromosomally-balanced gametes which, on fertilisation, will give viable offspring.

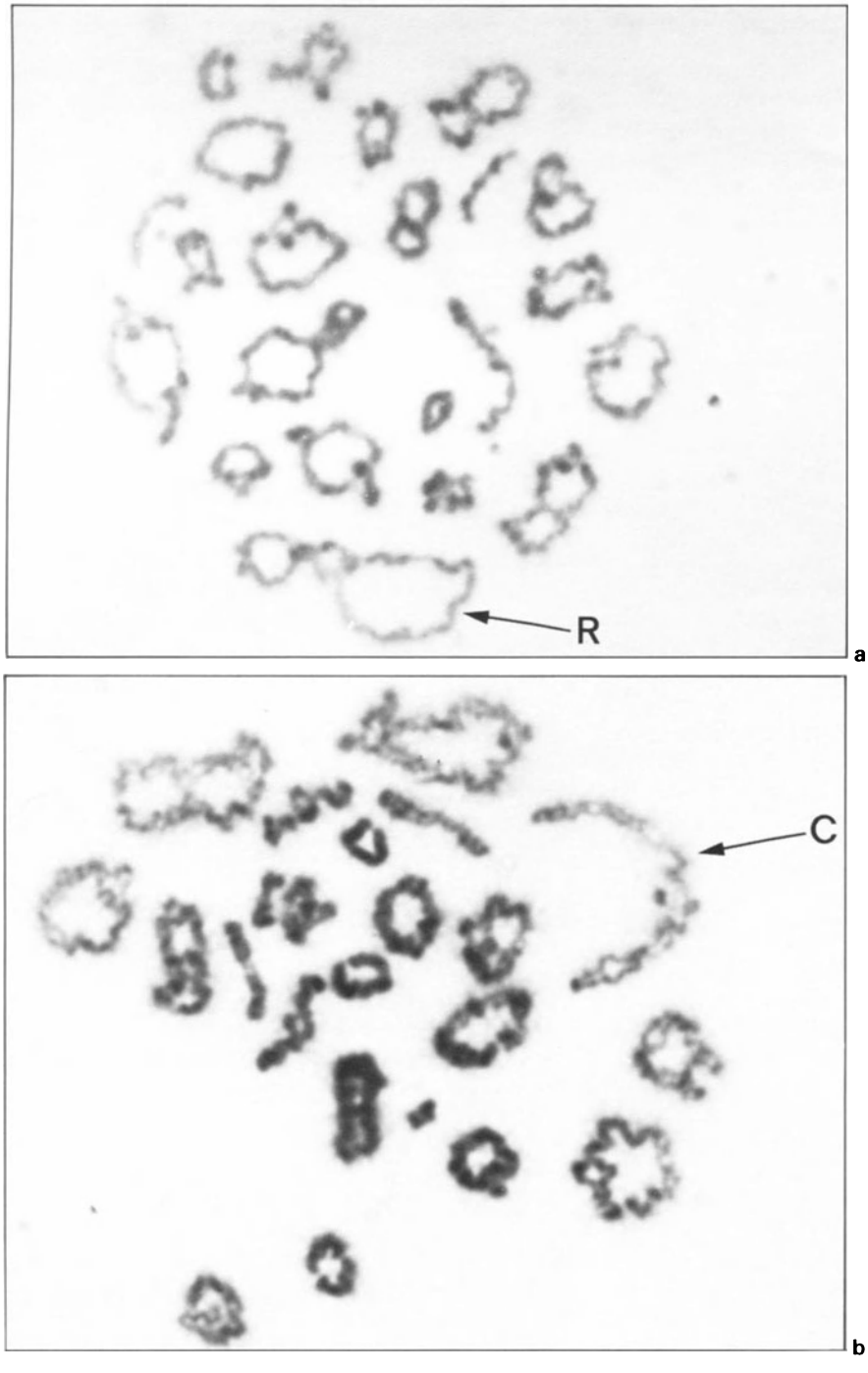

Fig. 8.1. Meiotic metaphase I preparations from two different translocation heterozygotes. **a** 46,XY,t(1q;18q) showing a typical 'ring' (*R*) quadrivalent. **b** 46,XY,t(9q;22p) showing a typical 'chain'. **c** quadrivalent

Chromosome Studies at Meiosis

Meiosis is an integral part of the process of spermatogenesis which takes place continuously in the adult male, giving rise, under normal circumstances, to a plentiful supply of spermatozoa.

The behaviour of chromosome abnormalities at meiosis in man has been the subject of a good deal of investigation over the past 20 years, ever since Ford and Hamerton made the first human meiotic studies in 1956. The techniques have been continually developing and improving since then and now a good deal is known of the likely meiotic picture to be found among the various chromosomally-abnormal situations. Furthermore, in some rare instances, abnormalities are observed in the chromosomes at meiosis when none can be seen in the somatic karyotype. This is because there are gene mutations which can act specifically on the meiotic process causing pairing or other disturbances, while producing no detectable damage to the chromosomes in the somatic tissues. Obviously, in these instances, a meiotic investigation would be required to detect the abnormality, but whether meiotic studies are always necessary to interpret and understand the behaviour of a chromosome abnormality at gametogenesis is doubtful. Meiotic chromosome studies can provide a strong additional tool in the diagnosis of infertility but even when they are not undertaken, certain predictions can still be made by reference to the somatic karyotype alone. For example, certain theoretical predictions about translocations could be made from a knowledge of breakpoint positions and postulated sites of chiasma formation. Thus, the types of translocation expected to yield high frequencies of chromosomally unbalanced gametes leading to recurrent abortion could probably be predicted and so also could those translocations likely to generate male sterility through spermatogenic impairment.

For the benefit of those who may be interested in undertaking meiotic chromosome investigations however, a brief account of some of the techniques employed will be given.

The Air-Drying Technique

In the early days of meiotic chromosome investigation, the usual procedure was to examine the chromosomes in simple squash preparations made from small pieces of testicular tissue. Now the squash method has been largely superseded by the use of air-drying techniques applied to germinal cells in suspension. The use of air-drying, combined with a short hypotonic pretreatment has brought about an enormous improvement in the quality of meiotic chromosome preparations and better spreading of the chromosomes with much clearer fixation have been achieved (Evans et al. 1964). Hand-in-hand with these developments has gone improvement in the staining techniques so that more precise analysis can now be carried out using the recently developed 'C-' and 'Q-' banding methods (Chandley and Fletcher 1973; Chandley 1977; Spowart 1979). Fig. 8.2a shows a human meiotic preparation in diakinesis/metaphase I stained by the C-band technique of Chandley and Fletcher (1973). Fig. 8.2b shows another prepared by the Q-band method of Spowart (1979). In both, chromosomes 1, 9, 16 and the Y-chromosome are particularly easy to identify on account of their staining reactions.

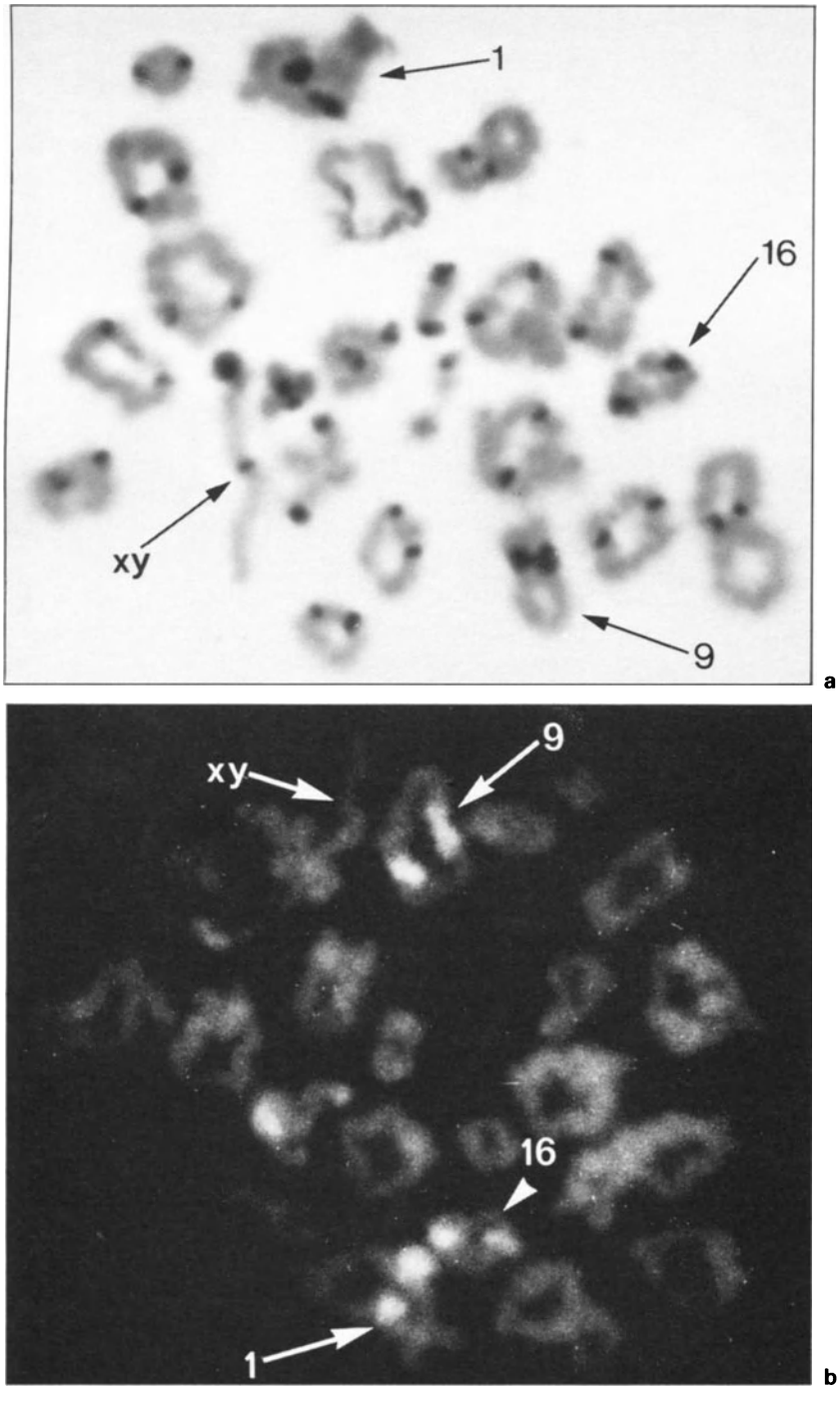

Fig. 8.2. Meiotic metaphase I preparations stained **a** by the 'C'-band technique, **b** by the 'Q'-band technique. Note in **a** the large heterochromatic stained blocks in chromosomes 1, 9, 16 and the Y chromosome. In **b** these same blocks show bright fluorescence

The air-drying method is also of great use for the study of chromosomes at meiosis in male carriers of reciprocal translocations and other chromosomal abnormalities. Fig. 8.1 shows the typical 'ring' and 'chain' quadrivalents observed at metaphase I in two different translocation heterozygotes. The formation of such configurations is dependent entirely on the positioning of chiasmata and this is ultimately determined by breakpoint positions.

Surface-Spreading

Perhaps the most interesting recent development in meiotic technique has been the application of a surface-spreading method, originally developed for use with insect spermatocytes (Counce and Meyer 1973), to the prophase spermatocytes of mammals including man (Dresser and Moses 1979; Fletcher 1979). Such spreading, followed by fixation and staining with an appropriate silver solution, permits visualisation of the proteinaceous axes of the autosomes and sex chromosomes. The behaviour of these closely parallels that of the prophase bivalent in synapsis and disjunction and therefore characteristics such as length, centromere position and stage of pairing can be taken directly from them. The silver-stained elements can be seen readily at the light microscope level (Fig. 8.3).

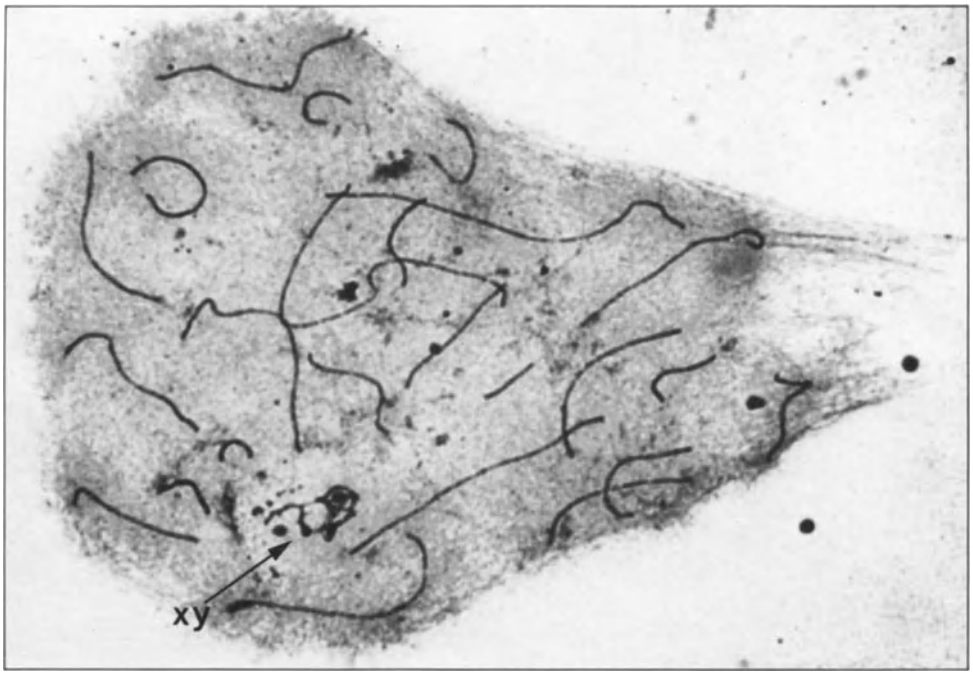

Fig. 8.3. Silver-stained surface-spread human pachytene preparation. The 22 autosomal elements show their varying lengths and the XY bivalent is typically twisted and folded

The great advantage of this method is that it is applied most effectively to cells at the pachytene stage of meiosis, a stage which is plentiful in any normal testicular suspension and yet one which does not yield satisfactorily to analysis by the air-drying or squash techniques. The method has been found, in mammalian species other than man, to be of particular benefit in the analysis of the behaviour at meiotic prophase of certain structural abnormalities. It is also of particular benefit for chromosomal analysis at the pachytene stage when maturation impairment at a stage preceding metaphase I has occurred, making analysis at metaphase I impossible (Laurent et al. 1982).

Meiosis in Semen

Although it is generally assumed that the testis is the source of cells for meiotic investigation, one novel account has been given of the use of ejaculated seminal fluid for chromosomal analysis at meiosis (Sperling and Kaden 1971). These authors estimate that 3%–5% of all cells in the ejaculate of men with normal sperm counts and 40% or more of cells in the ejaculate of some oligozoospermic men consist of spermatogonia, spermatocytes and spermatids. Preparations of meiotic chromosomes, sometimes of good quality, have been obtained from these immature cells (Sperling and Kaden 1971; Templado et al. 1980).

The method could prove to be of particular value in circumstances where, for one reason or another, testicular biopsy material cannot be obtained. Moreover, since the numbers of spermatocytes found in semen appear to be highest in oligozoospermic men, the method could be of particular value when applied to the study of meiosis in infertile men with spermatogenic impairment or arrest.

Chromosomes in Spermatozoa

On the subject of technique and the recent advances, mention should be made of the latest and perhaps most exciting development. This concerns the use of an in vitro fertilisation technique to analyse directly the chromosomal complements of human spermatozoa. The method (Rudak et al. 1978) involves the in vitro fertilisation of zona-free hamster eggs by human spermatozoa with activation of the human sperm to a point where the chromosomes of the male pronucleus can be seen and studied (see also chap. 5). With this technique, sperm chromosomes appear large, with parallel chromatids showing distinctive coils (Fig. 8.4). The technique holds out considerable potential for the future in that, for the first time, human sperm genomes can be analysed with the same precision as the chromosomes of somatic cells. As Rudak et al. (1978) have pointed out, 'the way is now open to studying directly the chromosome constitution of a population of spermatozoa from any given semen sample, and to assessing the effects of natural or experimentally induced phenomena on the chromosomes of human gametes'. The technical expertise required to carry out such in vitro fertilisations is, however, quite considerable and it may well be some time yet before the method comes into routine use.

Nevertheless, at least one large survey of human sperm genomes has already been successfully carried out (Martin et al. 1982). In this study, 240 Q-banded sperm chromosome complements from 18 males, eleven of proven and seven of

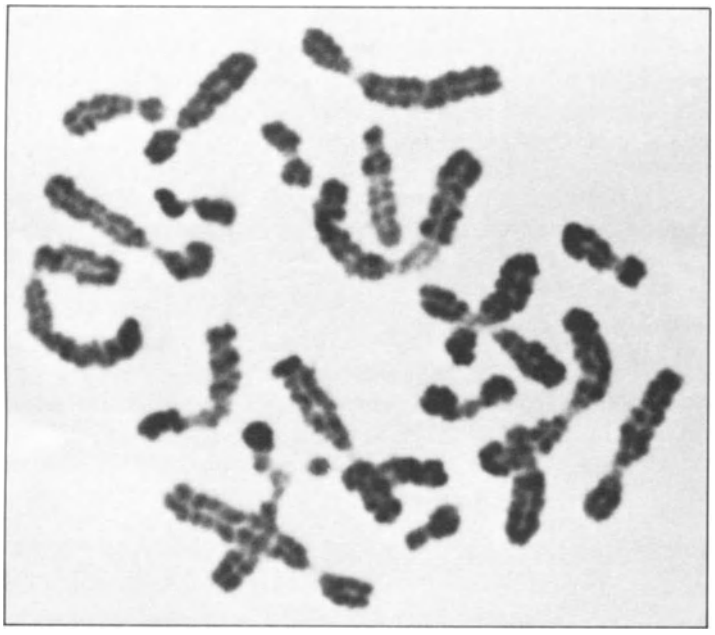

Fig. 8.4. The chromosomes of a human spermatozoon with a 23,Y complement (Rudak et al. 1978)

unproven fertility, were analysed. The frequency of sperm chromosome abnormalities in males of proven fertility was 6.9% compared to 12.5% in males of unproven fertility. A larger series is needed to determine if individual differences between men are significant.

Counselling of the Chromosomally Abnormal Infertile Male

It is seen from the foregoing that chromosome abnormalities can act to reduce the fertility of the human male in a variety of different ways. For the clinician offering advice and counselling to the infertile couple, it is important to have a thorough understanding of the processes involved and to be aware of the consequences of heterozygosity for a particular kind of chromosome abnormality.

Where a genetic or chromosomal cause underlies impaired gamete production, conventional therapy which attempts to raise the sperm count is probably of little value in treatment. Such men could be excluded from conventional therapy and offered alternatives such as AID before too much time and effort is wasted. Where the problem is one of recurrent abortion, the patient may be advised to keep on trying for a child, but each pregnancy should be carefully monitored in case the conceptus is chromosomally unbalanced. In this event, spontaneous abortion may occur but there is also a risk that the pregnancy might go to full term producing a child with perhaps multiple physical and mental abnormalities.

Where a male is oligozoospermic and a chromosomal abnormality is detected in the somatic karyotype, an important question may arise when the clinician is considering whether or not to encourage that patient to continue trying to father his own children. Could the few spermatozoa he is producing possibly carry abnormal chromosome complements? This is a question which will probably have to await the future development of the in vitro technique for direct analysis of spermatozoan chromosomes. Then the answer could be found in each individual case.

Finally, it is pertinent to repeat that if chromosome investigations are to be carried out only on selected male patients attending infertility clinics, priority should be given to men in the oligozoospermic and azoospermic categories since it is amongst these that the majority of chromosomal abnormalities will be found.

The overall frequency may not be high, but where a chromosomal factor does underlie the infertility, the clinician is provided with a diagnosis, and this can save a lot of further time-consuming consultation.

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Chapter 9

Auto-Immunity to Sperm

T. Hjort

Introduction

The history of sperm immunology goes back to the turn of the century when the immunogenicity of spermatozoa was demonstrated, first in heterologous systems independently by Landsteiner (1899) and Metchnikoff (1899) and shortly afterwards in a homologous system by Metalnikoff (1900). However, interest in auto-immunity to sperm as a possible cause of infertility was not evident until 1954, when Rümke (1954) and Wilson (1954) in independent studies detected sperm-agglutinating and -immobilising auto-antibodies in the sera of a few infertile men. In Wilson's study auto-agglutination of the spermatozoa in the patients' ejaculate and lack of penetration into cervical mucus was observed. Indeed, it calls for admiration that the main aspects of sperm immunology and immunological infertility could be outlined with such precision by studying only two patients!

Auto-immunity to sperm is of interest because: (a) it involves organ-specific auto-antigens of extraordinarily strong immunogenicity; (b) it can be studied in animal models and also after vasectomy which is a unique human auto-immune model; and (c) there is evidence that auto-antibodies to certain spermatozoal antigens can exert pathogenic effects causing infertility. In other words immune infertility' is a true auto-immune disease—and furthermore it is better understood than most other auto-immune diseases.

The aim of this chapter is to show that: (a) the occurrence of auto-antibodies to spermatozoa in men from infertile couples is not a rare phenomenon; (b) routine investigation will not necessarily detect antibodies; (c) anti-sperm antibodies can easily be diagnosed by simple tests; and (d) the mechanisms whereby antibodies may cause infertility are now understood to an extent that offers basis for rational attempts at treatment (to be discussed in Chap. 16).

Recent reviews on immunity to sperm—in males as well as females—have been given by Rümke and Hekman (1975), Shulman (1975), Jones (1976, 1980), Beer and Neaves (1978), Alexander and Anderson (1979), Rümke (1980, 1981), Menge (1980), and Jager (1981). A detailed description of an animal model for studying auto-immune aspermatogenic orchitis has been given by Voisin et al. (1974).

Immunogenicity of Sperm

The fact that about 70% of vasectomised men develop auto-antibodies to spermatozoa (Samuel and Rose 1980) demonstrates that spermatozoa must contain potent auto-antigens. An explanation for this is that spermatozoa, and thus sperm-specific antigens, are not yet present in the organism at that time of embryonic development when the immune cells' recognition of 'self' and 'not-self' is being developed, and consequently immunological tolerance to sperm-specific antigens may never be established. Such a lack of tolerance will have no immunological consequences as long as the spermatozoa and their progenitors remain secluded from the cells of the immune system.

After vasectomy a disruption of membranes, separating spermatozoa from the surrounding tissues may take place, either at the site of operation with formation of a spermatic granuloma (Schmidt and Morris 1973) or possibly by extravasation of spermatozoa into the interstitium of the epididymis, as observed in aged men with sperm antibodies (Rümke 1972). Similarly, after a testicular biopsy causing only brief damage to the membrane barrier, weak and transient immune responses have been detected, although mainly in patients in whom the biopsy had been performed once before (Hjort et al. 1974). On the other hand, the rare occurrence of the same antibodies in normal men may then be taken as evidence for the efficacy of the membrane barrier in preventing leakage of spermatozoa or spermatozoal antigens into the connective tissues as well as penetration of lymphoid cells into the tubular system.

Recently, this theory on sequestration of sperm antigens has been challenged by Tung (1980), because other potential autoantigens which originally were thought to be sequestered (such as thyroid antigens) have been proved subsequently not to be so. In animal experiments the rete testis has been found to form a weak spot in the membrane barrier (Johnson 1973) and possibly minute amounts of sperm antigens may leak out from this location establishing immunological tolerance. Certain results from animal studies do in fact suggest the existence of immunological tolerance, for example the strongly enhancing effect of complete Freund's adjuvant in developing experimental allergic orchitis (Johnson 1973) and the spontaneous development of auto-immune orchitis and anti-sperm antibodies in thymectomised Lewis rats (Lipscomb et al. 1979). However, if this theory of immunological tolerance is correct, it remains to be explained why the tolerance is circumvented so frequently.

Immunogens (Antigens) in Semen

Human semen has recently been described as 'an antigenic nightmare' (Jones 1980). The results of heterologous immunisation experiments, i.e. immunisation of one species with semen from another species, show that this is no exaggeration, as a multitude of different antigens can be recognised, not only in spermatozoa, but also in seminal plasma. However, if one considers only human auto-immunogens in the following discussion, a less perplexing picture appears.

First, the seminal plasma components can be ignored, as auto-antibodies to these never seem to have been demonstrated. This includes the so-called sperm-coating antigens which are seminal plasma components adsorbed so firmly to the surface of

spermatozoa that they cannot be washed away (for instance ABO blood group determinants and lactoferrin). The non-immunogenicity of these substances in men is remarkable, as sperm-coating antigens are among the most potent immunogens in heterologous immunisation (Weil 1960), and antibodies to sperm-coating substances have also been described in infertile women (Isojima et al. 1972). Occasionally women have developed anaphylactic reactions (type I hypersensitivity) to a glycoprotein in seminal plasma (Halpern et al. 1967).

Consequently, only spermatozoal antigens need be considered, but even these form a rather broad spectrum (Table 9.1). Normally, only surface antigens in living spermatozoa are accessible to antibodies and significant for male infertility. Auto-immune responses to sub-surface antigens may play a role, but the effect of such responses would be indirect and would occur only if spermatozoa degenerate

Table 9.1. Spermatozoal auto-antigens and their main characteristics

Antigens	Localisation and characterisation of antigen	Methods for detection of corresponding antibody	Increased incidence of antibody among males from infertile couples
1. *Membrane antigens*	Two glycoproteins in head as well as tail One antigen in tail end piece	Agglutination–, immobilization and cytotocicity tests Mixed antiglobulin reaction	Yes
2. *Sperm-specific enzymes:*			
LDH-X (LDH-C4)	Postacrosomal area of the plasma membrane and in seminal plasma	Electrophoretic RIA	Not known
acrosin	Acrosome, including equatorial region	Enzyme inhibition test	Not known—only few and preliminary data available
hyaluro-nidase	Acrosome	"	"
DNA-poly-merase	Nucleus and seminal plasma	"	"
3. *Nuclear proteins*	Protamines 1 and 2 in sperm nucleus	Immunofluorescent technique on swollen sperm heads	Yes
4. *Various sub-surface antigens, characterised by their localisation*	Acrosome (2 antigens) Equatorial segment Post-nuclear area Main tail piece Tail end piece	Antibody localisation techniques on fixed spermatozoa	Slight increase for some antibodies, no increase for other antibodies

and release their antigens in the male organism (leading to formation and deposition of immune complexes). Such a condition, with continuous release of sperm antigens, may exist after vasectomy. In vasectomised cynomolgus monkeys Alexander and Anderson (1979) demonstrated increased atherosclerosis, which they explained as a result of arteritis caused by circulating immune complexes composed of sperm antigens and anti-sperm antibodies. Whether such effects of auto-immunity to sperm may also occasionally occur in humans has not yet been determined, but attempts to demonstrate immune complexes in vasectomised men have so far revealed negative or inconclusive results (Hellema et al. 1979; Hess et al. 1979; Linnet et al. 1980) (see also chap. 17).

Table 9.1. (*Continued*)

Antigens	Antigen in living spermatozoa accessible for antibody	Auto-antibody of significance for male 'immune infertility'	References
1. *Membrane antigens*	Yes	Yes (when present in seminal plasma)	Rümke and Hellinga (1959) Kolk (1979) Poulsen and Hjort (1981) Ahuja et al. (1980)
2. *Sperm-specific enzymes:*			
LDH-X (LDH-C4)	Yes	Not known, but possible	Goldberg (1974) Erickson et al. (1975) Kolk (1977)
acrosin	No	Not known, but doubtful	Stambaugh and Smith (1976) Zaneveld et al. (1973a)
hyaluronidase	No	Not known, but doubtful	Erickson and Martin (1978) Zaneveld et al. (1973b)
DNA-polymerase	No (?)	Not known	Witkin et al. (1978)
3. *Nuclear proteins*	No	No	Kolk and Samuel (1975)
4. *Various sub-surface antigens, characterised by their localisation*	No	No	Hjort and Hansen, 1971; Tung (1975)

Surface Antigens

Although reactions involving sperm surface antigens—such as agglutination and immobilisation reactions—have been studied since 1954, little is known about these antigens. They are usually claimed to be organ-specific (Rümke and Hekman 1975). Isojima et al. (1972) were unable to absorb sperm-immobilising antibodies using extracts of liver and kidney, whereas Krogsrud et al. (1977) in similar absorption experiments with human sperm-agglutinating sera found evidence for cross-reacting determinants on brain and haemopoietic progenitor cells. In these experiments absorption with leucocytes did not reduce the agglutinating activity significantly, but recently an antigenic cross-reactivity of sperm and T-lymphocytes and reduced levels of T-lymphocytes in 'auto-immune' males with unexplained infertility were described by Mathur et al. (1980). On the other hand, Kissmeyer-Nielsen (1980) observed no cytotoxic activity on either T-lymphocytes or B-lymphocytes by selected sera with high levels of sperm-agglutinating and—immobilising antibodies. Therefore, the question of cross-reactivity cannot be considered settled, and additional experimental data are needed.

A major problem in the investigation of the membrane antigens is their insolubility in aqueous media. Detergents are needed for solubilisation, but in their presence the conventional tests requiring living cells (i.e. agglutination and immobilisation tests) cannot be employed. This leaves two alternatives: either to try to obtain soluble fragments of the antigens or to develop methods for detection of the antigens in the presence of detergents. Both lines have recently been followed. Kolk (1979) obtained soluble antigenic (antibody-neutralising) activity in spermatozoal extracts after treatment of the cells with papain, and D'Almeida et al. (1981) found soluble antigen fragments in sperm homogenates (apparently produced by the enzymes released from the spermatozoa). In this latter study antibody-binding material was also extracted with 8 M urea and absorption experiments gave evidence for the occurrence of two antigens involved in sperm agglutination (in head-to-head and tail-to-tail agglutination, respectively) and one antigen in immobilisation. Further techniques—such as indirect immunoprecipitation followed by polyacrylamide gel electrophoresis—are now available, allowing detection of detergent-solubilised antigens (see below).

It has generally been assumed—although without any experimental proof—that the various modes of agglutination observed in microscopic agglutination tests would have to reflect different antigen–antibody systems. In this case a minimum of three different antigens, corresponding to the main types of agglutinates (head-to-head, tail-to-tail and tail-tip-to-tail-tip), should exist. Recent results, partly based on experiments with labelled solubilised membrane antigens, have provided evidence for the existence of at least three auto-antigens in the membrane, but only one of these (the tail-tip antigen) seemed to be associated with a particular mode of agglutination, whereas the other two antigens could be involved in both head-to-head and tail-to-tail agglutination (Hjort and Poulsen 1981; Poulsen and Hjort 1981). One of the latter antigens could be detected by indirect immunoprecipitation with potent human antisera followed by SDS–polyacrylamide gel electrophoresis and was shown to be a glycoprotein. Preliminary data now indicate that the other antigen—also a glycoprotein—can be detected in detergent extracts from isolated sperm membranes (Ahuja et al. 1980). The unexpected finding that the same antigens may be involved in different modes of agglutination has not yet been

confirmed by other laboratories, neither has an explanation for the phenomenon been given.

The easiest way of mapping the membrane antigens would seem to be by means of antibody-localisation techniques but clear results have not emerged. Feltkamp et al. (1965) and Hamerlynck (1970), using the immunofluorescent technique, observed staining of spermatozoa—mainly of the acrosomal area, the posterior part of the head and the midpiece—by sera with high titres of sperm agglutinins, but there was no correlation between the staining patterns and the mode of agglutination. The more sensitive autoradiographic technique, applied by Fjällbrant (1970), disclosed that tail-to-tail agglutinins reacted not only with the tail, but also with the midpiece. Similar results have been obtained by the immuno-peroxidase technique, where tail-to-tail agglutinins were located on the tail and the posterior part of the head, whereas head-to-head agglutinins seemed to cover the entire membrane (Hjort 1976). Recently D'Almeida et al. (1980), also using the immuno-peroxidase technique, have reported that although head-to-head agglutinating sera would primarily stain the head area, tails were also stained to some extent, and vice versa.

So far, antibody localisation studies have been considered inconclusive, as they have not shown the localisation patterns of the antigens expected from the modes of agglutination. However, the recent results obtained with solubilised antigens seem to justify a re-evaluation of the results since they all indicated that the different antigens (except the tail-tip antigen) may not be as restricted to certain areas of the membranes as originally presumed.

Among the various spermatozoal enzymes (Table 9.1) only the sperm-specific lactate dehydrogenase LDH-X (or LDH-C4) seems to present itself on the surface of intact spermatozoa; i.e. on the membrane overlying the postacrosomal dense lamina (Erickson et al. 1975). But it is also released into seminal plasma, from which the human enzyme has been isolated (Kolk 1977). It is obviously a poor immunogen in males. So far, antibodies to LDH-X have not been detected in sera from males from infertile couples, but among 102 vasectomised men three were found to have anti-LDH-X in serum (Kolk 1977, 1979).

Sub-Surface Antigens

These may be sperm-specific enzymes, nuclear proteins or various other antigens characterised by their location (Fig. 9.1., Table 9.1). As stated above these antigens may be relevant during the formation of immune complexes following vasectomy but are unlikely to play a part in immune infertility in males.
Experimental data support this view because relatively high levels of some of the antibodies detectable by immunofluorescence techniques on methanol fixed spermatozoa may occur in fertile men. Furthermore, the occurrence of the various antibodies in men from infertile couples has been found not to differ significantly from antibody findings in male blood donors. Only when men from couples with unexplained infertility were compared with clinically normal men from infertile couples where the women had been found infertile was a difference noticed (i.e. titres ≥ 10 for antibodies against the equatorial segment and the main tail piece were more frequent in the former group than in the latter) Husted 1975a). Some of the antigens are not sperm-specific, but cross-reacting substances can be found in certain bacteria and human tissues (Tung 1975).

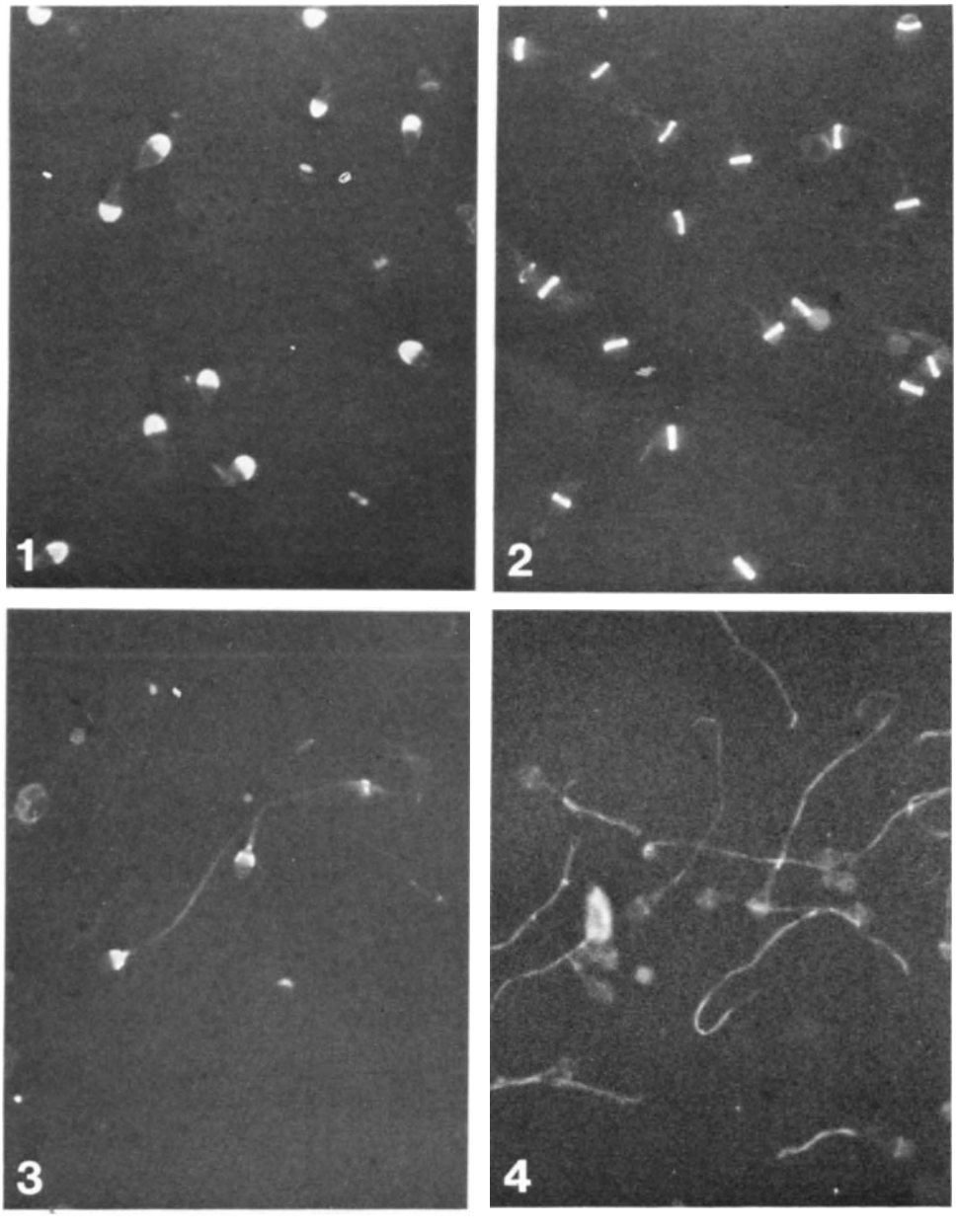


Fig. 9.1. Various sub-surface antigens detected by indirect immunofluorescence test on methanol-fixed spermatozoa, showing staining of: **1** acrosome; **2** equatorial segment; **3** post-nuclear area; **4** main tail piece. [Little is known about the nature and functions of these antigens. The antigens in the front part of the acrosome, the equatorial segment, and the post-nuclear area are apparently proteins, as they are easily destroyed by treatment with trypsin. (Hansen and Hjort 1971; Kolk et al. 1974)]

By using sperm heads, artificially swollen by treatment with dithiothreitol and trypsin, as antigen in immunofluorescent tests, Kolk et al. (1974) detected auto-antibodies to normally hidden nuclear antigens in some male sera. The antigens were subsequently shown to be protamine 1 and 2 (Kolk and Samuel 1975). Sera with antibodies to swollen sperm heads practically always also contain antibodies to membrane antigens, even though the two categories of antigens are not cross-reacting.

The Immune Response to Sperm Antigens and its Pathological Effects in the Male Organism

Humoral Immunity

Most studies in humans on auto-immunity to sperm membrane antigens have dealt with humoral immunity by detection of sperm agglutination, immobilisation or cytotoxicity. In male *sera* the antibodies causing these phenomena belong mainly to the IgG class (Boettcher et al. 1971; Fjallbrant 1969; Husted 1975b). For sera giving tail-to-tail agglutination this seems a rule, with few exceptions, whereas the head-to-head agglutinins—being relatively rare in male sera—have been found in some cases to be of the IgM class (Friberg 1974a,b). Analysis of selected sera by means of immuno-affinity chromatography confirmed the predominance of IgG, but apparently minor contributions of IgA and IgM were present in some sera (Ingerslev et al. 1979).

Most of the serum proteins in *seminal plasma*, including IgG and IgA, seem to reach the semen by transudation in the prostate (Rümke 1974a). However, it is possible that antibodies to antigens occurring in semen could also be actively secreted, in which case a predominance of IgA would be expected. Several investigations have indicated that a major proportion of the sperm agglutinins in seminal plasma belong to the IgA class and only a minor proportion to the IgG class (Coombs et al. 1973; Friberg 1974c; Husted and Hjort 1975). Recently, Jager et al. (1980a) have succeeded in demonstrating by means of the mixed antiglobulin reaction that the antibodies from seminal plasma, bound to the surface of spermatozoa in the ejaculate, are predominantly of the IgA class. At which level in the genital tract these antibodies enter the semen is not known for infertile men, but in vasectomised men undergoing vasovasostomy to recover fertility high levels of sperm agglutinins have been detected in the epididymal fluid (Linnet and Fogh-Andersen 1979).

Cell-Mediated Immunity

The role of cell-mediated immunity to spermatozoa is far from clear. In animal experiments, where immunisation is performed with spermatozoa or sperm antigens in complete Freund's adjuvant, cell-mediated immunity seems an essential part of the immune response which in these cases usually leads to experimental auto-allergic orchitis (or maybe more correctly epididymo-orchitis) with subsequent aspermatogenesis (Voisin et al. 1951; Mancini 1976). Similar experiments

with injection of autologous or homologous testicular antigen preparations in complete Freund's adjuvant have been carried out in male volunteers, i.e. men with prostatic carcinoma who were to be castrated for therapeutic purposes (Mancini 1976). In some cases the patients were hemicastrated, the testis used for preparation of antigen, and biopsies were obtained from the remaining testis 20–90 days after immunisation. Some of the patients receiving complete Freund's adjuvant developed mild transient histological lesions in the testis with congestion and serous oedema accompanied by sloughing and cytolysis of germinal cells. Furthermore, delayed-type hypersensitivity skin reactions to testicular extract were recorded. Thus, cell-mediated immunity and auto-immune orchitis can be induced in humans in exceptional circumstances with little relevance to cell-mediated immunity and testicular lesions as part of naturally occurring auto-immune responses to sperm.

Several studies with either lymphocyte transformation tests or leucocyte migration inhibition tests have provided evidence for the occurrence of cell-mediated immunity to sperm in 20%–50% of men from infertile couples, vasectomised men or men with various testicular disorders, whereas few normal men revealed positive reactions (El-Alfi and Bassili 1970; Nagarkatti and Rao 1976; Thestrup-Pedersen et al. 1976; Dondero et al. 1980). However, these techniques are still far from ideal, notably because very complex antigen preparations have had to be used, i.e. whole spermatozoa, frozen and thawed spermatozoa, spermatozoal extracts or testis extracts. This implies that contamination with leucocytes cannot be avoided, and lymphocyte stimulation might therefore be expected if allogeneic antigens are used. That such—in this context false—reactions may occur was stressed by Boettcher et al. (1979), who observed stimulation by whole semen, but not by a pure suspension of motile spermatozoa. Similarly, Thestrup-Pedersen et al. (1976) recorded fewer positive reactions in lymphocyte transformation tests with autologous spermatozoa than with allogeneic sperm, but positive reactions were also observed with the true auto-antigen.

Pathological Effects

The pathological effects of the immune response in the target organs, i.e. testis and epididymis, have been difficult to evaluate, because tissue from these organs is not usually available for examination, so the evaluation has to be made mainly on the basis of the ejaculate. Semen samples from men with even high levels of antibodies to sperm membrane antigens often appear quite normal at the routine examination, i.e. the number of spermatozoa, the percentage of motile cells and the percentage of abnormal forms are within the normal ranges; but the presence of auto-antibodies may be noticed by the formation of agglutinates in the ejaculate. In a follow-up study on men with sperm-agglutinating antibodies (Rümke et al. 1974) a group of 137 normospermic men apparently remained normospermic during the observation period of up to 16 years in spite of the persistence of the antibodies. Friberg and Kjessler (1975), studying testicular biopsies and sperm agglutinins in 59 patients with azoospermia or cryptozoospermia, found complete spermatogenesis in all eight patients with tail-to-tail agglutinins (referred to by them as head-to-tail agglutinins), suggesting that the presence of mature spermatozoa is a prerequisite for the spontaneous production of these antibodies. These findings justify the important conclusion that the mere presence of anti-sperm antibodies does not

necessarily affect spermatogenesis, but they do not exclude the possibility that an auto-immune response could under certain circumstances induce pathological effects in the testes in those of patients (about two-thirds) with sperm agglutinins, who have been found to have oligozoospermia or azoospermia (Rümke 1980).

Associations between testicular pathology and auto-immunity to sperm may be analysed in two ways; by examining patients with orchitis for auto-immune reactions and by studying testicular biopsies from patients with auto-immune responses for evidence of immune reactions displayed in the tissue (viz. lymphocytic infiltration or deposition of immunoglobulin). Using the first approach, Cruickshank and Stuart-Smith (1959) detected sperm agglutinins in two of six patients with granulomatous orchitis, and Hendry et al. (1979) have recently described another case with focal round-cell infiltration of seminiferous tubules and a high titre of sperm agglutinins. Andrada et al. (1977), investigating 70 patients with mumps orchitis, observed delayed-type hypersensitivity reactions after skin testing with ultrasonicated spermatozoa in 17 of the patients, but anti-sperm antibody findings were rather scarce. Sperm agglutinins were detected in low titres in only five of 35 patients tested (in two of 28 acute and three of seven chronic cases).

Immunofluorescence tests on testicular biopsies from patients with various testicular disorders, mainly oligozoospermia, have disclosed deposits of immunoglobulin—in most cases IgG—in 14%–22% of the patients (Isidori et al. 1973; Donat and Morenz 1979; Jadot-van de Casseye et al. 1980). The deposits were most commonly located on the tubular walls, but staining of germinal cells and—in rare cases—Leydig cells or Sertoli cells was also seen. Again, the most striking result may be that the great majority of the patients revealed no signs of auto-immune orchitis; these studies therefore lend little support to the assumption that auto-immunity to sperm could, by impairment of the spermatogenesis, be a common cause for oligozoospermia or azoospermia. However, auto-immune reactions in the rete testis or epididymis might block sperm transport, thereby causing oligozoospermia (Rümke 1980). This has not yet been investigated in humans, since biopsies are not usually taken from the rete testis. It should be noticed that rete testis is the 'weak spot' in the membrane barrier (Johnson 1973), that the epididymis is involved in the lesions of experimental auto-allergic orchitis (Mancini 1976) and that anti-sperm antibodies in high concentrations have been found in epididymal fluid from some vasectomised men (Linnet and Fogh-Andersen 1979). Therefore, there are good reasons for paying more attention to these sections of the genital tract in future investigations.

Detection of Antibodies to Sperm Surface Antigens

For routine purposes antibodies to sperm surface antigens are usually detected by various agglutination tests or complement-mediated cytotoxic reactions (immobilisation or cytotoxicity tests), but several new techniques, such as mixed antiglobulin reaction (MAR) and cellular radio-immunobinding, have recently been developed. It is not the intention to give a complete description of the various techniques here, but merely to summarise the principles on which they are based and discuss some of their advantages, disadvantages and difficulties. More detailed

descriptions and discussions have been given by Rose et al. (1976); WHO Reference Bank (1977a,b); Hellema (1978); Shulman (1978) and Mettler et al. (1980).

Agglutination Tests

Since the *gelatin agglutination test* (GAT) of Kibrick et al. (1952), originally designed for studies of rabbit antisera to spermatozoa, was introduced for use in humans by Rümke (1954) it has been widely used, particularly for studies of male sera. In this test, fresh semen, adjusted to a sperm concentration of 20 million/ml in a gelatin solution, is mixed with dilutions of inactivated serum or seminal plasma (usually starting with a 1:4 dilution). The mixtures are transferred to narrow test tubes and incubated at 37°C for 2 h. Agglutination can then be observed macroscopically by the appearance of white floccules along with clearing of the suspending medium (Fig. 9.2). With properly selected high quality semen samples the test is easy to carry out and agglutination reactions are usually clear-cut. A practical problem is that the test requires relatively large volumes of semen, so that only a limited number of sera can be tested with each ejaculate.

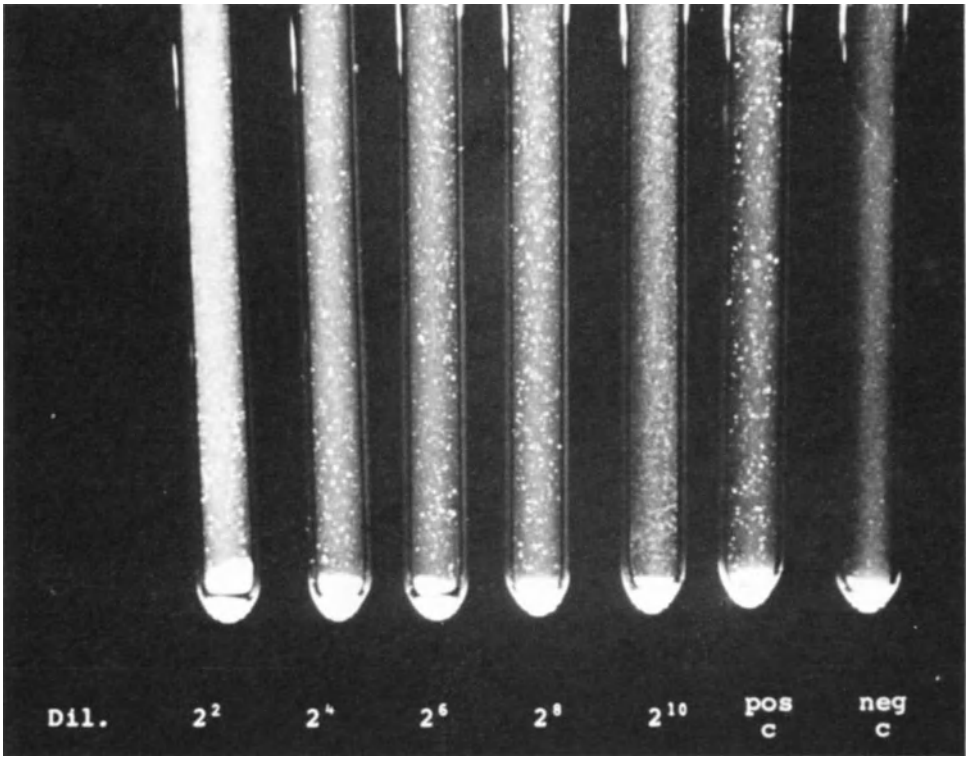


Fig. 9.2. Gelatin agglutination test on seminal plasma revealing a titre of 2^8 (± reaction in the 2^{10}-fold dilution) *pos C*, positive control; *neg C*, negative control

In order to overcome this problem Friberg (1974d) developed a microtechnique—*the tray agglutination test* (TAT)—in which the agglutination reaction is carried out on trays with microchambers in a drop under liquid paraffin (5 μl serum dilution +1 μl sperm suspension with 40 million spermatozoa per ml). After 4 h at room temperature or—as we prefer in our laboratory—after 2 h at 37°C, the reactions are read under an inverted microscope at low magnification (×60) for evaluation of large agglutinates and also at a higher magnification (e.g. ×400) for detection of small head-to-head agglutinates and for recording of the mode of agglutination (Fig. 9.3). Usually only titres of 8 or more are considered to be positive reactions. Head-to-head agglutination, caused by a non-immunoglobulin factor (see below), is thereby largely eliminated. The possibilities of testing a large number of samples with the same ejaculate and of recording the mode of agglutination at the same time, has made the tray agglutination test the most widely used technique today. Some experience is required for evaluating reactions in low dilutions of serum where clumps of spermatozoa may occur around leucocytes, and a distinction between such clumps and specific agglutinates therefore has to be made. Non-specific clumping can be eliminated by harvesting and using only motile spermatozoa, but this on the other hand reduces the sensitivity of the test system, at least for tail-to-tail agglutinins (Hellema and Rümke 1978).

Finally, the agglutination test of Franklin and Dukes (1964)—now commonly referred to as *the tube–slide agglutination test*—is still being used, although mainly for the study of female sera. A mixture of serum and spermatozoa is incubated at 37°C in a test tube, and drops from this mixture are transferred to a

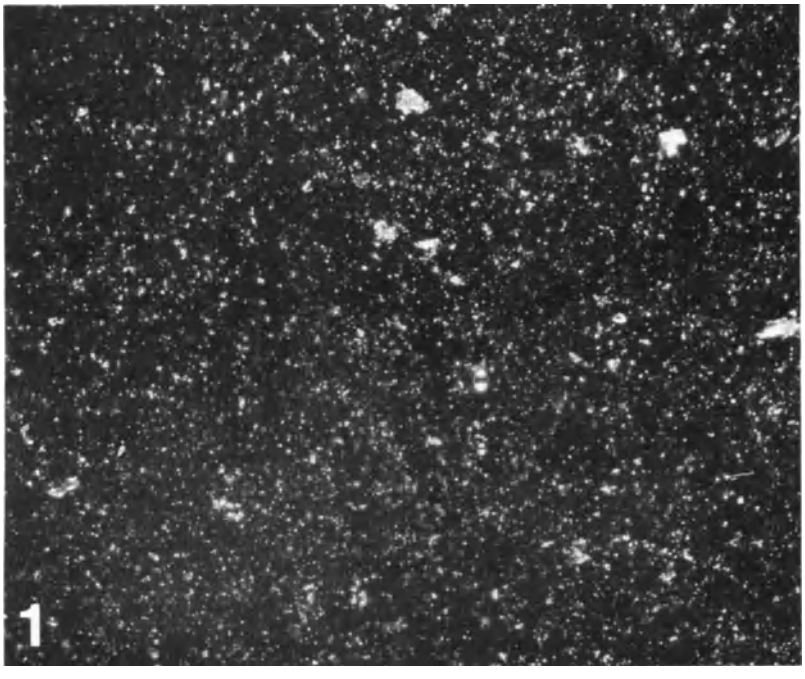
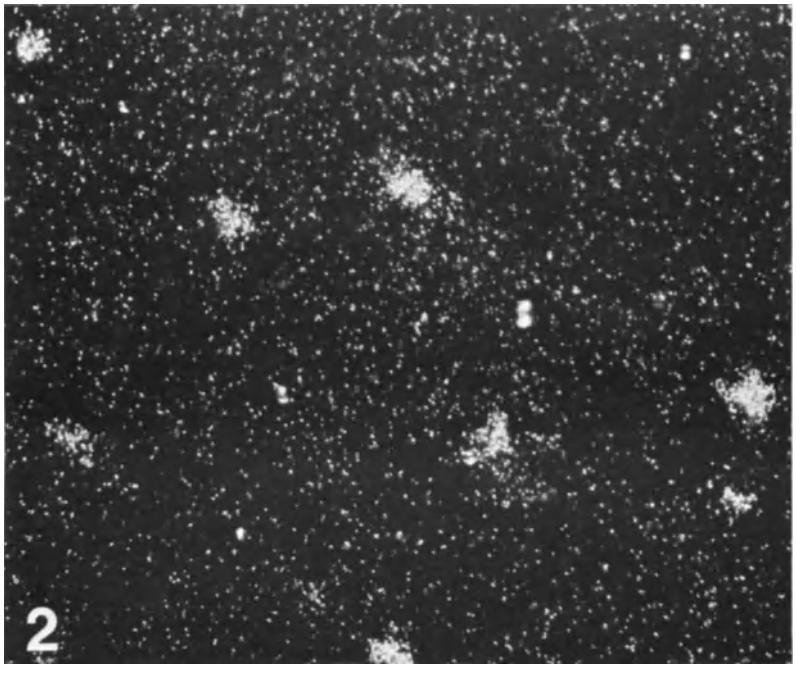


Fig. 9.3. Tray agglutination test as seen at low magnification. **1** Negative reaction—a few clumps but no specific agglutinates.



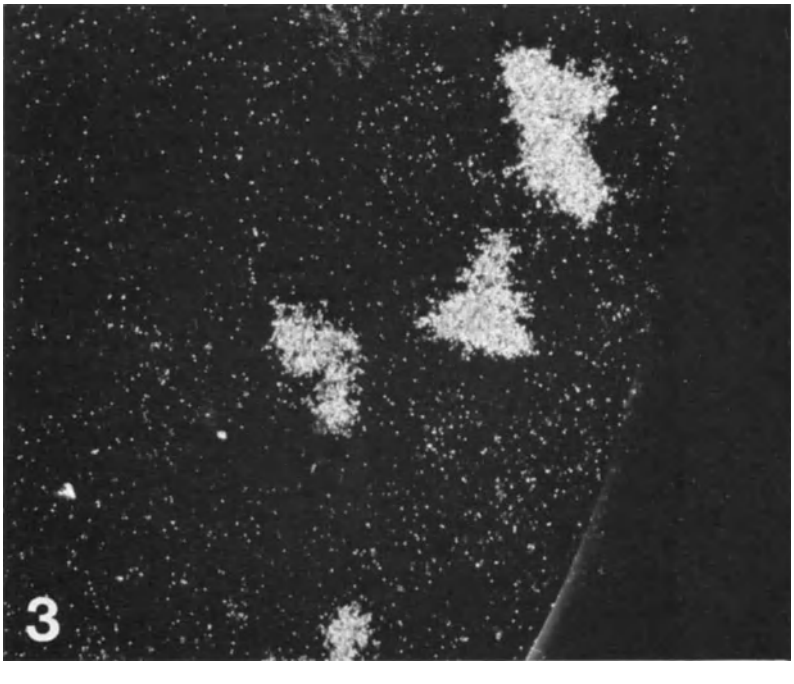


Fig. 9.3. (*Continued*) **2** Weakly positive reaction (tail-to-tail agglutination). **3** Strongly positive reaction (tail-to-tail agglutination)

slide for immediate examination under the microscope. Apparently some agglutinates, particularly tail-to-tail agglutinates, which are the most common with male sera, are disrupted by the transference to the slide, and the technique therefore almost exclusively reveals head-to-head agglutination. Furthermore, as the result is evaluated by counting the agglutinates, titration is laborious, and testing is therefore usually carried out only with undiluted serum or a 1:4 dilution. However, in such low dilutions of serum head-to-head agglutinates may be formed not only by antibodies, but also by a non-immunoglobulin, high-molecular-weight component, sometimes referred to as β-agglutinin, since it co-migrates with the β-globulin in electrophoresis (Boettcher et al. 1971; Ingerslev 1979). This agglutinin seems to occur mainly in female sera. Since the introduction of the tray agglutination test there is little justification for use of the tube–slide agglutination test, and if used it should be supplemented by one of the other techniques, e.g. the gelatin agglutination test (Shulman 1978).

Immobilisation and Cytotoxicity Tests

The *sperm immobilisation test* was introduced by Isojima et al. in 1968. After incubation of a mixture of inactivated serum (from the patient), spermatozoa and guinea pig serum as complement source for 1 h at 32°C, the percentage of immotile cells is determined and compared with the corresponding percentage in a mixture with normal human serum. The test is recorded as positive if at least half of the initially motile cells have become immobilised. Usually a titre is determined as the highest dilution giving positive reaction, but the dilution immobilising exactly 50% of the motile cells can also be determined (Isojima and Kyoama in Rose et al. 1976). A mixture of patient's serum and inactivated complement is included as a control; thus, the test provides a guarantee that the immobilisation is complement-dependent and therefore antibody-mediated; but, since IgA does not activate complement, the test only detects IgG and IgM antibodies. The main problem of this test is the spermotoxic effect of most guinea pig sera. A careful selection of sera without such effect is therefore required.

Cytotoxic reactions are, in principle, like the immobilisation test; but instead of counting the percentage of immotile cells, dye is added, e.g. eosin or trypan blue. This will stain cells in which the plasma membrane has been damaged by complement activation and the percentage of stained cells is then counted (Hamerlynck 1970). It is generally agreed that immobilisation and cytotoxicity represent the same phenomenon, recorded in different ways. The immobilisation technique is usually preferred because it is easier to carry out and it may even be slightly more sensitive than the cytotoxicity test (WHO Reference Bank 1977a).

Recently new and more sophisticated modifications of the cytotoxicity test have appeared. Suominen et al. (1980) have described an ATP-release assay in which the decrease in ATP release (compared with the release from normal cells) provided an objective measure for the cytotoxic killing of spermatozoa. Mathur et al. (1981) have developed a microcytotoxicity test with double staining of the cells, so that living and dead spermatozoa will appear different colours, for easy microscopic reading of the test. An exceptionally high percentage of sera from infertile males were found to be positive, but as pointed out by Shulman (1981) the clinical significance of a positive reaction in this test—and in any new test system—is difficult to evaluate as long as a comparison with the classic test systems has not been made.

Reproducibility and Sensitivity of Agglutination and Immobilisation Tests

Earlier scepticism about the above techniques now seems unjustified. In the early days of sperm immunology results from different laboratories were often incompatible, apparently because test systems were not always standardised and the investigations were sometimes characterised more by enthusiasm than by immunological expertise. Recently, results have been in much better agreement. From an international comparative study some years ago, in which several laboratories tested the same panel of sera by the various techniques, one of the conclusions was: 'that gelatin agglutination, tray agglutination, immobilisation and cytotoxicity tests are highly reproducible and reliable techniques if carried out properly' (WHO Reference Bank 1977a).

The observations that sera with sperm-immobilising and cytotoxic activity have always shown agglutinating activity at the same time—at least in the tray agglutination test (Husted 1975b; WHO Reference Bank 1977a,b)—provide strong evidence that the same antibodies are being detected in the various test systems, although not with the same sensitivity. Based on the results from the above-mentioned international study (WHO Reference Bank 1977b) (Table 9.2), the tray agglutination test was found to be sensitive in all three main modes of agglutination. The gelatin agglutination test revealed a similar sensitivity for tail agglutinins, but was less sensitive for head-to-head agglutinins, possibly because these agglutinates are often so small that they cannot be observed with the naked eye. Immobilisation and cytotoxicity reactions were generally less sensitive and seemed not to detect tail-tip agglutinins.

Since weak agglutination reactions with sera are of doubtful clinical significance and head-to-head agglutinins are rare in male sera, it does not matter which of the three tests—i.e. gelatin agglutination, tray agglutination or immobilisation—is used for male sera. However, for testing of seminal plasma the agglutination tests are preferred, not only because they will detect the IgA antisperm antibodies, but also because even low concentrations of antibody may be of clinical significance here.

Table 9.2. The relative sensitivities of the various tests for the detection of sperm anti- antibodies of different reactivities

Mode of agglutination	Gelatin agglutination test	Tray agglutination test	Tube–slide agglutination test	Immobilisation and cytotoxicity test
Head-to-head	+	++	++	+
Tail-to-tail	++	++	−	+
Tail-tip-to-tail-tip	++	++	−	(−?)

New Techniques

The *mixed antiglobulin reaction* (MAR) is not really new, as it was first used for detection of anti-sperm antibodies by Coombs et al. in 1973. However, only recently has it been studied in detail and perfected for detection, not only of IgG, but also of IgA antibodies (Jager et al. 1978, 1980a). Antisperm antibodies can be

detected in sera by an indirect version of the technique, where spermatozoa are initially incubated with serum and after washing applied in the mixed agglutination system. However, the test system is much more suited for demonstration of antibodies bound to the surface of spermatozoa in the ejaculate; in this *direct MAR test*, semen, erythrocytes sensitised with an IgG antibody or coated with IgA are mixed with mono-specific anti-IgG or anti-IgA, respectively, and studied under the microscope. If spermatozoa carry immunoglobulin of the given class on their surface, a mixed agglutination reaction can be observed by the attachment of erythrocytes to motile spermatozoa, and the result may be quantitated by estimating the percentage of motile spermatozoa involved in mixed agglutinates (MAR%). Latex particles coated with IgG or IgA can also be used. The detection of IgA antibodies is apparently technically more difficult than detection of IgG antibodies, but in the hands of experienced investigators both test systems seem reliable.

Not unexpectedly, the IgG MAR% was found to be related to the IgG antisperm antibodies in serum. On the other hand, the percentage of motile spermatozoa with IgA was not closely related to the sperm-agglutinating activity, either in serum or in seminal plasma; but it was roughly proportional to the percentage of spermatozoa showing the 'shaking phenomenon' in cervical mucus (i.e. shaking movements without forward progression) (Jager et al. 1980a). If these findings are confirmed in other laboratories, the MAR test may become the method of choice for detection of auto-immunity to sperm in the male infertility clinic because (a) it detects the antibodies where they really should be looked for, i.e. on the target cells and (b) the test can be carried out with commercially available reagents in routine semen analysis. One serious limitation is that the test depends on adequate motility of the patient's sample.

Another new technique is *passive haemagglutination* combined with a Coombs' test (Mathur et al. 1979). Erythrocytes are coated with antigen obtained by sonification of spermatozoa and with seminal plasma. In Mathur's study about one-third of the 50 males from couples with unexplained infertility showed 'elevated Coombs' autoantibody titres', but the antibodies detected in this system seem to be different from those detected by the conventional tests, because they also react with erythrocytes coated with seminal plasma.

Radioimmunoassays have now also been developed for detection of antibodies to surface antigens in sperm. A highly sensitive and quantitatively acccurate technique, employing ^{125}I–labelled protein A for detection of IgG bound to the cell surface has been described by Han and Tung (1979), but has so far been tried only on guinea pig testicular cells and spermatozoa. A somewhat similar assay with human spermatozoa was recently investigated by Haas et al. (1980a). The spermatozoa were incubated with undiluted human sera and after washing, IgG on the surface of the cells was detected by means of radiolabelled rabbit anti–IgG. In the testing of 83 sera the radiolabelled antiglobulin test revealed nearly twice as many positive reactions as the gelatin agglutination test, but only a few sera with sperm agglutinins were negative in the former (possibly not due to IgG antibodies). An indirect solid phase micro-radioimmunoassay, on the other hand, appeared to be less sensitive than agglutination tests but correlated more closely with the immobilisation test (Young 1978). The explanation for the relatively low sensitivity of these radioimmunoassays may be the presence of Fc receptors for IgG on human spermatozoa (Sethi and Brandis 1980) causing some non-specific binding of IgG with all sera tested.

New techniques for labelling and solubilising the membrane antigens are now available and should allow pure preparations of the antigens to be prepared. It should therefore be possible to develop readioimmunoassays for each of the different sperm surface antigens.

Which Test to Use

In choosing among the various techniques special attention should be given to the following three qualities:

1. All kinds of antibodies to sperm-specific membrane antigens (i.e. antibodies binding primarily to head, tail or tail-tip) should be detected with a relatively high sensitivity.
2. Antibodies of all three main immunoglobulin classes should be determined in order not to miss the IgA antibodies in seminal plasma.
3. The antibody levels should be determined in a quantitative or semiquantitative way, for instance by titration.

At present the tray agglutination test (TAT) and the gelatin agglutination test (GAT) seem to fulfil these criteria. Independent testing by both techniques using different sperm samples increases the credibility of results. New techniques such as the MAR-test where the antisperm antibodies are detected directly on spermatozoa or radioimmunoassays with whole cells or purified antigens will soon be available for routine purposes and some of them may replace conventional tests.

Occurrence of Antibodies to Sperm Membrane Antigens and their Clinical Significance

Incidence

Since the first study on the incidence of sperm agglutinins in sera from fertile and infertile males by Rümke and Hellinga (1959), a series of similar studies (Table 9.3) have been carried out. The results clearly indicate that sperm agglutinins in titres of 32 or above are very rarely found in sera from fertile males, whereas they are observed in 3%–13% of the sera from infertile males. Most investigations have included males from couples with unexplained as well as explained infertility. Therefore, these studies cannot tell whether the presence of antibodies is the cause of infertility or merely reflects other abnormalities in the genital tract which can cause infertility by themselves. This latter possibility was already evident from the study by Rümke and Hellinga (1959), as a particularly high incidence of sperm agglutinins was recorded among the patients with occlusions of the efferent ducts.

Antibodies and Infertility

To find evidence for a direct fertility-reducing effect of antibodies, it is necessary to

Table 9.3. Sperm-agglutinating antibodies in sera of men from fertile and infertile couples

Individuals tested			Result of antibody testing		
—Investigator	Technique	Number investigated	Negative	Weakly positive	Strongly positive
Fertile men (husbands of pregnant women)					
Rümke and Hellinga (1959)	GAT	416	412 (99%)	4 (1%)	0[a] (0%)
Fjällbrant (1968a)	GAT	500	487 (97.4%)	9 (1.8%)	4[b] (0.8%)
Hargreave et al. (1980)	TAT	100	90 (90%)	10 (10%)	0[b] (0%)
Men from infertile couples (explained + unexplained infertility)					
Rümke and Hellinga (1959)	GAT	1913	1836 (96%)	15 (0.8%)	62[a] (3.2%)
Sobbe et al. (1966)	GAT	150	?	?	9[a] (6%)
Fjällbrant (1968a)	GAT	400	373 (93.3%)	10 (2.5%)	17[b] (4.3%)
Husted (1975a)	GAT	657	613 (93.3%)	20 (3.0%)	24[b] (3.7%)
Shulman (1978)	GAT	381	348 (91.3%)	33 (8.7%)	
Hendry et al. (1978)	GAT	591	524 (88.7%)	17 (2.9%)	50[a] (8.5%)
Hargreave et al. (1980)	TAT	300	226 (75.3%)	35 (11.7%)	39 (13%)
Men from couples with unexplained infertility					
Friberg (1974d)	TAT	134	103 (76.9%)	9 (6.7%)	22[b] (16.4%)
Husted (1975a)	GAT	165	145 (87.9%)	4 (2.4%)	16[b] (9.7%)

[a] titre ≥ 32
[b] titre ≥ 64

GAT = gelatin agglutination test
TAT = tray agglutination test

study males from couples with so-called unexplained infertility (defined as a condition where both partners appear normal, not taking the results of postcoital tests into consideration). As seen in Table 9.3, the two investigations of such patients have revealed a relatively high incidence of antibodies—as one might expect if they have an anti-fertility effect. In the study of Husted (1975a) the 165 patients with unexplained infertility formed a sub-group of the 657 men from infertile couples. Thus, while nearly 10% of the men from couples with unexplained infertility showed high titres of sperm agglutinins, this was the case for only 1.6% of those where a likely explanation for the infertility was found.

Even more persuasive evidence for a fertility-reducing effect of sperm agglutinins was presented by Rümke et al. (1974b) from a study on the fertility of men with sperm agglutinins in serum over an observation period of up to 16 years: among 137 normospermic men there was a highly significant inverse relationship between antibody levels and fertility rates. Thus, 15 (48%) out of 31 patients with titres of 16 or less succeeded in inducing pregnancy, whereas only 15 (16%) of 95 with titres between 32 and 512, and none of 11 men with titres of 1024 or more, did so.

Significance of Antibodies in the Seminal Plasma

Obviously, an anti-fertility effect of antibodies to sperm membrane antigens cannot be ascribed to the circulating antibodies, but must be due to antibodies in seminal plasma. The level of detectable anti-sperm antibodies in seminal plasma will be determined by the transudation from serum of mainly IgG antibodies, the active

secretion of IgA antibodies in semen, and the amount bound to the spermatozoa (and thus absorbed from the seminal plasma). Nevertheless, in men from infertile couples there is generally a relatively close relationship between the titres in serum and seminal plasma, the latter being in most cases at least two titre steps lower than the former, and, almost without exception, sperm agglutinins are only found in seminal plasma if also present in serum (Friberg 1974e; Rümke 1974b; Husted and Hjort 1975; Jager et al. 1978). However, in individual patients there may be wide variations and antibodies may be undetectable in seminal plasma in spite of relatively high titres in serum. This is most clearly seen in vasectomised men, where sperm agglutinins rarely occur in seminal plasma and only when there are very high titres in the serum, therefore apparently representing only transudated immunoglobulins (Linnet and Hjort 1977; Hellema et al. 1979). However, after vasovasostomy anti-sperm antibodies often appear in seminal plasma (Hellema 1978; Linnet and Fogh-Andersen 1979).

The clinical significance of various titre levels of anti-sperm antibodies in seminal plasma has so far not been fully studied. In a series of 26 men with sperm agglutinins in serum three out of five men with titres in seminal plasma of four or less induced pregnancies, whereas this occurred for only one of six men with titres of 16 in seminal plasma and for none of 15 patients with higher titres (Husted and Hjort 1975). Rümke (1980) reported that he has never seen a fertile man with a titre of sperm agglutinins in seminal plasma higher than 16[a]. Thus, it seems that even low levels of anti-sperm antibodies in seminal plasma may be closely associated with infertility or reduced fertility.

This point of view was further stressed when we recently studied the fertility of 20 previously vasectomised men, who had undergone vasovasostomy for restoration of fertility. During an observation period of 19–33 months, among seven men with sperm agglutinins in seminal plasma, only one (with a titre of 4) succeeded in inducing pregnancy, whereas 11 of 13 men without detectable antibodies in seminal plasma had done so. Since the two groups had nearly identical sperm counts, the difference in fertility rate could apparently be due only to the presence of anti-sperm antibodies in the one group, although most of the patients had titres in seminal plasma of only 4 or 16 (Linnet et al. 1981).

Association of Antibodies with Genital Tract Disorders

The occurrence of antibodies to sperm membrane antigens in patient groups other than men from couples with unexplained infertility and vasectomised men, has attracted relatively little interest. Generally speaking, an increased incidence of antibodies has been recorded in men with complete or incomplete occlusions in the genital tract, e.g. caused by bilateral absence of the vasa or epididymal obstruction (Rümke and Hellinga 1959; Schoenfeld et al. 1978). Infections other than those which cause occlusion also seem to play a role. In a study by Fjällbrant and Obrant (1968) 16 of 22 men with sperm agglutinins and concomitant pathological conditions were found to have prostatovesiculitis; Fattah et al. (1980) have recently confirmed this by finding sperm agglutinins in eight of nine patients with prostatitis. In this study an increased incidence of antibodies was also recorded in patients with urinary schistosomiasis. A decrease in anti-sperm antibody titre with subsequent

[a] There appear to be exceptions to this rule, as two laboratories have recently reported one case each.

conception has been observed in cases of chronic prostatitis (Fjällbrant and Nilsson 1977). Finally, patients with bilateral varicocele apparently reveal a slightly increased occurrence of sperm agglutinins.

Effects of Antibodies to Sperm Membrane Antigens on the Reproductive Processes

The preceding discussion has led to two conclusions which may at first seem incompatible; viz., that spermatogenesis and sperm transport may continue unaffected in the presence of anti-sperm antibodies, but that, when present in seminal plasma, the antibodies seem nevertheless to cause infertility or reduced fertility. However, the explanation is simple: The antibodies to sperm membrane antigens do not execute their effects in the male, but in the ejaculate and in the female. There are at least four mechanisms to be considered:

1. *Auto-agglutination in the ejaculate* may take place, so that most of the spermatozoa are entrapped in agglutinates, leaving few cells free to migrate into cervical mucus. Spontaneous agglutination can most easily be observed by microscopic examination of semen (Wilson 1954), but in certain cases it may also be seen macroscopically by observing settling of the spermatozoa at the bottom of the test tube after 1–2 h (Fjällbrant 1965). The phenomenon will only occur if the sperm density in the ejaculate exceeds about 10 million/ml, and it should only be recorded if 15%–20% of the spermatozoa are seen by microscopic examination of semen to be caught in pure agglutinates (to be distinguished from clumping around leucocytes and epithelial cells). Using such strict criteria a very close association between auto-agglutination and the presence of auto-antibodies is found (Husted 1975a). Friberg (1980) has recently observed that men with head-to-head agglutinins in serum showed no or only weak auto-agglutination in the ejaculate, whereas about 40% of the patients with tail-to-tail agglutinins in serum revealed strong or complete spontaneous agglutination.

2. *Impaired penetration into cervical mucus* by spermatozoa covered with antibodies to membrane components was also first observed by Wilson (1954). Obviously the postcoital test will give the clinically relevant information about sperm penetration into cervical mucus, but in cases with poor postcoital tests the cause of the impaired penetration may be analysed and quantified by means of in vitro penetration tests. The most useful clinical test is the crossed hostility test (Morgan et al. 1977; see also Chap. 16).

Two different in vitro techniques have been developed for studying sperm–cervical mucus interactions. In the capillary mucus penetration test (Kremer 1965; Fjällbrant 1968b), which forms a direct in vitro correlate of the postcoital test, the distance of sperm penetration into a capillary tube filled with cervical mucus obtained at mid-cycle is determined after incubation at 37°C for 2 or 3 h. Fjällbrant (1968b) observed a negative correlation between antibody titres in serum and the penetration of the patient's spermatozoa into normal cervical mucus. In nearly all cases with sperm agglutinins in serum at titres of 64 or more there was a very poor penetration, whereas the spermatozoa from patients with titres of 32 or less usually showed fair or normal penetration. Not unexpectedly, the results of

postcoital tests were closely correlated with the in vitro sperm penetration. Thus, this study stresses the importance of taking the titre levels of the antibodies into consideration.

Fjällbrant (1968b) also studied the penetration of spermatozoa into a drop of cervical mucus on a slide and noticed that spermatozoa from patients with high titres of sperm agglutinins or immobilising antibodies remained in the periphery of the drop. Later Kremer and Jager (1976) modified this technique by mixing the drop of semen and cervical mucus before studying the behaviour of the spermatozoa. This was named the sperm–cervical mucus contact test or SCMC test. While normal spermatozoa move freely when mixed with normal cervical mucus, spermatozoa from patients with antibodies in seminal plasma show no forward progression although they remain actively motile for a long time, i.e. they show what has been called the 'shaking phenomenon'. If more than 75% of the motile spermatozoa reveal this phenomenon, the reaction is considered positive. A similar reaction is observed if normal spermatozoa are mixed with cervical mucus from a patient with anti-sperm antibodies in the mucus. Further studies by Kremer et al. (1978) have clearly shown that IgA antibodies play a major role in this reaction although IgG antibodies may also participate (Jager et al. 1980b), and that the shaking phenomenon is closely correlated with a poor postcoital test. It is thought that spermatozoa bind to glycoprotein micelles of the cervical mucus through the Fc parts of the antibody molecules. This means that the shaking phenomenon may specifically indicate antibody-mediated reaction, but more studies are needed to find out whether non-specific factors can occasionally cause similar effects. In fact Jager et al. (1979) observed positive SCMS-tests with normal donor semen and normal cervical mucus in a few cases where the reaction was apparently not due to the presence of antibodies.

3. *Complement-mediated immobilisation* of spermatozoa covered with antibodies is another possibility, particularly if the spermatozoa succeed in overcoming the cervical mucus barrier and reach the Fallopian tube or the peritoneal cavity. Thus Landsteiner (1899) detected the anti-sperm antibodies in immunised guinea pigs by their immobilisation of spermatozoa injected into the peritoneal cavity. Low levels of complement have been detected in the cervical mucus (Price and Boettcher 1979), but as the major part of the anti-sperm antibodies from seminal plasma are usually of the IgA class (and thus not complement-fixing), the importance of sperm immobilisation is difficult to evaluate. The studies with the SCMC-test indicate that spermatozoa with antibodies from seminal plasma attached to their surface antigens may remain motile in cervical mucus for several hours.

4. *Blocking of sperm–ovum interaction* could be expected to occur if the species-specific receptors on the spermatozoa are covered by the antibodies. The demonstration of such an effect would require zona-coated human eggs, but such experiments have not yet been performed with spermatozoa from patients with anti-sperm antibodies. However, Menge and Black (1979) observed that the ability of human spermatozoa to penetrate zona-free hamster eggs was reduced by treatment with heterologous antisera, and a similar effect has been demonstrated with human sera (Dor et al. 1981; Friberg 1980). It was observed that the inhibitory effect appeared with head-to-head agglutinating sera, but apparently not with tail-to-tail agglutinating sera. Penetration into the eggs was only inhibited by dilutions of sera in which there was still strong agglutination, and since in

head-to-head agglutination much more than in tail-to-tail agglutination nearly all spermatozoa are involved in small agglutinates, the agglutination phenomenon itself might explain the findings. However, a similar inhibition has been recorded with non-agglutinating Fab fragments from heterologous antisera, suggesting that the effects observed are not simple consequences of agglutination.

Rational attempts at treatment of 'immune infertility' will have to be based on a detailed knowledge and understanding of the mechanisms whereby anti-sperm antibodies exert their anti-fertility effect; hence the importance of further research.

Who Should be Examined for Anti-Sperm Antibodies?

Until recently the demonstration of 'immune infertility' in a couple with otherwise unexplained infertility was of interest mainly for evaluating the chances of conception. However, as there are now possibilities for treating such patients, the demonstration of antibodies to sperm membrane antigens may be decisive in the choice of treatment, and testing for such antibodies should therefore be considered part of any full investigation of infertile couples where no other explanations for the infertility have been found.

As 10% of the men from couples with unexplained infertility seem to have anti-sperm antibodies that could explain infertility, it is justifiable to test this group. This is conveniently done by examining serum and subsequently testing seminal plasma from all patients with positive serum. Difficulties in obtaining fresh ejaculates of sufficiently high quality as test substrate may limit such testing programmes. In this situation testing can be confined to couples with unexplained infertility and a poor postcoital test, since the patients with a clinically significant level of anti-sperm antibodies should obviously be found in this group. Another indication is spontaneous agglutination in the ejaculate but usually these patients will belong to the group with poor postcoital tests.

As the mixed antiglobulin reaction (MAR test) seems to have been satisfactorily developed for detection of both IgG and IgA antibodies, patients with clinically significant levels of anti-sperm antibodies could possibly be most easily identified by performing the direct MAR test on fresh ejaculates as part of the routine examination of semen. More experience is still needed with this test system before it can be used as the only screening procedure. But even if it should prove as useful as it seems at present, there will still be a need for testing also for free antibodies as this is quantitatively more informative.

In vasectomised men who want to have a vasovasostomy done in order to become fertile again, testing for anti-sperm antibodies may also be helpful. In this situation the testing of seminal plasma is of little value because the antibodies do not usually appear in the seminal plasma until after operation. It seems that patients who develop agglutinins in seminal plasma after operation are those who have high titres in serum before the operation ($\geq$64). Thus, determination of the levels of sperm agglutinins in serum before vasovasostomy may be helpful in assessing the chance of conception after a technically successful operation although positive tests should not prevent any patient from having vasectomy reversal or the surgeon from doing it.

Concluding Remarks

A review on auto-immunity to sperm cannot come to an end without mentioning that immune responses to sperm may also occur in women (Isojima et al. 1972; Shulman 1978). The incidence of clinically significant levels of anti-sperm antibodies in infertile women seems to vary in different parts of the world, but is apparently significantly lower than in males—at least in the western hemisphere. However, when present in cervical mucus, antibodies to sperm membrane antigens may hinder migration of spermatozoa into cervical mucus just as effectively as antibodies originating in the male partner and located on the surface of the spermatozoa (Kremer et al. 1978). This adds a new dimension to sperm immunology, as immunisation of women may be a realistic possibility for fertility regulation (WHO 1978).

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Chapter 10

The Female Partner

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Although this book is about male infertility and this chapter confines itself to the investigation of the female, in clinical practice the emphasis is always on the management of the couple. The following guide lines are helpful in the management of any couple complaining of infertility:

1. After initial assessment of both partners which should include full explanation and reassurance to dispel any misapprehensions, an attempt should be made to reach a clinical diagnosis.
2. When a diagnosis has been reached or investigations are complete the results should then be discussed with both partners, so that they are clear about their options and about the plan of treatment.
3. At this and at later stages in treatment there should be a continuing willingness on the part of the doctor to discuss their prognosis frankly with the couple and there should be no hesitation in advising them when it is appropriate to discontinue treatment and try to accept their infertility.

Investigation of the Female

In order that a rational protocol of investigation can be evolved, it is worth considering the process of conception step by step. In this way it should become clear which investigations are valid and likely to lead to clinically useful information, while it also underlines the areas of uncertainty, where inadequate clinical tests can further confuse the picture.

The process of conception has been divided into the following steps:

1. Insemination
2. Sperm transport
3. Ovulation
4. Oocyte pick-up and transport
5. Fertilisation
6. Implantation

Each of these is discussed in turn.

Insemination

Following ejaculation, semen immediately forms a gel which is subsequently liquefied. Spermatozoa leave the seminal plasma, which is deposited in the region of the external os, and enter the cervical mucus. Only motile sperm achieve this step. This stage can be investigated clinically by obtaining a full sexual history with special emphasis on the success and frequency of intercourse, and then by the examination of several masturbated semen specimens as described in Chap. 4. The assumption from this line of investigation is that adequate numbers of spermatozoa are able to reach the external cervical os. If there is any doubt about the adequacy of intercourse from the history, or if the husband is unable to produce a masturbated specimen for any reason, then a postcoital test can be organised. The finding of spermatozoa in the cervical mucus indicates that intercourse has been successful, although valid conclusions about the quality of the ejaculate cannot be made.

Sperm Transport

Only motile spermatozoa reach the cervical mucus and are thus, even at this stage, selecting themselves, a process which continues throughout the female tract. Morphological assessment of spermatozoa found at the site of fertilisation indicates that the spermatozoa themselves, rather than the female tract, are chiefly responsible for sperm selection (Mortimer et al. 1982). It is thought that spermatozoa reach the fallopian tube well within 2 h of ejaculation and remain fertile, or at least motile, there for approximately 48 h (Hafez 1976). However, spermatozoa have been found in the tubes 5 min after artificial insemination (Settlage et al. 1973) suggesting that the female tract aids in the transport of spermatozoa to the site of fertilisation, although the competency of such sperm must be in doubt as capacitation is thought to require at least 4 h in the tract (Edwards et al. 1970). Unfortunately, there is no clinical test to show that spermatozoa have reached the site of fertilisation and current clinical tests of sperm transport are confined to the in vivo or in vitro assessment of the interaction between spermatozoa and cervical mucus. While such tests correlate reasonably well with subsequent pregnancy rates, many patients have consistently poor tests and yet achieve pregnancies, while other patients with good tests and no other apparent abnormality remain infertile. The most common method of assessing cervical function is the postcoital test. A small amount of mid-cycle cervical mucus is aspirated 6–12 h after intercourse and examined under the high power lens for the presence of motile sperm. The test is graded according to the number of progressively motile sperm seen in each high power field (Moghissi 1976). It is often assumed that if sperm are present in sufficient numbers to negotiate the cervical barrier they are likely to reach the site of fertilisation. However, this is not always the case (Templeton and Mortimer 1980); conversely, spermatozoa have been found in the pouch of Douglas fluid and the fimbrated ends of the tubes in patients with poor or even negative postcoital tests (Asch 1976, 1978; Templeton and Mortimer 1980).

The chief problem with the postcoital test is poor timing in the cycle. In some patients the cervical mucus production may be normal but last only for a period of 2 days and unless the timing of the test is precise, a poor result can be consistently obtained and the infertility attributed to the cervical factor. The only way to be

certain that mid-cycle mucus has been obtained is by serial sampling of the cervical mucus for 4 or 5 days around mid-cycle. Ideally the timing in the cycle should be confirmed by serial measurements of plasma LH so that the mid-cycle peak can be identified. Where this has been done (Matthews et al. 1980) the incidence of poor penetration in an in vitro system (Kremer's test) is exceedingly small. Recent sources quote the incidence of cervical problems in infertile patients at around 5% (Speroff et al. 1978). Nevertheless, there can be no doubt that where extensive cervical cautery or conisation has taken place the mucus production may be severely affected and infertility may result.

Ovulation

In general terms women who are menstruating regularly are also ovulating regularly. The further they are from the extremes of reproductive age, i.e. from the menarche and from the menopause, the more likely this is to be true (Metcalf 1979, Mecalf and Mackenzie, 1980). Following ovulation the corpus luteum secretes progesterone and it is the measurement of this sex steroid, or of one of its biological effects, that provides the clinical evidence that ovulation is likely to have occurred. In practice most centres have facilities for the measurement of plasma progesterone or urinary pregnanediol—unquestionably the best indicators of ovulation. The best known biological effect of progesterone is the biphasic temperature response in which the basal body temperature rises following ovulation and is sustained until 1–2 days prior to menstruation. Unfortunately, such temperature charts cannot always be interpreted with certainty (Johansson et al. 1972), particularly in infertile patients (Lenton et al. 1977) and there is no doubt that their completion does cause some patients considerable inconvenience and even distress.

The main function of progesterone is the preparation of the endometrium for implantation and the histological recognition of secretory endometrium at endometrial biopsy in the second half of the cycle has in the past been a widely used indication that ovulation has occurred. The endometrial biopsy is carried out less commonly now but it can easily be done if the patient is undergoing another investigation, e.g. hysterosalpingography or laparoscopy during the second half of the cycle. If the patient is being subjected to laparoscopy the corpus luteum itself can be visualised, usually without difficulty.

In a small proportion of patients, ovulation occurs but the development of the corpus luteum is inadequate and progesterone levels are lower in the luteal phase than expected (Jones and Madrigal-Castro 1970). The classical diagnosis of inadequate luteal phase was originally based on endometrial biopsy dating (Noyes et al. 1950), the condition being thought to exist where the endometrial biopsy was 2 days behind the menstrual dating (Noyes 1956). However, the condition is difficult to diagnose and infertility should not be attributed to this cause until all other investigations have been completed. A persistently inadequate luteal phase is probably not a frequent cause of infertility and its successful management, where it does exist, is a matter of controversy.

Similarly the 'trapped oocyte syndrome' (Marik and Hulka 1978; Koninckz et al. 1978) is said to be a cause of infertility in a small group of patients. In this condition the oocyte is said to be retained inside the follicle which subsequently becomes

luteinised. It is easy to see that if the ovaries are surrounded by adhesions then the oocyte could be trapped in these while the corpus luteum develops normally, but whether such a condition exists in an ovary that looks normal on inspection is debatable.

Oocyte Pick-up and Transport

Following ovulation the oocyte surrounded by its cumulus mass is discharged onto the surface of the follicle and if the fimbrated end of the fallopian tube is adjacent at that time, the egg is wafted into the infundibulum by the cilia. This action continues until the egg reaches the ampullary–isthmic junction where it is arrested. Egg transport to this point is rapid (within a few hours) and is achieved mainly by the abovarian ciliary action of the tubal endothelial cells; in addition, segmental contractions of the tubal musculature facilitate transport. Although, the cilia play a key role in egg transport, pregnancies still occur in patients with defective cilial action, e.g. in Kartagener's syndrome.

Tests of tubal function have until recently been dependent on the demonstration of tubal patency either by air or carbon dioxide insufflation or by hysterosalpingography after a trans-cervical injection of radio-opaque dye. Tubal insufflation (Rubin's test) will confirm that at least one tube is patent, but can give no information about peritubal adhesions, or ovarian problems. Similarly the hysterosalpingogram will confirm that the uterine cavity has a normal outline and that tubal patency with an apparently normal tubal structure exists. Occasionally, it is also possible to discern the existence of pelvic adhesions from the pattern of dye excretion from the end of the tube, but there is an appreciable incidence of false positive and false negative results following hysterosalpingography (Corson 1979). For this reason laparoscopy, which allows direct visualisation of the pelvic organs, is being increasingly used to assess the genital tract in infertile women. Laparoscopy in the hands of an experienced investigator will detect minor pelvic abnormalities which are known to interfere with oocyte pick-up, such as peritubal adhesions or ovarian endometriosis. These abnormalities could not be detected by a test that is solely aimed at confirming tubal patency. Laparoscopy is of course a more invasive procedure, requiring a general anaesthetic, and many centres retain the use of hysterosalpingography, only resorting to laparoscopy if the infertility remains undiagnosed for long.

Fertilisation

Unless pregnancy occurs there is no indication that fertilisation has been achieved. Even where sperm meet a mature oocyte in the tube under appropriate conditions, there is no way of assessing clinically whether the egg is fertilisable or whether the sperm have the capacity to fertilise the egg. Thus, while it is likely that problems do arise in this area, we are unable to investigate it in vivo. However, the finding by Yanagimachi et al. (1976) that zona-free-hamster eggs can be penetrated by human sperm has allowed the development of clinical test of the fertilising ability of human sperm in an in vitro system. Several authors have assessed the clinical value of this test (Rogers et al. 1979; Barros et al. 1979; Aitken, Chap. 5), and from these studies it is evident that apparently normal spermatozoa from infertile men can

have decreased fertilising ability when compared with spermatozoa from normal, fertile controls. In fact, in a small group of individuals where routine investigations are entirely normal, the continuing infertility can be attributed to defective sperm fertilising ability. There is no doubt that continuing use of this in vitro system will allow the determination of those features in the routine seminal analysis that are relevant as far as fertilisation of the oocyte is concerned and will provide further insights into this important aspect of sperm function.

Implantation

After fertilisation the developing zygote is transported down the isthmic end of the fallopian tube into the uterine cavity where after several days it achieves implantation. This delicate process is governed by a precise but as yet undefined neural and endocrine control (Croxatto and Ortiz 1975) and its complexity is underlined by the fact that in embryo transfer work the biggest single problem is successful uterine implantation of the blastocyst (Edwards et al. 1980). There can be no doubt that there is an appreciable loss of embryos in and around the time of implantation (Hertig 1975; Short 1979), but whether this is due mainly to abnormalities in the developing zygote or whether implantation loss is a recurrent feature in the human and therefore a cause of infertility, is uncertain. Unfortunately, there is no unequivocal clinical test to indicate that the process of conception has failed because of failure of implantation. The β subunit of hCG becomes detectable in blood within a few hours of implantation but it is still uncertain whether it is produced by the developing zygote prior to implantation. One study has reported early pregnancy levels of hCG in the late luteal phase in five patients with unexplained infertility (McBain and Pepperell 1980), but in a small group of similar patients, we could not confirm this (Corker, Kerr and Templeton, unpublished data).

Basic Investigations

From the above description of the processes involved in conception, it should be clear which tests are clinically justifiable in that they provide a rationale for treatment or have some prognostic value. However, there are still many areas which cannot be investigated in practical, clinical terms and thus it is hardly surprising that in 25% of couples attending an infertility clinic, no definite cause for the infertility can be identified (Templeton and Penney 1982). The basic assessment of the female which should be carried out during the investigation of the couple together should ideally follow the following guidelines.

History and Examination

At the initial interview, for which at least half an hour should be set aside, a thorough history is taken. The duration of infertility is defined and an assessment made of the time the couple have spent together and hence the possibility of exposure to pregnancy. The sexual history should pay particular attention to the success and frequency of intravaginal ejaculation, and occasionally it is helpful to

interview the husband and wife first together and then separately. Sometimes it will be found that their perception of a particular problem differs considerably and that one partner has attempted to belittle an area of concern. This opportunity of separate interviews can also be used to enquire whether there is a history of previous pregnancies of which the partner has no knowledge. In the woman's case this is particularly important as tubal damage following post-abortal or post-natal infection is a frequent cause of secondary infertility. The menstrual history should include enquiry about the frequency and duration of menstrual bleeding and any episodes of amenorrhoea should be noted. Intermenstrual and postcoital bleeding should be excluded, and if there is dysmenorrhoea it should be ascertained whether this is premenstrual and hence suggestive of endometriosis or whether it is spasmodic and usually taken to indicate that ovulation has occurred. A full medical history should include an enquiry about previous pelvic infection or abdominal conditions, such as appendicitis or peritonitis, that could lead to salpingitis and possible tubal damage. In addition any previous gynaecological procedure should be carefully documented and particular attention paid to cervical cautery, uterine sling and ovarian wedge resection, all of which can be associated with subsequent subfertility. A drug history is taken although it is uncertain whether any drug that does not directly affect ovulation or menstruation is associated with female subfertility.

A family history should be taken, particular note being made of a history of TB, diabetes or thyroid disease.

A contraceptive history is essential so that the period of infertility can be assessed and any complications associated with the method of contraception can be documented. The previous use of oral contraception is almost universal in patients attending an infertility clinic and they are often anxious to know whether this has contributed to their problem. The best evidence that the oral contraceptive pill is not implicated comes from the epidemiological work of Vessey et al. (1978), who showed that in patients stopping the pill with the intention of becoming pregnant, there was a slight delay of a few months only before their return to the fertility pattern that would be expected of a group of women who had never used contraception and were trying to conceive. Nevertheless, secondary amenorrhoea following the use of oral contraceptives will undoubtedly cause infertility and up to 40% of patients who complain of amenorrhoea have previously used oral contraception (Jacobs et al. 1977). It is not yet certain whether the oral contraceptive is an aetiological factor or whether it can merely mask a developing amenorrhoea due to another cause. Some reports (e.g. Jacobs et al. 1977) question the existence of post-pill amenorrhoea as a disease entity, and suggest that it should be taken as seriously as amenorrhoea at any other time. If the duration is more than 6 months, then full endocrinological assessment, including skull x-ray, and FSH, oestradiol and prolactin measurements, is indicated.

Full examination of the woman should then be undertaken. Her general physical condition should be noted with particular reference to examination of the thyroid gland, to her breasts and to the exclusion of galactorrhoea. Her abdominal examination should be thorough and any scars that are not explained by her history should be noted.

Pelvic examination should be carried out. A smear is only necessary if one has not been done in the previous 2 years. On speculum examination the appearance of the cervix should be carefully noted and if the patient is at mid-cycle the appearances of the os and the cervical mucus can be described. The presence of any

vaginal discharge or inflammation of the vaginal epithelium should also be recorded and a high vaginal swab should be sent for culture and sensitivity if infection is suspected; common vaginal infections, viz trichomonas and monilia are not thought to be associated with infertility. The size, shape and position of the uterus should be estimated by bimanual palpation, and an assessment should be made of its mobility and whether its movement causes the patient discomfort. The adnexal structures should then be palpated and a careful note made of any swellings or discomfort. The ovaries are not usually palpable, except in thin or very relaxed women.

If the patient is reluctant to be examined or if she cannot relax sufficiently to permit speculum examination or bimanual palpation, then no attempt should be made to pursue the issue at the initial assessment. Equally, however, the matter should not be left in the air and the reasons for her reluctance should be gently explored.

Having carried out this initial history and examination it is usually appropriate to carry out an examination of the male partner and arrange for a series of seminal analyses as described in Chap. 3. After the results of the seminal analyses are available it is our practice to see the couple again at the clinic. They are then given the results and where appropriate the next step in the basic investigations is explained. These are directed at the female and consist of a test to confirm ovulation, and a test of tubal function.

Ovulation

As was stated above, patients in their 20s or 30s who are menstruating regularly are usually ovulating regularly. This can be confirmed by drawing blood for assay of plasma progesterone in the second half of the menstrual cycle, ideally between days 19 and 23 of a 28-day cycle. If the progesterone level is 20 nmol/l or more, then there is a corpus luteum and hence presumptive evidence that ovulation has occurred. Ideally this should be done in two consecutive cycles. If a progesterone assay is not available then urinary pregnanediol will give the same information. The minimum level that confirms the existence of a corpus luteum is 2.0 mg pregnanediol in a 24-h urine collection. If the patient is to be admitted to hospital in the luteal phase for a test of tubal function then an endometrial biopsy can easily be taken with a Sharman curette and histological examination will provide evidence of ovulation. It is important not to carry out full curettage otherwise the menstrual cycle may be disturbed making it impossible to date the biopsy.

In a proportion of patients basal body temperature charts will give possible evidence that ovulation has occurred, but the tests described above are far more precise. Examination of the cervical mucus, which increases in quantity and changes in character in response to the mid-cycle oestrogen surge, will also not confirm that ovulation has occurred in that cycle.

If the cycle is short, around 21 days, then it is usually the follicular phase that is shortened, the luteal phase being nearer the usual length of 12–14 days. In these circumstances the blood or urinary test should be arranged for around day 16 or 17. If the cycle is irregular or longer than usual, 35 or 42 days, then it may be necessary to arrange for a series of blood tests at weekly intervals from the 21st day of the cycle until menstruation occurs. Sometimes this can be difficult to organise but it is important to know whether patients with irregular cycles are ovulating as subsequent treatment will depend very much on this evidence. If the patient is

amenorrhoeic or severly oligomenorrhoeic, then it is appropriate to carry out a full endocrine assessment of the hypothalamic–pituitary–ovarian axis (Baird 1979).

Test of Tubal Function

Tubal insufflation has now been replaced by hysterosalpingography or laparoscopy. Hysterosalpingography has the advantage that it gives an image of the uterine cavity and allows some assessment of tubal patency and mobility. It can, where conditions are appropriate, be carried out without anaesthesia although many women find the procedure uncomfortable. Unfortunately, when the results are subsequently compared with laparoscopy there are many misleading assumptions (Corson 1979). For this reason there is an increasing tendency to use laparoscopy earlier in investigations, and several clinics, including our own, now use it for the primary assessment of the female tract (Templeton and Kerr 1977). The procedure is generally carried out under general anaesthesia and has an exceedingly low morbidity in experienced hands (Working Party, RCOG 1979). It can provide information about peritubal adhesions which, although minor, might interfere with oocyte pick-up, and about ovarian or pelvic endometriosis or other ovarian abnormalities; at the same time, the feasibility of corrective surgery can be assessed. Tubal patency is confirmed under laparoscopic vision by the transcervical injection of methylene blue dye, and where there is tubal blockage the exact level of the blockage can be determined.

Laparoscopy can be an out-patient procedure, although some patients experience discomfort afterwards and wish to spend the post-operative night in hospital. It can be carried out in the follicular phase when there is no danger of disturbing an implanted zygote and less possibility of obtaining an apparent but spurious block at the isthmic end of the fallopian tubes. If it is carried out in the luteal phase then the opportunity can be used to take an endometrial biopsy and also to inspect the ovaries for the existence of a normal corpus luteum. The uterine shape can be inspected at laparoscopy; but of course the investigation will give no information about the contour of the uterine cavity and the existence of any minor structural abnormalities. For this reason some sources have suggested combined laparoscopy and hysterosalpingography, while more recently combined laparoscopy and hysteroscopy has been advocated (Cumming and Taylor 1980). However, in the author's experience minor uterine abnormalities are rarely associated with infertility, presenting more often as a cause of recurrent abortion.

Causes of Infertility

Using this schedule of (a) history and examination, (b) seminal analysis, (c) test of ovulation, and (d) diagnostic laparoscopy, we investigated 400 consecutive couples attending the infertility clinic, Royal Infirmary, Edinburgh. The findings from this study are briefly summarised in Table. 10.1. It can be seen that in 22% of couples the problem could be attributed chiefly to the male. In 27% of cases the problem could be attributed chiefly to the female either because of anovulation and infrequent ovulation (8.5%) or tubal problems (18.8%). However, it is worth noticing that almost 24% of patients became pregnant either during or just after

Table 10.1. Causes of infertility in 400 consecutive new couples attending the Infertility Clinic, Royal Infirmary, Edinburgh

	Primary infertility (%)	Secondary infertility (%)	Total (%)
	n = 292	n = 108	n = 400
Azoospermia	11.3	3.7	9.2
Other insemination problems	15.1	7.4	13.0
Ovulation problems	9.6	5.5	8.5
Tubal problems	17.5	22.2	18.8
Pregnancy during or just after investigations	22.9	37.9	27.0
Results of investigations normal; no pregnancy	23.6	23.2	23.5

investigations before any treatment could be instituted; this should be borne in mind, especially when assessing new forms of treatment.

It is likely that our results do not reflect the actual rates of the various causes of infertility in the community, but are more a reflection of the clinic's particular interests and referral sources. For example, most clinics would show a higher rate of ovulation problems than we have recorded in our clinic and this could be attributed to our easy access to an endocrine clinic that has a reputation for its treatment of such disorders. However it is humbling to note that in 24% of couples no identifiable cause could be found for the continuing infertility. These couples, where the aetiology of the infertility is unknown and who are commonly referred to as 'normal infertiles', are a particularly difficult group of patients to manage and provide the justification for our continuing search for more critical and functional methods of investigating the infertile couple.

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PART II

CLINICAL PROBLEMS

Chapter 11

Varicocele

S. Nilsson

Introduction

But when the disease has spread also over the testicle and its cord the testicle sinks a little lower, and becomes smaller than its fellow, in as much as its nutrition has became defective.

This statement concerning varicocele was made by Amelius Cornelius Cellsus who lived from 42 B.C. to 37 A.D. and implies that the varicocele can give rise to atrophy of the testicle. The association between varicocele and subfertility became a matter of interest when Wilhelm (1937) and Hammen (1944) found that varicocele could influence spermatogenesis and when Tulloch (1952) reported the restoration of fertility to an azoospermic man. We still do not know the mechanisms by which a varicocele affects testicular function nor how often this occurs in the male population. Several theories, including increased scrotal temperature, retrograde flow of substances from the adrenal gland down the spermatic vein, and hypoxia of the germinal epithelium, are discussed in the literature. It is also a common belief that varicocele is associated with specific abnormalities of spermatozoal morphology. A marked improvement in semen quality and high rates of pregnancy after testicular vein ligation in many studies have been regarded as evidence in support of the role of varicocele in infertility. Recently, however, the efficacy of varicocelectomy in subfertile patients has been questioned.

The incidence of varicocele in the general population has been reported to be as high as 22.6% (Uehling 1968) but is probably 10%–15% (Clarke 1966). Many patients with varicocele have normal fertility. Hirsch and Pryor (1981) found varicoceles in 26.3% of 190 men with proven fertility presenting for vasectomy and they also found varicoceles in 23.8% of 240 male partners of infertile marriages. The high incidence of varicocele in infertile men might reflect the fact that patients with a known varicocele are frequently referred to urologists with a particular interest in male infertility problems.

Anatomy

Varicocele occurs as a result of incompetent internal spermatic vein valves, with reflux of venous blood into the pampiniform plexus. Incompetence of these valves

is much more common on the left side than on the right, where the internal spermatic vein usually drains obliquely into the vena cava. It has been found that the anatomical conditions allowing retrograde flow down the left testicular vein exist in approximately 50% of cases (Ahlberg et al. 1968); this figure is greater than the incidence of varicocele, indicating that retrograde flow may occur without producing overt signs of varicocele. Varicoceles are unilateral and left-sided in 80%–98% of cases. Although unilateral right-sided varicoceles are rare (up to 2%), bilateral varicocele has been reported in up to 20% of cases. In 50% of such cases the right internal spermatic vein entered the right renal veins as demonstrated by internal spermatic venography. Ahlberg et al. (1968) found that the right internal spermatic vein entered the right renal vein in 10% of autopsy specimens. Bilateral varicocele can also result from crossed circulation from the left to the right side in cases of left-sided valvular incompetence. Crossed circulation between the left and the right side at the level of the pampiniform plexus has been confirmed by Brown et al. (1967) and Etriby et al. (1975) using venography methods. In the studies by Etriby et al. (1975) the anastomosis of the internal spermatic vein with the ipsilateral deep pelvic veins was seen to be at the highest level, just above the internal spermatic ring.

To produce a bilateral depression of spermatogenesis the 'toxicity' has to reach both testes. The demonstration of collaterals connecting the venous systems of the two testes has provided the necessary link in this theory.

Pathophysiology

Temperature

The mechanism by which a varicocele depresses male fertility remains obscure. It is well documented that the testes are extremely sensitive to increases in temperature but the question of whether a varicocele produces higher testicular temperature has met with conflicting reports.

Under normal conditions an elaborate mechanism is responsible for maintaining the temperature of the testes. The testes are situated outside the body cavity in the scrotum, where the muscular components of the scrotum and testes play a particular role in temperature regulation. Contraction of the cremasteric muscle and the dartos bring the testes closer to the body, thereby increasing testicular temperature and, conversely, relaxation of the muscular elements removes the testes from the body surface increasing the heat-radiating area and lowering the temperature of the testes.

Hanley and Harrison (1962) reported that the normal scrotal temperature was approximately 2.5° C lower than the rectal temperature and that a large varicocele reduced the temperature differential to as little as 0.1° C. Hanley (1966) found that removal of a moderately large varicocele generally lowered the intrascrotal temperature by about 1° C; he concluded that: 'in all cases so far where a demonstrable change in temperature differential has been achieved, provided that the testicles are reasonably well developed, there has also been some improvement in fertility'. However, Tessler and Krahn (1966) were unable to demonstrate that a

varicocele produced any disturbance in testicular temperature regulation and the average testicular temperature of 34.0° C and 34.1° C were recorded from the left and the right testis, respectively. The average testicular temperature in 14 patients with left varicocele was found to vary less than 0.2° C from that of control subjects. Stephenson and O'Shaughnessy (1968) measured the temperature of the scrotal septum using a thermistor probe. They found the average intrascrotal temperature to be 0.6° C less than that of the rectum in control patients while the corresponding difference in five patients with varicocele amounted to 0.9° C.

Following operation on left-sided varicoceles, Agger (1971b) found that the average temperatures of the septum and the left testis fell by 0.3° C and 0.2° C respectively, whereas the rectal and right testicular temperatures remained identical.

Thermography has been found valuable in the diagnosis of varicose veins and venous insufficiency (see Chap. 2). The method has been applied in infertile men for diagnosis and in order to gain information on the thermoregulatory mechanisms of the scrotum. Koromano et al. (1970) did thermographic studies on twenty-four 20-year-old servicemen. In the ten normal subjects the temperature of the scrotal surface was almost equal to or slightly lower than that of the thigh. In the 14 subjects with varicocele the surface temperature of the scrotal skin covering the left testis or of the left inguinal area was higher than that of the rest of the scrotum. The warm area, however, differed in location and extent. In their study a clear relationship was not always seen between the extent of the varicocele as determined by palpation, the warmer area of the thermograph and increased temperature over the testes. The surface temperature was not always higher even when the varicosities were located close to the scrotal skin.

Lewis and Harrison (1979) reported that in 30 infertile patients without clincial evidence of varicocele there was a temperature difference between the two sides of the scrotum; they stated that this relative difference was more important than the absolute scrotal temperature. They found unilateral increase in temperature in seven of 14 patients with non-palpable varicoceles and three of these seven patients also presented seminal stress patterns.

The bilateral effect of a unilateral varicocele has been emphasised by several investigators. If the difference in temperature is accepted as the diagnostic criterion for identifying a varicocele it is difficult to diagnose bilateral varicoceles. The lack of specificity of scrotal thermography has been pointed out by Comhaire et al. (1976). In their comparison of scrotal thermography with selected spermatic venography they found that only one of five patients had normal thermograms and reflux. Positive thermograms were found in patients without reflux. These findings indicate that scrotal thermography is doubtful adjunct in the diagnosis of varicocele.

Results of thermographs and of seminal analysis were correlated by Gasser et al. (1973) in 37 men with left-sided varicocele and 23 control subjects. The thermographs in the controls were characterised by a symmetry of temperature over the scrotal area with an average temperature reduction of 2° C over the scrotum compared to the medial upper thigh skin. In all cases of varicocele, however, there was a temperature differential between the two sides of the scrotum. The area of the varicocele was either warmer or equal in temperature compared to the upper thigh skin and the suprapubic region. After operation there was both an increased number of patients with lowered scrotal temperature and of patients with improved sperm motility.

Although retrograde blood flow is characteristic of varicoceles, not all men with a varicocele have poor semen quality. It is possible that variation in distribution of the warmer areas is responsible for the variance in effect between individual cases. If the testis itself is not reached by the retrograde blood flow, spermatogenesis may be unaffected. The extent of the venous reflux as well as the efficiency of venous collaterals carrying it off before it reaches the testis may influence the pathogenesis of varicocele.

The complex arrangement of the pampiniform plexus around the testicular artery was suggested by Claude Bernard to form the anatomical basis for a countercurrent exchange; the cool blood returning in the venous network can be expected to reduce the temperature of the blood flowing to the testes, which consequently will lower their environment temperature. It is likely that in men this mechanism of countercurrent exchange contributes to the temperature control of the testes; the consequences of interruption of the pampiniform plexus during varicocelectomy must therefore be kept in mind.

Adrenal Metabolites

MacLeod (1965, 1969) summed up the effects of antispermatogenic compounds on spermatogenesis in men and reported a depression in sperm count, leading to azoospermia within 60–90 days. A severe inhibition of sperm motility accompanied the decline in sperm count as well as an increased incidence of morphological aberrations, with tapering sperm being the most common. A similar seminal picture was found in many infertile men including those with varicocele, which led MacLeod (1965) to propose that an abnormal chemical environment of the testes may be the causative factor in the depressed spermatogenesis associated with varicocele. Because of the entrance of the suprarenal vein into the renal vein and the retrograde passage of blood from the renal vein into the testicular vein, chemical products arising in either the kidney or the adrenal glands may pass retrogradely into the testes and so exert a depressive effect on spermatogenesis. Anatomical studies have shown, however that in only a few cases are the relative positions of the suprarenal vein and testicular vein sufficiently close to account for the possibility of venous effluent from the adrenal glands reaching the testes. Generally, the testicular vein enters the renal vein lateral to the entrance of the suprarenal vein which reduces the likelihood of adrenal blood refluxing towards the testis.

In order to test the hypothesis that factors of renal or adrenal origin may be responsible for the seminal abnormalities associated with varicocele, Lindholmer et al. (1973) studied the possibility of high concentrations of renin in the left testicular vein. They were unable to show that varicocele caused a dysfunction in the testes or epididymis by raising the concentration of renin in the testicular vein. Comhaire and Vermeulen (1974) investigated the levels of catecholamines originating in the adrenal medulla in the left testicular veins of men with varicocele and of control subjects; no absolute difference existed between the catecholamine concentrations in the controls and the patients with varicocele. Several authors (Koumans et al. 1969; Agger 1971a; Lindholmer et al. 1973; Comhaire and Vermeulen 1974) have reported a generally lower concentration of cortisol in the testicular vein compared to peripheral plasma.

Prostaglandins

It has not been proved that reflux of toxic adrenal substances down the spermatic vein into the testicular system has resulted in abnormal spermatogenesis. However, elevated prostaglandin levels (PGF2α) in the spermatic vein were found in 40% of patients with varicoceles by Desai (1978) and Cohen (1979). PGF2α has previously been shown to reduce sperm motility when the testis is exposed to increased levels. Increased levels of spermatic vein serotonin were found in 27 of 40 patients with palpable varicoceles (Caldamone et al. 1980). However, no significant difference was found between the positive and negative groups of patients with regard to semen volume, sperm density and percentage of immature and tapered forms. In patients with elevated spermatic vein serotonin levels, the mean sperm motility was reduced. Serotonin is capable of inhibiting androgen synthesis in vitro and is thought to be inactivated for the development and maintenance of spermatogenesis. Increased excretion of serotonin and 5-hydroxyindole acetic acid, a major metabolite of serotonin, was found in patients with reduced sperm concentration (Segal 1975). Using cyproheptadine, an anti-serotonin agent, he reported successful treatment of patients with idiopathic oligozoospermia. No control trials with prostaglandin or serotonin inhibitors are, however, available.

Hormones

Attempts have been made to relate changes in testosterone levels accompanying varicocele to suppressed fertility. No relationship has, however, been found between sperm count and testosterone concentration within the group of varicocele patients or between the testosterone levels and the size of the varicocele. In a few studies a lower concentration of testosterone has been found in the testicular vein but in these investigations testosterone concentration may have been considerably less than that present at the level of the testis because the blood samples were taken from the testicular vein at a distance of 20 cm from the testes. Another possibility might, however, be a general Leydig cell dysfunction, demonstrated by Weiss et al. (1978a) in men with varicocele and sperm concentration reduced to less than 10 million/ml. However, the levels of peripheral vein testosterone, FSH and oestradiol were normal in subfertile men with varicoceles. Also the testosterone and oestradiol levels in blood drawn from the spermatic vein were normal in such patients.

The relationship between sperm count and steroid levels in the spermatic and cubital veins of patients with varicocele was studied by de la Torre et al. (1978). Their results supported previous conclusions that pregnenolone sulphate and testosterone sulphate are secreted by the human testis. The peripheral levels of 17-hydroxypregnenolone, dehydroepiandrosterone, 17-hydroxyprogesterone and testosterone were significantly higher in seven patients exhibiting sperm count above 25×10^6/ml than in those with low sperm count (less than 25×10^6 sperm/ml). They also found that the progesterone concentration in the spermatic vein of the normospermic subjects was significantly higher than that of patients with low sperm count. From their studies and those of Jönsson et al. (1975) it is reasonable to assume that approximately 97% of the testosterone and almost 75% of the 17-hydroxyprogesterone present in peripheral circulation of men is of gonadal origin. It is safe to conclude that the individual levels in the spermatic vein

blood are invariably higher than those in the peripheral vein blood with respect to testosterone, 17-hydroxyprogesterone, androstenedione and progesterone, constituting fairly strong proof that these steroids are major secretory products of the human testes. This strongly suggests that continued studies on the steroid profile of spermatic and peripheral blood in normospermic and oligozoospermic subjects combined with the simultaneous study of the in vitro metabolism of progesterone and other key steroids may provide information on the complex interrelationship between testicular steroidogenesis and spermatogenic function under normal and abnormal conditions. In the above study, no control series of men with low sperm count without varicocele was investigated.

Morphology

The first comprehensive study of semen quality in men with varicocele was published by MacLeod in 1965. He described the seminal pattern in subfertile men with varicocele as being characterised by varying degrees of decreased sperm count, reduced motility and an increase in tapered and amorphous sperm. This complex of signs has became known as the 'stress pattern' and may appear after certain infectious diseases (MacLeod 1951), after acute allergic reactions (MacLeod 1962b), after the administration of antispermatic agents (MacLeod 1962a), or after environmental stress (MacLeod 1964). The cytological response of human testes to a variety of trauma, varicocele included, is thus not specific; it is characterised by a shift in spermatozoal headshape from the oval to the tapered and amorphous forms (MacLeod 1969). Despite this information, many recent publications have stated that increased numbers of tapered and amorphous spermatozoa are pathognomonic or characteristic of varicocele. No data have, however, been provided by these authors to support their statements. No studies have been published comparing the morphology of spermatozoa in fertile and infertile men with or without varicocele.

To elucidate this, a patient population comprising 312 men was investigated; 271 with infertile marriages and 41 with proven fertility requesting vasectomy. The diagnosis of varicocele was established in 82 'infertile' men by examination of the patient in the upright position at room temperature (22°C). In all subjects, the varicose veins were visible and seen on the left side of the scrotum. Men with azoospermia, with antisperm antibodies and with grossly elevated FSH levels were excluded. In the female partners the occurrence and adequacy of ovulation were evaluated during at least four consecutive menstrual cycles. Emphasis was placed on basal body temperatures and on the observation of a cervical mucus of ovulatory character, being clear without clumps of turbid areas, and having low viscosity and a *spinnbarkeit* of at least 50 mm as well as a strongly positive ferning test. Bilateral tubal occlusion was not diagnosed in any female partner of infertile men entering the study. Medical therapy had not been given to any husband. All infertile couples had tried to conceive for at least 2 years, in some instances for as long as 9 years. The methods of sperm analysis were the same as in previous investigations (Nilsson et al. 1979). No significant difference in the incidence of spermatozoa with normal morphology was found between infertile men with or without varicocele (Table 11.1). The proportion of tapering forms varied between 1% and 5% of the total number of spermatozoa and no difference was found

Table 11.1. Frequency distributions of percentage of normal spermatozoa in 82 infertile men with varicocele and 189 infertile men without varicocele

Per cent normal	Varicocele[a]	No varicocele[a]
<30	18% (15)	17% (32)
30–40	45% (37)	50% (95)
40–50	21% (17)	18% (34)
<50	16% (13)	15% (28)

[a] Number of subjects in parentheses

between the two series. The mean sperm density and mean total sperm count of men with varicocele did not differ from those of men without varicocele (Table 11.2). Furthermore, the incidence of spermatozoa with normal morphology (>50% normal forms) was the same in men with or without varicocele and no correlation between varicocele size and sperm morphology was detected. The patterns of spermatozoal morphology in the presence or absence of varicocele were almost identical. The ability of sperm in vitro to penetrate in the Kremer test was identical in the two series—severely reduced. The results of this preliminary report confirm the observations by Nilsson et al. (1979) that the mucus invasion test of Kremer provides the closest correlation with proven fertility and that evaluation of semen quality from one or more of the crude variables such as sperm count and sperm morphology is often given too much significance.

Testicular biopsies of patients with varicocele have shown characteristic changes with decreased spermatogenesis, desquamation of germ cells with obliteration of the lumen and an increased number of Leydig cells (Charny 1962; Etriby et al. 1967; Dubin and Hotchkiss 1969). These changes were bilateral, despite the fact that the varicocele was always on the left side. Johnsen and Agger (1978) studied

Table 11.2. Seminal characteristics of prevasectomy men, infertile men with varicocele, and infertile men without varicocele

	Sperm density (million/ml)	Total count (million)	Morphology		In vitro penetration (μm/s)
			Oval heads (%)	Tapered (%)	
Prevasectomy men (n = 41)	78 ± 30	196 ± 87	56 ± 4	1	4.2 ± 0.8
Men with varicocele (total) (n = 82)	69 ± 18	178 ± 93	34 ± 6	2.5 ± 1	2.6 ± 1.3
Small varicocele (n = 24)	72 ± 19	192 ± 72	34 ± 6	2 ± 0.5	2.9 ± 1.6
Medium varicocele (n = 38)	68 ± 15	181 ± 82	32 ± 7	2 ± 0.5	2.6 ± 0.8
Large varicocele (n = 20)	67 ± 20	156 ± 108	36 ± 6	4 ± 0.5	2.2 ± 1.2
Infertile men without varicocele (n = 189)	61 ± 17	132 ± 24	31 ± 9	2 ± 0.5	2.4 ± 1.5

the effect of varicocelectomy on the testicular biopsy mean score, finding that after varicocelectomy it increased significantly in a group of men with an age range of 25–44 years. They also showed that preoperatively the semen quality of younger men with varicocele was considerably better than that of older men. However, improvement was based on increase in total sperm count and no differences were found between large and moderately-sized varicoceles. This observation is in contrast to that of Fritjofsson and Åhren (1967) who found the greatest beneficial effect after operation on large varicoceles.

The change in the testicular biopsy score count after operation is small, particularly in relation to the high conception rates reported after operation. MacLeod (1965) and Dubin and Amelar (1975) found that the most marked change after varicocelectomy was in sperm motility. Therefore it was postulated that varicocele had an effect on the function of the epididymis and the vas deferens. These organs share the vascular supply of the testes and spermatozoa remain in them for a mean of 12 days.

In their controlled study Nilsson et al. (1979) found no significant change in motility and motile sperm count following varicocelectomy when preoperative sperm counts were less than 10 million/ml in spite of follow up of 36–74 months. A slight, but statistically insignificant, increase in sperm motility was observed in the group of patients with preoperative sperm counts greater than 40 million/ml. No difference was found between varicocele patients subjected to varicocelectomy and controls.

Diagnosis

With the patient in the supine position, varicoceles secondary to incompetent internal spermatic vein valves collapse while varicoceles caused by pathological obstruction of the internal spermatic vein remain dilated regardless of the position of the patient. The patient must therefore be standing during examination if incompetent valves are suspected.

It is commonly thought that the size of the varicocele has no bearing on the rate of improvement in semen quality following ligation of the testicular vein in subfertile males; thus, it is necessary to search for small and subclinical varicocele. If a small varicocele is present, a sudden rush of venous blood will be felt in the pampiniform plexus when the patient performs a sudden Valsalva maneouver while in the standing position.

Venography

Spermatic vein venography can be helpful in providing additional information about testicular venous drainage. Etriby et al. (1975) demonstrated contralateral venous anastomosis at the level of the symphysis pubis, through the inguinal canal on the anterior abdominal wall, the gluteal veins and contralateral femoral vein communication; ipsilateral anastomosis occurred at the level of the pubic bone with the saphenous and femoral veins. They concluded that correction of varicocele must be done above the inguinal canal. Later, Hill and Green (1977) found cross-communication through the retropubic and sacral veins. They also demon-

strated varicoceles of the cremasteric vein system and mixed varicoceles involving the internal spermatic and cremasteric veins. In patients where surgical correction of varicocele had been unsuccessful, cremasteric vein insufficiency was verified by Sayfan et al. (1980), and following cremasteric vein ligation three patients showed significantly improved semen parameters. These reports highlight the possibility of cremasteric incompetence, high cross-collateralisation or multiple internal spermatic veins as causes of surgical failure.

In clinical practice, routine use of venography is unwarranted because spermatic vein ligation above the internal inguinal ring gives fairly good success rates. The place for venography is in the patient previously operated upon but with secondary persistent varicocele, in order to find the anatomical position of persistent insufficient veins.

Doppler Test

Non-invasive tests are available to confirm the presence of varicocele suspected on the basis of venous thrill. To detect non-palpable varicocele Greenberg et al. (1979) used the Doppler ultrasonic stethoscope. In a group of 28 patients with palpable varicoceles they found that all had positive regurgitation on Doppler examination; five of 13 oligozoospermic patients without palpable varicocele also had positive Doppler test. Two of five patients submitted to ligation of testicular veins still had positive Doppler test.

In a recent study Hirsch et al. (1980a) showed that the Doppler technique was able to accurately identify varicoceles associated with significant elevation of scrotal temperature as determined by computer-assisted infra-red scrotal thermography. The Doppler test was, however, unable to distinguish between the presence of competent internal spermatic veins and internal spermatic vein reflux.

Operative Procedure

Incisions for supra-inguinal and high inguinal approaches currently start about 2 finger-breadths medial to and below the antero-superior iliac spine. This incision has replaced the original one advocated by Palomo (1949). Based on his experiences on 4470 varicocelectomies, Ivanissevich (1960) emphasised that the site of choice for division and ligation of the internal spermatic vein is at the internal inguinal ring. If the spermatic vessels are approached below the level of the inferior epigastric vessels too many venous channels are encountered while above this level usually only one or two channels are found. For the inexperienced surgeon difficulties may arise in identifying the internal spermatic vessels through the supra-inguinal approach. Gentle pulling of the spermatic cord by grasping it in the scrotum indents the peritoneum thereby identifying the internal spermatic vessels in the retroperitoneal location. Dubin and Amelar (1977a) modified the Ivanissevich procedure by dividing and ligating the vein at the level of the internal inguinal ring, approaching the vein through the external oblique aponeurosis. Palomo originally performed division and ligation of the internal spermatic vessels en masse including the spermatic artery. A modified Palomo procedure without division of the artery is now used by many surgeons.

Selective injection of sclerosing solutions into the internal spermatic veins has also been used.

Operative Failures and Complications

If the original varicocele was large, persisting fullness of the scrotal veins may persist and should not be confused with failure of varicocelectomy. True surgical failures with persistent postoperative venous reflux occur in about 5% of cases. In such cases persistent postoperative venous reflux can be demonstrated by venography.

In a series of 114 operations for varicocele using the high ligation technique, Wallijn and Desmet (1978) noticed hydrocele formation on the ipsilateral side in 7% of cases and Dubin and Amelar (1977b) reported an incidence of 3% Hydrocele must therefore be considered a complication which may be due to damage caused by ligature of the lymphatic vessels running with the spermatic veins.

Results

Greenberg (1977) reviewed the results of more than 1300 varicocelectomies performed by various surgeons. He found that the semen quality improved in 58%–71% of patients and pregnancy occurred in 25%–55% of the female partners. Improved semen quality was reported in 70% of 986 varicocelectomies performed by Dubin and Amelar over a period of more than 12 years, and they also reported a pregnancy rate of 53% (Dubin and Amelar 1977b). In their series, 354 (85%) of 416 men with preoperative sperm counts greater than 10 million/ml had improved semen quality and 70% impregnated their wives. When the preoperative sperm count was less than 10 milliom/ml, varicocelectomy improved the semen quality in 50 (35%) of 143 men and the conception rate was 27%.

These results of varicocelectomies in subfertile men provide strong arguments for the view that ligation of the testicular vein has a definite place in the treatment. The efficacy of varicocelectomy in subfertile patients has, however, been questioned by Rodriguez-Rigau et al. (1978a) who found that when therapy was given only to the female partner of the varicocele patient, the pregnancy rate was 51%. Following varicocelectomy the pregnancy rate was only 45%. Their study stresses the importance of a complete evaluation of the wife before subjecting the patient to surgery, a point underlined by Nilsson et al. (1979) who found that there was no statistically significant improvement in semen variables after surgery and that the pregnancy rate in the wives of men who were submitted to surgery was less than half of that in the wives of those who had no surgery.

Conclusions

The treatment of infertile men with oligo-teratozoospermia and/or reduced sperm motility is not very satisfactory. If the disorder is concomitant with varicocele however, improvement in semen quality has frequently been noted following

varicocelectomy. Although this improvement is either modest or questionable, impressive pregnancy rates have been reported. Although it is generally accepted that ligation of the left testicular vein has a beneficial effect, the mechanism of action is a tantalising problem. The most puzzling phenomenon has been that although varicoceles are almost entirely on the left, the spermatogenic reduction is identical in both right and left testes (Agger and Johnsen 1978). Furthermore, the duration of varicocele has no effect on spermatogenesis (Johnsen and Agger 1978) nor has it been possible to correlate the size of the varicocele with the reduction in spermatogenesis (Agger and Johnsen 1978). Many men with a varicocele have normal semen quality.

Tulloch (1952) reported a case of an azoospermic man who fathered three children following bilateral varicocele ligation. This case was well documented by testicular histopathology and semen analysis both before and after operation and proves that the operation can be of benefit in selected cases. Recently, the efficacy of varicocele ligation has been questioned because no significant improvement in semen variables or pregnancy rate was found in a controlled trial of surgery (Nilsson et al. 1979) and because meticulous attention to therapy for the wife produced a greater pregnancy rate than varicocele ligation for the husband (Rodriguez-Rigau et al. 1978a). These two studies indicate that in many cases varicoceles do not cause infertility but are an incidental finding, and that in many cases pregnancy following varicocele ligation is unrelated to treatment. Further work is needed to define the part varicoceles may play in male infertility. Before infertility is ascribed to the husband's varicocele, it is important to be sure that the results of the wife's tests are normal and in particular that there is no defect of ovulation.

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Chapter 12

Azoospermia

J. P. Pryor

Introduction

Azoospermia, or the complete absence of spermatozoa from the semen, is one of the few causes of sterility that can be detected by seminal analysis. The investigation of azoospermic patients aims to establish the aetiology of the abnormality in order that attempts at its correction may be made. In many instances the available treatment is ineffective and in those circumstances it is important to discuss the prognosis so that the couple may make a realistic choice of the alternative methods of management. Adoption or artificial insemination with donor semen (AID) are essential options available for such couples (see Chap. 18).

There is no universal classification of azoospermia and the meaning of 'primary testicular failure' depends upon whether the physician is an endocrinologist, andrologist or gynaecologist. In infertility practice it is useful to classify azoospermia according to the level of plasma FSH. This is in accordance with the older concept of primary testicular failure when the abnormality was considered to be due to an intrinsic failure of the testis rather than an extrinsic factor such as pituitary failure.

In 1972 Franchimont et al. and de Kretsner et al. demonstrated that plasma FSH levels were higher in patients without spermatogenesis and suggested that this was concomitant with spermatogenesis not proceeding beyond production of spermatocytes. This suggestion was confirmed in a clinical study of 261 men (Pryor et al. 1976) and the association between elevated levels of plasma FSH and a gross impairment of spermatogenesis is of basic importance in the management of azoospermic men. A classification based upon plasma FSH levels and histological appearances of the testis is shown in Table 12.1.

Evaluation of Azoospermic Men

A history is first taken from the patient to identify any aetiological factors that are known to be associated with azoospermia (Table 12.2). Clinical evidence of

Table 12.1. Classification of azoospermia based upon the plasma follicle stimulating hormone (FSH) level and the histological appearance of the testis

I. Plasma FSH level elevated
1. Anorchism, cryptorchidism
2. Klinefelter's syndrome
3. Germinal aplasia, Sertoli-cell-only syndrome
4. Testicular atrophy/focal sclerosis
5. Hypergonadotrophic hypogonadism
II. Plasma FSH level 'normal'
1. Spermatogenic arrest (hypogonadotrophic hypogonadism)
2. Normal spermatogenesis

Table 12.2. General conditions that may be associated with azoospermia (some of these factors may cause reversible azoospermia)

Genetic disorders
e.g. Klinefelter's syndrome
intersex problems
Congential disorders
e.g. cystic fibrosis
myotonia dystrophia
bronchiectasis
Hormonal defects
e.g. pituitary
adrenal
thyroid
Severe systemic illness or malnutrition.
Infection
e.g. mumps
smallpox
bilharzia
Drug therapy
e.g. cytotoxic drugs
Irradiation

hormonal abnormalities may be observed and those men with a light beard, a female pattern of pubic hair and small testes are usually found to have Klinefelter's syndrome; gynaecomastia is present in only half of such patients. Testicular size is an excellent guide to the spermatogenic function of the testes. Prader (1966) graded testicular size by volume with 13 sizes ranging from 0.5 ml to 25 ml. This system was designed for paediatric use and is impracticable in the routine assessment of the infertile man. In infertility practice it is more convenient to compare the length of the testis with ovoids 3, 4 or 5 cm in length (Figure 12.1). Some clinicians find it useful to carry an ovoid 4 cm long for a rapid comparison should there be any doubt as to testicular length. The correlation between testicular size and the histological assessment of spermatogenesis in 261 azoospermic men is shown in Table 12.3. Examination of the genitalia should not be confined to examination of the testis as valuable information may be obtained by examining the epididymis (thickening or nodularity being associated with obstruction), vas deferens and rectum as has been described in Chap. 2.

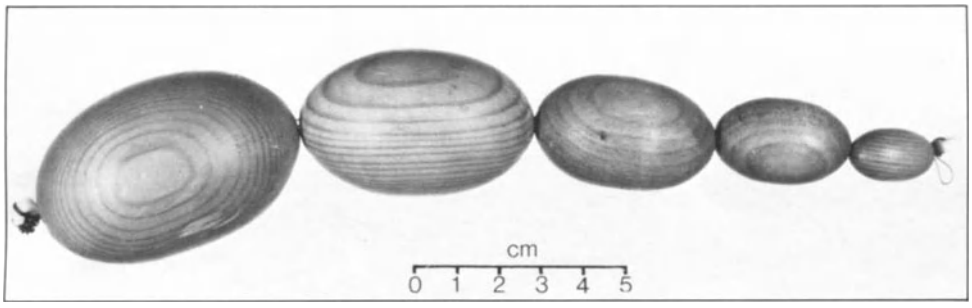

Fig. 12.1 Rosary of wooden ovoids 2, 3, 4 and 5 cm in length, which are used to determine testicular length. (In clinical practice, and with experience, only the 4 cm ovoid need be carried)

Table 12.3. Relationship between testicular length and spermatogenesis in 398 testicular biopsies from 261 azoospermic men (Pryor et al. 1978a)

Testicular length (cm)	Number (%) of biopsies mean Johnsen score			
	<2.0	2.1–7.9	>8.0	Total
2	11(100)			11(100)
3	36(51)	34(48)	1(1)	71(100)
4	39(33)	35(30)	44(37)	118(100)
5	11(6)	35(18)	152(77)	198(100)

The plasma FSH level correlates well with the histological assessment of spermatogenesis (Table 12.4). The ready availability of plasma FSH assay has reduced the need for testicular biopsy in azoospermia to those men in whom the plasma FSH level is no greater than twice the upper limit of normality (Pryor et al. 1978c). This permits plenty of latitude and allows for occasional errors based on the results of a faulty laboratory test. Table 12.5 summarises the indications for testicular exploration in azoospermic patients.

Testicular exploration is preferable to a testicular stab biopsy as the plasma FSH level correlates well with the histological assessment of spermatogenesis. Fur-

Table 12.4. Relationship between the plasma FSH level and spermatogenesis in 214 azoospermic men (Pryor et al. 1978b)

FSH level	Number (%) of biopsies mean Johnsen score			
	<2.0	2.1–7.9	>8.0	Total
Normal		42(33)	82(67)	123(100)
Mild elevation		18(58)	11(42)	29(100)
Gross elevation[a]	1	16(64)	8(32)	25(100)

[a] The normal ranges varied according to the reference preparation used and 'gross elevation' of the plasma FSH level was considered to have occurred when the value was greater than twice the upper level of the normal range. Most azoospermic patients with grossly elevated levels did not undergo exploration and biopsy.

Table 12.5. Testicular length and plasma FSH level determine the initial management of the azoospermic patient

Testicular length (cm)	FSH level	Management
2, 3, 4	Gross elevation[a]	Counsel[b]
2, 3, 4	Mild elevation	Counsel (operation)[c]
2, 3, 4	Normal	Repeat FSH (operation)
4	Normal	Operation
5	Any	Operation

[a] Gross elevation occurs when the plasma FSH level is greater than twice the upper limit of the normal range

[b] Counsel with regard to the hopeless prognosis and discuss AID and adoption

[c] At operation inspect epididymis, perform vasography, biopsy the testicle and carry out any reconstructive procedure

thermore, the demonstration of normal spermatogenesis does not mean that surgical correction of the obstruction is necessarily possible. The epididymis is inspected for dilated tubules containing opalescent fluid; when these are seen the testicular histology always confirms that spermatogenesis is occurring. In those patients where there is proximal obstruction, i.e. near the urethra, the epididymal tubules may not be so noticeably dilated and may be overlooked. Occasionally, dilated epididymal tubules are seen, containing a clear fluid; this appearance is associated with the absence of spermatogenesis or with a block within the testes in addition to a more proximal block in the vas deferens.

The normal appearance of the epididymis, in azoospermic men without greatly elevated levels of plasma FSH, is associated with the variable histological appearance of the testes. Testicular biopsy of 116 testes from such men showed essentially normal spermatogenesis in 40; in these patients there was presumably a block within the testes. Twenty testicular biopsies showed a complete absence of spermatogenesis and in the remaining 56 biopsies the histology indicated hypospermatogenesis or spermatogenic arrest.

A small testicular biopsy is taken at the time of the testicular exploration and placed in Bouin's Solution (see p. 114). The biopsy is assessed using the semi-quantitative technique of Johnsen (1970) (see p. 115). The normality of the vasa, seminal vesicles and ejaculatory ducts is verified by vasography at the time of testicular exploration. Vasography may be performed by directly puncturing the lumen of the vas deferens with a 25 gauge needle and injecting 2–5 ml of 45% hypaque solution towards the bladder. Entry into the lumen of the vas is facilitated by placing a cat gut sling around the vas deferens. A normal vasogram shows that the lumen of the vas deferens is not dilated, the seminal vesicles are symmetrical and the ejaculatory duct is outlined, with a free reflux of contrast into the bladder. The injection of saline or methylene blue along the vas deferens is not a satisfactory method for excluding pathology in the vas or seminal vesicles, as in some patients there may be congenital abnormalities such that obstructive lesions are overlooked. In order to make a radiological diagnosis of obstruction it is essential to observe dilatation of the vas deferens. The many and varied appearances of vasograms are to be found in Boreau (1974) but they are not of any great clinical significance.

Azoospermia with Elevated Levels of Plasma FSH

Grossly elevated levels of plasma FSH are detected in the absence of spermatogenesis and are associated with the absence of the feedback mechanism inhibiting FSH production. There may be an additional defect in Leydig cell function and in such instances the plasma LH level will also be elevated and the plasma testosterone level low.

Anorchidism, Cryptorchidism

It is often difficult to distinguish between these two conditions. Normal testicular descent is essential for spermatogenesis to occur and maldescended testes show histological abnormalities from a very early age (Scorer and Farrington 1971). It is for this reason that the early treatment of testicular maldescent is recommended otherwise the patient remains sterile (see p. 135). There are only two reported instances whereby an orchiopexy carried out in adult life has improved fertility. (Britton 1975; Comhaire et al. 1978) In both patients the testes were palpable in the inguinal canal but the testicular histology at the time of orchiopexy was not reported. The preoperative plasma FSH level was elevated in the second patient but fell to just above the normal range following the operation.

Patients with testicular maldescent have an increased risk of developing a testicular neoplasm; thus attempts should be made to locate and operate upon those azoospermic patients with undescended testes. It is difficult to distinguish between anorchidism and cryptorchidism and a rise in the plasma testosterone level following stimulation with human chorionic gonadotrophic hormone (De Kretsner et al. 1975; Levitt et al. 1978) may be useful in detecting the presence of Leydig cells (Rivarola et al. 1970). The test may be carried out by giving 5000 units of hCG by intramuscular injection on three consecutive days and measuring the rise of plasma testosterone level on the fourth day. The role of radiological and scanning techniques before surgical exploration remains uncertain (Redman 1980). Patients with anorchidism require androgen replacement therapy either by 6-monthly implantation of a subcutaneous pellet of 600 mg of testosterone, by monthly intramuscular injection of 1 ml of Sustanon 250, or by oral therapy with 10 mg of sublingual testosterone each day. All patients with undescended testes should undergo operation even though the operation is unlikely to improve fertility if the testes are intra-abdominal. The operation should be performed through an inguinal incision when the testis is palpable; otherwise, particularly if the maldescent is bilateral, it is best carried out through a transverse lower abdominal incision. The inguinal canal is inspected in the operation to establish the presence of a spermatic cord and, when this is absent, the rectus sheath should be incised in the midline and the rectus abdominis muscles separated to search for the missing testis in the peritoneal cavity or retroperitoneal tissues. The testes are commonly found on the side wall of the pelvis and even after extensive mobilisation of the testicular vessels and vas deferens it may be impossible to place the testes in the scrotum. It is important to preserve at least one testis in order to maintain the endogenous production of androgens, and to facilitate the orchiopexy it may be necessary to divide the vas deferens or testicular vessels. If a successful orchiopexy has been carried out on one side then the other testis may be excised. Silber and

Kelly (1976) have successfully carried out auto-transplantation of the undescended testis to the scrotum using a microvascular technique. More remarkable was the successful transplantation of a functioning testis from one twin to the other (Silber 1979). A more practical solution to the problem is androgen replacement therapy, testicular prostheses for cosmetic reasons, and AID.

Klinefelter's Syndrome

This occurs approximately once in every 400 births; men with Klinefelter's syndrome usually present with azoospermia, very small testes (approx. 2 cm in length) and a female distribution of hair. This syndrome is more fully discussed in other chapters (chromosomes p. 147, endocrinology p. 97, pathology p. 132).

Men with Klinefelter's syndrome may require androgen replacement therapy as they grow older. There is no treatment for the infertility and the couple should be advised with regard to adoption or AID.

Germinal Aplasia and Sertoli-Cell-Only Syndrome

Patients with these syndromes usually have normal levels of LH and testosterone but the testicular histology shows an absence of spermatogenesis. Testicular exploration is unnecessary as the testes are usually small and the plasma FSH level is grossly elevated. On rare occasions the plasma FSH level is normal as a result of an additional defect in pituitary function. The aetiology of this condition is unknown, no treatment is required and the couple should be counselled about AID or adoption.

Testicular Atrophy

The germinal epithelium is more sensitive to damage than the Leydig cells which are remarkably resistant to injury (see p. 122). Spermatogenesis may be inhibited by any severe acute viral infection and the patient may develop temporary azoospermia. It is uncertain whether the effect is due to the pyrexia or the systemic metabolic disturbance. Mumps orchitis occurs in 20% of patients with parotiditis occurring after puberty and is bilateral in half of these patients. There is a recovery of spermatogenesis in most patients but a case could be made for active immunisation during a mumps epidemic.

Acute epididymo-orchitis may cause the destruction of the germinal epithelium but this is usually on a patchy basis and the infection may be accompanied by obstruction due to oedema and subsequent scarring. Cytotoxic drugs and irradiation may arrest spermatogenesis but the severity and permanence of this damage depends upon the dosage (cytotoxic drugs are discussed on pages 13, 98 and 131).

Damage to the germinal epithelium is accompanied by elevation of the plasma FSH level and its recovery is accompanied by a fall in this hormone level although this may not occur for up to 5 years. No specific therapy is available for men with testicular atrophy and the Leydig cell function is usually preserved.

Hypergonadadotrophic Hypogonadism

Elevated levels of gonadotrophin are found when there is an intrinsic failure of the testis to produce androgens and spermatozoa. The androgen deficiency produces the clinical manifestations of hypogonadism and the patients usually seek advice for this problem. Patients with hypergonadotrophic hypogonadism are rarely encountered in an infertility clinic although male pseudohermaphrodites are occasionally seen. Such men have usually undergone extensive surgery in infancy and then ceased to receive regular medical supervision. In hypergonadotrophic hypogonadism the plasma FSH and LH levels are elevated. The patients may require androgen replacement therapy but they should be counselled with regard to AID or adoption on account of their infertility.

Azoospermia due to Spermatogenic Arrest

Spermatogenesis may be arrested at any stage of the spermatogenic cycle and histological studies show that the majority of the seminiferous tubules are arrested at the same stage of spermatogenesis. Spermatogenic arrest at the spermatogonia level (Johnsen step 3 (see p. 116)) is unusual and is usually accompanied by an elevated level of plasma FSH. The aetiology of this condition is unknown and no treatment is available.

Spermatogenic arrest at the spermatocyte state is more common; some of the tubules may even contain spermatids. The condition is seen in hypogonadotrophic hypogonadism and in such instances the associated androgen deficiency also requires treatment. This type of spermatogenic arrest occurs following pituitary ablation (Macleod et al. 1966) and may be also seen in patients with an isolated deficiency of FSH and in some patients with bilateral varicoceles.

Spermatogenic arrest at the spermatid stage occurs infrequently and is of unknown aetiology.

Hypogonadotrophic Hypogonadism

Hypogonadotrophic hypogonadism is characterised by low plasma levels of FSH and LH and may be due to pituitary or hypothalamic abnormalities (Table 12.6) (see p. 92). The delayed puberty of the patient may be due to a lack of androgens; or the deficiency of gonadotrophic hormones may be part of a syndrome associated with a lack of other pituitary hormones arising from pituitary abnormality or in association with primary or secondary pituitary failure. The diagnosis of hypogonadotrophic hypogonadism is suggested on clinical grounds by the lack of androgenisation. Other abnormalities, such as hare lip, cleft palate, anosmia such as occurs in Kallman's syndrome (Chap. 5), or testicular maldescent, may be seen. In hypogonadotrophic hypogonadism the plasma levels of LH and FSH are usually at the lower level of sensitivity for the assay. The response to gonadotrophin releasing hormones (GnRH) is variable and depends on whether the defect is pituitary or hypothalamic in origin. Testicular biopsy is unnecessary where the testes show the features of immature or prepubertal testes, as the diagnosis may be established by the clinical and hormonal evidence.

Table 12.6. Classification of hypogonadotrophic hypogonadism

I. Hypothalamic disorders
Idiopathic
Traumatic
Neoplastic: benign, e.g. craniopharyngioma
malignant, e.g. metastatic carcinoma
II. Pituitary disorders
Primary, e.g. adenoma
pituitary ablation
Secondary, e.g. meningioma
internal carotid aneurysm
haemochromatosis

The importance of hypogonadotrophic hypogonadism is twofold. First, the defect may be a result of hypothalamic or pituitary tumours which require treatment, and secondly, the condition is usually reversable. Treatment commences with 1500 units of human gonadotrophic hormone (hCG) by intramuscular injection 3 times each week. The treatment continues for 3 months and if there is no clinical response the dosage should be increased. When a satisfactory response is still not obtained it is possible to add FSH to the treatment regime by giving increasing doses of 'Pergonal' (75 IU FSH and 75 IU LH), or by using gonadotrophin releasing hormones. The effects of treatment are unpredictable and it is necessary to monitor the therapy on the basis of the clinical response (degree of androgenisation), plasma hormone levels and seminal analysis. It should be remembered that such patients rarely present to a fertility clinic.

Isolated FSH Deficiency

Nine in a series of 311 azoospermic men were found to have histological evidence of a spermatogenic arrest—usually at the spermatocyte level of spermatogenesis (Pryor et al. 1978c). The plasma LH and testosterone levels were normal but the plasma FSH level varied. Those patients with low levels of plasma FSH may have an isolated deficiency of that hormone—if so, they will respond to treatment with gonadotrophins (Pryor et al. 1976). Such an isolated deficiency is rare (Bell et al. 1975; Maroulis et al. 1977; Hägg et al. 1978), and in view of the paucity of reports the case history of one such patient is described.

J.M., a healthy 27-year-old man, was referred to the Infertility Clinic with a 3-year history of infertility. He had a normal distribution of hair and his testes were of normal size (approximately 5 cm in length) and consistency, and no other abnormality was detected by examination of his external genitalia. Repeated seminal analyses confirmed the finding of azoospermia. At the time of testicular exploration the epididymes appeared normal and vasography failed to demonstrate any abnormality. A testicular biopsy was placed in Bouin's solution and assessed by the Johnsen technique (1970). No abnormalities were seen in the Leydig cells but spermatogenesis was only proceeding to the spermatocyte stage in the majority of tubules (55.5% of the tubules were at step 5, 43% at step 6 and the remainder at step 7).

The level of immunoreactive FSH in peripheral plasma was estimated by radioimmunoassay and repeated analyses showed that it was at the lower end of sensitivity of the assay. The plasma levels of LH and testosterone were normal. The patient was initially treated with clomiphene (50 mg daily for 3 months) with no improvement of the azoospermia. He then commenced therapy with one ampoule of Pergonal administered by intramuscular injection three times weekly. Spermatozoa appeared in the ejaculate after 2 months of treatment and the highest sperm density recorded was 3 million/ml with a semen volume of 2.2 ml. It was also necessary to treat his wife for an ovulation defect. After 9 months of therapy she conceived and was subsequently delivered of a normal male infant. Throughout the period

that the patient was receiving Pergonal therapy the level of plasma FSH remained low and 6 weeks after the cessation of therapy an LH–RH test was performed. This showed a normal response for the release of LH but no response for FSH.

Not all patients with a spermatogenic arrest respond to treatment with gonadotrophic hormones and it is possible that in some patients the defect is enzymatic rather than hormonal.

Bilateral Varicocele

The different aetiological types of varicocele and the effect on fertility remain the subject of much discussion and speculation (Chap. 10). It is therefore not surprising that there is no uniform view as to the histological changes occurring in the testes of patients with varicocele or the improvement that might follow the ligation of the varicocele of an azoospermic patient. Tulloch (1955) describes the onset of spermatogenesis in a 27-year-old azoospermic man following the bilateral ligation of varicoceles. The testicular histology showed a bilateral spermatogenic arrest at the time of the original operation and normal spermatogenesis when a further biopsy was taken after fertility had been established. Ibrahim et al. (1977) observed bilateral spermatogenic arrest in four men with varicoceles, and Mehan (1976) reported the restoration of spermatogenesis in four men following varicocelectomy in a group of ten men with bilateral varicoceles and azoospermia. The histological appearance of the testes in azoospermic men with varicoceles varies considerably and ranges from germinal aplasia to almost normal spermatogenesis. In the latter case, patients may have an obstructive lesion in addition to a varicocele. One patient (A. B., aged 29 years) was found to have the histological appearance of a spermatogenic arrest at the spermatogonia level in November 1978. The spermatogonia showed a considerable degree of nuclear pleomorphism and in July 1980 he presented with a malignant teratoma of the same testis. The finding of abnormal germinal cells, carcinoma in situ of the testis, and testicular malignancy is discussed in Chap. 6 and further information may be found in the proceedings of a recent symposium on the early detection of testicular cancer, which was held in November 1980 (Skakkebaek et al. 1981).

Obstructive Azoospermia

Azoospermia with normal spermatogenesis is due to an obstructive lesion between the seminiferous tubules and the orifice of the ejaculatory duct. The cause of the obstruction may be suggested from the history or by clinical examination of the patient.

The testes in obstructive azoospermia are usually of a normal size and the plasma FSH level is within the normal range. In a series of 84 consecutive patients the level of obstruction was found to be pre-epididymal in 20, epididymal in 44 and vasal in 20 (Pryor et al. 1978b). There is no satisfactory treatment for obstruction within the testes although improvement may occasionally be observed following treatment with a low dose of steroids (5 mg prednisolone three times daily), or a prolonged course of antibiotics. In these instances obstruction is usually due to oedema.

Epididymal Obstruction

The observation of dilated tubules in the epididymis, and of the level at which such dilated tubules are no longer visible, provides clear evidence for the site of epididymal obstruction and is superior to any radiological method. The obstruction may be congenital in origin or the result of previous non-specific infection or infection due to gonorrhoea, tuberculosis or smallpox (Phadkee et al. 1973) or schistosomiasis (Girgis and Wassef 1980). The histological appearance of the epididymis in obstruction varies considerably. McConnell (1981) reported that in some patients the vasa efferentia were grossly thickened and this itself caused an obstructive element. In some patients there is evidence that small areas of acute inflammation lead to obstruction and, associated with these lesions, spermatozoa leak into the interstitial tissue to form sperm granulomata and subsequent fibrosis. In some specimens there was no evidence of sperm granulomata or acute inflammation and in these rupture of the dilated vasa efferentia was associated with the spillage of cholesterol material into interstitial tissue with the subsequent formation of a foreign-body-type granulomatous reaction. This caused thickening around the duct with consequent narrowing of the lumen and interstitial fibrosis.

Hodges and Hanley (1966, 1968) drew attention to the presence of microcysts in the caput epididymus in those patients where the obstruction was considered to be of a congenital origin and suggested that there might be a failure of fusion between the genital and urinary tracts. Young (1970) noted that there was an association between epididymal obstruction and bronchiectasis and it has been suggested that this might be due to a defect in the cilia. However, Hendry et al. (1978) were unable to confirm this.

Epididymovasostomy

Epididymal obstruction may be corrected by performing a short-circuiting operation which attempts to anastomose the mucosa of the vas deferens with that of the epididymis. The results of epididymovasostomy vary greatly and whilst many reputable surgeons state that they have never achieved a successful outcome other surgeons have reported a patency rate of 73% and pregnancies in 31% of couples (Schmidt et al. 1976). Hanley (1955) emphasised that the results obtained for congenital obstruction were much inferior to those obtained when the obstruction followed infection. Schoysman (1981) has drawn attention to the better prognosis in post-gonorrheal obstruction and has been able to demonstrate the different patency and pregnancy rate when anastomosis is made at different levels of the epididymis (Table 12.7).

Schoysman (1981) also states that there is little hope of restoring patency in those patients where the obstruction has been present for more than 10 years. The results of a questionnaire which was sent to 150 urologists in the United States showed that 13% of surgeons never performed the operation and that 49% had success rates of less than 1%. Four surgeons stated that they had success rates in the order of 50%–70% (Getzoff 1973). This variability of results is confirmed in the collected review of Schmidt et al. (1976).

Epididymovasostomy is best performed at the time of testicular exploration and is carried out under a general anaesthetic. The scrotal skin and tunica vaginalis are incised over the testis, which is allowed to prolapse out through the wound. The

Table 12.7. Effect of the site of epididymal anastomosis to the vas deferens on patency and subsequent conception rates (Schoysman 1981)

Site of anastomosis	Number	Patency rate (%)	Conception rate (%)	
			Total	Patent
High (caput)	72	75	8	11
Medium	54	52	20	39
Low	24	38	25	66
Total	150	61	15	25

level of epididymal obstruction is determined by visual inspection for dilated tubules containing opalescent fluid. The vas deferens is manipulated until it lies beneath the tunica vaginalis which is then incised to allow a catgut sling to be placed around the vas. The lumen of the vas deferens may be punctured with a 25-gauge needle and vasography performed to ensure that there is no proximal obstruction. The injection of 5–10 ml of saline or methylene blue, although easy, is not a satisfactory method of demonstrating vasal patency as it may fail to demonstrate a cystic lesion. The anastomosis between the vas and the epididymis should be performed as near the cauda epididymis as the level of obstruction permits. The distended part of the epididymis is incised at the proposed level of anastomosis and any solid granulomatous tissue is excised. The fluid that exudes from the vas may be examined under the microscope in the operating theatre in order to confirm the presence of spermatozoa—although the observation of opalescent fluid is usually sufficient to permit the operation to proceed. When no opalescent fluid exudes from the incision into the epididymis a further incision(s) is (are) made towards the caput until a satisfactory site for the anastomosis is found.

The vas deferens is gently mobilised, taking care to interfere as little as possible with the blood supply, and part of the anterior surface excised with a fine pair of ophthalmic scissors. This is facilitated by the catgut sling placed around the vas and by the use of magnifying spectacles. Once the lumen of the vas has been identified, a pair of fine straight scissors is used to incise the lumen for 10–15 mm. A lateral anastomosis is then made with fine, non-absorbable sutures between the mucosa of the vas and the incised epididymis. This direct anastomosis (Humphreys and Hotchkiss 1939; Bayle 1950) is preferred to the technique of burying the incised vas deferens in the epididymis (Hanley 1955). The use of a nylon stent confers no apparent advantage, but the direct end-to-side anastomosis of the lumen of the vas to a dilated epididymal tubule may lead to much better patency rates (Silber 1978, 1981). Such an operation requires the use of an operating microscope. On completion of the vasoepididymal anastomosis it is advisable to anchor the vas deferens to the epididymis with a 3/0 suture taking care to ensure that the vas deferens rests snugly on the epididymis and that there is no kinking or tension at the site of the anastomosis. The operation is usually performed on both sides at the same time.

Seminal analyses are performed at 3-monthly intervals following the operation and it is well recognised that spermatozoa may not appear in the ejaculate for up to 2 years. Patients having an unsuccessful result should be encouraged to consider AID, particularly if the appearance of the epididymis is unfavourable or the partner is over 30 years of age. Reoperation is seldom successful and should be

discouraged except in those patients where the findings were favourable at the first operation and the obstruction is considered to be a result of infection.

Vasal Obstruction

The level of vasal obstruction is determined radiologically and the possibility of reconstruction depends upon the number, length and location of the block. The reversal of vasectomy is described elsewhere (Chap. 16) and is associated with an excellent chance of subsequent patency (80%–90%) and fertility (40% within 2 years). A similar outcome is to be expected in those patients in whom the vas deferens has been injured at the time of a herniorrhaphy although the results depend upon the length of the interval before the repair is undertaken.

Blocks close to the epididymis, in the convoluted part of the vas, are best dealt by epididymovasostomy and it is of interest that the results in these patients are much better than in those patients with epididymal obstruction. A common site for high vasal obstruction is near the junction of the vas with the duct from the seminal vesicle. The surgical correction of this lesion is not practicable and the couple are advised with to consider AID or adoption.

A long area of vasal sclerosis is not amenable to correction and the couple are best advised to resort to AID or adoption. For those couples who refuse to accept the absence of satisfactory treatment, it is possible to attempt to interpose a substitute tube (silicone or venous); or to create an incubated vasostomy, similar to that described by Davis for the ureter; or one of the techniques used in vasa aplasia may be tried. Neither consistent nor satisfactory results have been obtained by these techniques.

Vasa Aplasia

The vas deferens and ureter both develop from the mesonephros (Wolffian duct) and unilateral absence of the vas occurs in 0.5%–1% of the male population (Michelson 1949). The unilateral absence of the vas is often associated with an ipsilateral absence of the kidney (Ochsner et al. 1972) or renal abnormalities (Lukash et al. 1975). Bilateral vasa aplasia has been found in 2% of infertile patients (Dubin and Amelar 1971) (see p. 20), but occurred in 20% of a recent series of patients with obstructive azoospermia (Pryor et al. 1978c). In patients with bilateral vasa aplasia the defect occurs at a later stage of development and in some patients it may be associated with cystic fibrosis (Holsclaw et al. 1971, Lukash et al. 1975).

The diagnosis of vasa aplasia is made by clinical examination of the spermatic cord. The vas deferens is usually easily palpable and any doubt about its presence should alert the clinician to the possibility of vasa aplasia. Unfortunately, the diagnosis of vasa aplasia is often overlooked by clinicians failing to specifically palpate the vasa. The defect is often associated with an absence of the seminal vesicle and this is suggested by a low semen volume; but it is unnecessary to resort to the measurement of seminal plasma fructose level in order to confirm the diagnosis.

The treatment of vasa aplasia is unsatisfactory as there is no satisfactory replacement for the vas deferens. Hanley (1955) created an artificial spermatocele

with amniotic tissue; he was able to recover spermatozoa and successfully used artificial insemination of the wife to obtain a pregnancy. Occasional successes have been obtained using the saphenous vein or tunica vaginalis (Schmidt et al. 1976) but these have been replaced by the artificial spermatocele. Wagenknecht (1977) successfully used the artificial spermatocele in the bull and Kelami has developed a similar prosthesis and obtained pregnancy in clinical practice (Kelami et al. 1981). The results remain unsatisfactory and AID is a more reliable method for obtaining a conception.

Obstruction of the Ejaculatory Ducts

This condition is only diagnosed by vasography although it may be suspected in those patients with low semen volume when the semen does not contain fructose—an estimation of limited value to the investigation of infertile men. The condition is usually congenital in origin and may be associated with cystic remnants, but it may also occur as a result of inflammation.

Dilatation of the ejaculatory duct is difficult and rarely rewarding although a conception does occasionally occur. The dilatation is facilitated by injecting methylene blue into the vas deferens. Endoscopic uncapping of a cyst or the obstructed duct orifice may occasionally be effective in treating the obstruction and this may be followed by the appearance of spermatozoa in the ejaculate (Wintraub 1980).

Conclusions

Azoospermia is of grave prognostic significance with regard to fertility. Almost half the patients are found to have a primary failure of spermatogenesis, which will not improve with medical treatment. Most of the remaining patients have obstructive azoospermia; these patients have normal-sized testes and a normal plasma level of FSH. Correction of the obstructive lesion is possible in a small proportion of patients and a conception rate of approximately 5% is to be expected. The conception rate is higher after correction of post-gonorrhoeal obstruction.

Secondary testicular failure—characterised by normal or low plasma levels of FSH—occurs in approximately 3% of azoospermic patients and half of these patients may respond to treatment with human chorionic or menopausal gonadotrophic hormone. Sympathy and tact are necessary in the management of the azoospermic patient and the couple should be counselled about the alternative methods for obtaining parenthood. Many couples when faced with a realistic appraisal of the chances of conception will gladly choose the option of AID.

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Chapter 13

Non-Specific Treatment to Improve Fertility

T. B. Hargreave

Introduction

Specific abnormalities causing defective spermatogenesis are the exception rather than the rule. Certain clinics may attract a biased population; thus, an endocrinologist may find that a large percentage of his patients are infertile because of an endocrinological abnormality but this will reflect referral based on cases of delayed or abnormal puberty rather than infertility. In a general infertility clinic no difference has been found between fertile and infertile men with regard to 17-hydroxyandrogens and oestrogens (Walker et al. 1975) and in the dynamic response to hCG (Traub 1981). Seven only of the last 662 couples we have assessed at our clinic had established endocrinological problems. We have searched for abnormal hormonal profiles (Hargreave et al. 1977), isolated FSH deficiency (Hargreave et al. 1979) and for hyperprolactinaemia (Hargreave et al. 1981) without success. The only common positive finding is an association between falling sperm density, abnormal testicular histopathology and elevated FSH levels; thus in the majority of cases we cannot identify an hormonal basis for the problem.

Some men are found, on clinical examination, to have undescended testes or varicocele and laboratory tests may indicate antisperm antibodies; but after these groups have been selected out we are left with the majority of cases where some sperm are present and the wife's situation may or may not be known. It may often seem appropriate to begin treatment before the wife has been fully investigated, e.g. in the older couple (one or both partners over 30) where the wife's menstrual cycle is regular and the husband's semen analysis on several samples shows one or more poor measurements. Starting treatment should not blind one to the possibility of a concurrent abnormality of ovulation or tubal patency (Table 1.4).

The difficulty in evaluating the results of infertility treatments for either partner is compounded by the frequent occurrence of abnormal findings in both partners; this presents the medical statistician assessing the results of treatment with some of the most complex problems of any branch of medicine. To evaluate treatment for the husband, controlled trials are necessary and if such trials are not to be a waste of time the results of investigation of both partners must be known. Nearly all reports of treatment in the male fail in one of three respects: (a) there is inadequate information about the wife, (b) there is inadequate information about the husband, or (c) a control population is not studied.

Assessment of Fertility

If comparison is made between semen analyses from large populations of fertile and infertile men then normal ranges may be defined, but it must be remembered that there is overlap between the infertile and fertile range. Nelson and Bunge (1974) reported that 20% of men presumed fertile undergoing vasectomy had sperm densities of less than 20 million/ml. Van Zyl et al. (1975) reported 14 pregnancies in 27 couples in whom sperm density was less than 10 million/ml with some pregnancies occurring in couples where the husband had a sperm density of less than 5 million/ml. Macleod and Gold (1951) found that 9% of infertile men had sperm motility of 30% or lower; thus low density and poor motility have been shown to occur more frequently in the infertile population, but just because a man has these characteristics he cannot be termed sterile. Similarly, elevated FSH levels are said by some to indicate sterility but this is not our experience (Table 1.2). Mucus penetration tests (Insler 1977) and egg-white penetration tests (see Chap. 4) may correlate with infertility when a fertile population is examined alongside an infertile population and there is hope that in vitro fertilisation tests may also give prognosis about fertility. It is not surprising that information about the trying time (duration of involuntary infertility) gives better prognostic information than the results of most currently used laboratory test (Fig. 4.6).

Who to Treat?

It is our policy to advise men with severely abnormal test results that the chances of pregnancy are low and that they would be wise to consider AID or adoption (Table 13.1). It is sometimes appropriate in such cases to try a course of treatment to allow the couple time to adjust to their poor fertility prognosis and also to give

Table 13.1 When to treat the husband

	Best result from 3 fresh samples	Primary course of action	Further action
Very poor results	Density < 2 million/ml Motility < 20% Abnormal forms > 70% Volume < 0.5 ml	Advise AID or adoption early	A 6–9 month course of treatment may allow time for the couple to adjust to the poor prognosis especially if the couple are young or if the duration of involuntary infertility is less than 12 months
Abnormal semen results	Density < 20 million/ml Motility < 60% Abnormal forms > 40% Poor mucus penetration Poor zona-free egg penetration Excess of white cells in semen hyperviscosity	Investigate wife and offer husband treatment	Advise AID or adoption when duration of involuntary infertility exceeds 36 months if treatment fails
All results normal		Investigate wife	If wife's tests are normal advise adoption if duration of involuntary infertility exceeds 36 months. ?? AID

them psychological support in that something is being done. In all other cases the wife should be investigated either before any treatment is prescribed for the husband or, if there are reasons for treating the husband quickly, at the same time.

Treatment may be given primarily to improve sperm quality (presumably by promoting spermatogenesis) or to improve sperm motility. In a small number of cases treatment is given to overcome presumed genital tract infection or because there is hyperviscosity of semen.

General Measures

It is traditional to advise moderation in smoking and drinking, plenty of exercise, adequate holidays and the wearing of loose underwear. There is no evidence other than anecdote that these measures help fertility.

Treatments to Improve Spermatogenesis

The majority of reported methods of treatment of male infertility fall into this category. Many have been tried but there is little proof that any are effective, although the occasional striking example of improvements suggests that they sometimes work. The problem is to try to predict more accurately those cases where treatments may work; basal hormone profiles and semen analysis have so far been unrewarding in this respect.

There follows a summary of the various 'best guess' treatments that have been used. In most cases, there is no justification for publication of any further uncontrolled studies of the more commonly used compounds.

Clomiphene

This compound interferes with the feedback by sex steroids on the pituitary, resulting in an increase in the secretion of pituitary gonadotrophins. Some results of treatment reported in the literature are shown in Table 13.2. Paulson (1977) tried to predict which patients were likely to respond; he found that those with depressed spermatogenesis and normal gonadotrophins were more likely to respond than those with abnormal testicular biopsies or elevated FSH levels. However, the results of a recent multicentre trial using a similar regime to Paulson showed no group or sub-group who benefit from clomiphene when compared with vitamin C (Scottish Infertility Group 1982).

Clomiphene therapy may carry risks and until there is clear evidence of benefit it should not be prescribed other than during the course of a clinical investigation. Heller et al. (1969) pointed out that clomiphene might exert a direct damaging effect on the seminiferous tubules. Our own practice is not to use clomiphene in cases where there is a history of previous or present testicular undescent because (a) this group do not respond to treatment and (b) a potentially powerful hormone could possibly stimulate latent malignancy. Clomiphene has side effects of nausea and headaches and occasionally visual disturbance and possibly cataract. If there is a family history of cataract this preparation is best not used. The USA Food and Drug Administration allow the use of clomiphene solely for the induction of

Table 13.2. Clomiphene treatment for male infertility

Reference	No. in sample	Regime	Improved semen analysis	Pregnancy	Controlled trial	Wife evaluated
Mellinger and Thompson 1966	13 men with oligozoospermia	Variable 25 or 50 mg daily for 30–35 days	10	0	No	Not stated
Heller et al. 1969	5	50 mg per day	4 improved			
	3	100 mg per day	2 depressed 2 improved	These authors conclude that at low doses benefit is seen because of enhanced testosterone levels whereas at high doses clomiphene damages spermatids.		
	4	200 mg per day	2 depressed			
	2	400 mg per day Between 2 and 12 months	2 depressed			
Palti 1970	69 men without endocrinopathy	12.5, 25, 50 or 100 mg daily for either 20, 30 or 60 days	47% improved	5	No	Not stated
Wieland et al. 1972	11 men with density less than 20 millions/ml.	5 or 10 mg for 12 weeks	No consistent changes		Yes	Not stated
Foss et al. 1973	114	100 mg daily for 10 days each month for 3 months	No consistent changes	No conclusions. 19 recorded	Randomised double-blind study	Yes
Reyes and Faiman 1974	16 men without endocrinopathy	1 mg per day for 3–9 months	Most improved	3	No	Yes

Schellen and Beek 1974	17 azoospermic 45 (0–5 million/ml) 20 (5–10 million/ml) 19 (10–20 million/ml)	50 mg for 40, 60 or 90 days	0 19 8 16	19	No	Not stated
Paulson 1977	10 men primary germinal hypofertility 47 men with normal FSH but oligozoospermia	25 mg for 25 days for 6 months	None Most improved	None 20 (42%)	Partly No	Yes
Rönnberg 1980	30	50 mg daily for 3 months Placebo	Significant improvement No change	3 1	Yes	Yes
Ross et al. 1980	179 men without endocrinopathy	100 mg three times a week	66% improved	26%	No	Not stated
Scottish Infertility Group 1982	179 men without endocrinopathy	50 mg for 25 days each month for 6 months or 200 mg vitamin daily	No consistent changes	Clomiphene 17% Vitamin C 13% N.S.	Yes	Yes

ovulation in women. Similarly, in the UK clomiphene is licensed for use in women only.

Gonadotrophins

Gonadotrophins are necessary for continued spermatogenesis and spermatogenesis can be restarted by the administration of gonadotrophins following pituitary ablation. In most cases, however, there is no evidence, either from releasing factor tests or basal hormonal profiles, of pituitary insufficiency. Thus, gonadotrophin therapy is presumably given to stimulate damaged spermatogenesis. Lunenfeld (1978) reports a collected series of reported results of gonadotrophin treatment of 275 men with sperm density less than 10 million/ml. Improvement as shown by semen analysis occurred in 72 men (26%) but only 20 pregnancies (7%) were reported. He also reports the results from a collective series of 82 men with sperm densities between 11 and 20 million/ml. Here 40 (49%) had improvement in semen analysis and 17 (21%) of wives became pregnant. These result are not encouraging and in view of the expense of the preparations used they are best reserved for those with specific endocrine abnormality, where results are very good (Lunenfeld 1978).

Androgen Therapy

There is no evidence that administration of testosterone to men with normal peripheral blood levels will have any beneficial effect. Steinberger (1977) showed that the intratesticular concentration of testosterone necessary for spermatogenesis is between 50× and 100× the plasma concentration; the high amounts within the testis are maintained by protein binding. In order to achieve levels in the blood equivalent to the normal intratesticular concentration, very high and possibly toxic oral doses would have to be given. This, however, would not help unless there were extra androgen binding protein available in the testis. The orally active testosterone derivatives are hepatotoxic and may cause jaundice which will usually disappear quickly after therapy is stopped.

Androgens have been used in three ways: (a) continuous androgen therapy, (b) rebound therapy and (c) mesterolone therapy.

Continuous Androgen Therapy

Macleod (1965) suggested that poor sperm motility in the presence of good semen density might in some cases be the consequence of deficiency of the androgen-dependent epididymis or seminal vesicles. Oral methyltestosterone is hepatotoxic and so the orally active androgen fluoxymesterolone has been used in doses of 10 mg daily with apparent improvement in sperm motility in 30 out of 58 men (Brown 1975). However, motility estimation is notoriously subjective and this work needs repeating using an objective technique for estimating motility such as that described by Makler (1978).

Rebound Therapy

Androgens are given in large enough doses to suppress secretion of gonadotrophins and cause cessation of spermatogenesis. When therapy is stopped the surge of

Table 13.3 Testosterone rebound therapy for male infertility

Reference	No. in sample	Regime	Observation
Heller et al. (1950)	20 residents of an institution for the mentally handicapped	20 mg testosterone propionate i.m. for 24–96 days	Suppression of spermatogenesis followed by improved spermatogenesis as judged by serial testicular biopsy
Heckel et al. (1951)	5 men with sperm densities between 31 and 63 million/ml	50 mg testosterone propionate i.m. 3 times per week until azoospermic	Suppression of spermatogenesis followed by improvement as judged by semen analysis
Charny (1959)	168	50 mg testosterone propionate i.m. 3 times per week until azoospermic	34 improved. 123 not improved. 11 made worse
Rowley and Heller (1972)	157 men given 163 courses	Norethandrolone 10 mg orally b.d. plus testosterone enanthate 200 mg i.m. on days 1, 21 and 42	110 improved. 24 not improved. 13 made worse. 67 conceptions
Lamensdorf et al. (1975)	131	100 mg depotestosterone cypionate i.m. weekly	40 improved. 45 not improved. 38 conceptions. 2 permanently damaged semen analysis

gonadotrophin causes renewed spermatogenesis. In Table 13.3 some reported results of this therapy are shown. The results are difficult to evaluate because the definitions of 'oligozoospermia' and 'an infertile marriage' vary from report to report. In the Rowley and Hellen (1972) series 67 courses of treatment were given to men with pretreatment sperm densities of 10 million/ml and 20 conceptions resulted. However, the preparation norethandrolone used by Rowley and Heller has been withdrawn from the market and these results have not been confirmed elsewhere.

Mesterolone Therapy

Mesterolone is a synthetic androgen which cannot be aromatised to oestrogen. This property is thought to account for the small effect mesterolone has on negative feedback, as feedback is attributed in part to oestrogens. The other advantage of mesterolone is a lack of hepatotoxicity. Clinicians who have used this preparation have an impression that some cases benefit but these have not yet been defined and the reported results from unselected cases give no grounds for optimism (Table 13.4).

Bromocriptine

There are two questions to be answered before this treatment can be recommended: (a) Are there significant numbers of men with hyperprolactinaemia to be found who will respond to specific treatment with bromocriptine? (b) In the

Table 13.4. Mesterolone therapy for male infertility

Reference	No. in sample	Dose	% with improved sperm density	Pregnancies	Controlled trial. Partner evaluated
Mauss (1974)	110	50 mg	36%	–	Controlled trial
	99	Placebo	19%	–	
Guillon (1975)	141	30, 50,	31%	13.5%	–
Nikkanen (1978)	42	75 mg	93%	–	–
Jackaman et al. (1977)	40	100 mg	35%	–	–
Szöllösi et al. (1978)	42	25 mg	57%	26%	–

absence of hyperprolactinaemia does non-specific treatment with bromocriptine promote fertility? In our experience significant numbers of men with hyperprolactinaemia are not found (Hargreave et al. 1981); we do find the occasional man with levels slightly above the laboratory normal range in both infertile and fertile populations. Treatment of infertile men with slightly elevated prolactin levels and of other infertile men with normal prolactin levels in a randomised trial gave no beneficial result in terms of semen analysis or pregnancy (Table 13.5).

Table 13.5. Results from a randomised study of five modes of treatment in 92 cases of male infertility; in all cases follow-up was for 9 months after the start of treatment. (Unpublished data from the Infertility Clinic, Western Infirmary, Glasgow; 1977)

	Mean pretreatment sperm density					
	<10 million/ml			>10 million/ml		
	n	*P*	%	*n*	*P*	%
No treatment for 6 months	3	0	0	15	3	20
Mesterolone 50 mg b.d. for 6 months	10	0	0	32	5	16
Clomiphene 25 mg at night for 5 nights for 6 months[a]	–	–	–	10	1	10
Clomiphene and mesterolone	5	0	0	5	1	20
Bromocriptine 2.5 mg daily	2	0	0	10	3	30

n = no. of men; *P* = no. of pregnancies

[a] This treatment was not included among the random options for men with a mean pretreatment sperm density of less than 10 million/ml

Tamoxifen

Tamoxifen is thought to increase the release of gonadotrophin releasing hormone (GnRH), resulting in testicular stimulation by endogenous LH and FSH. Vermeulen and Comhaire (1978) reported increases in sperm count in those patients with density of less than 20 million/ml and a normal basal FSH. They also showed that tamoxifen, in contrast to clomiphene, appears to increase the gonadotrophin response during a releasing-factor test.

Δ-Testolactone

This substance inhibits the conversion of androgens to oestrogens. Vigesky and Glass (1981) reported treatment of ten men with idiopathic oligozoospermia and found an increase in circulating androgen, decrease in oestrogens and increase in sperm density.

Corticosteroid Therapy

Following observations in rats that cortisone increased testicular size and improved spermatogenesis (Gaunt et al. 1953) and Finnerty (1954), this treatment was tried by Wilkins and Cara (1954) for male children with congenital adrenal hyperplasia. They found an improvement in spermatogenesis as judged by testicular biopsy in children over 7 years old. However, when this treatment was tried in adult men without endocrine disturbance results have been disappointing and in many cases a temporary depression in semen count and motility was recorded (Table 13.6). In view of the possibility of favourable results of corticosteroid therapy in cases of antisperm antibodies this possible damage to spermatogenesis must be remembered.

Table 13.6. Corticosteroid therapy

Reference	No. in sample	Regime	Observation
Maddock et al. (1953)	4 men with rheumatoid arthritis	500–100 mg cortisone qds for 23–25 days	No change in testicular biopsy. Decreased sperm density in 2 men. Increased urinary gonadotrophins in 4
Michelson et al. (1955)	7 oligozoospermia 3 azoospermia	25–50 mg cortisone daily for 35–63 days	One patient improved sperm density. Depression of spermatogenesis with return to pretreatment levels after cessation of therapy in the others. Rise in urinary gonadotrophins. One pregnancy, by man in whom no improvement in semen parameters was found
McDonald and Heckel (1956)	11	75 mg cortisone daily for 23–334 days	No change in semen parameters and no change in testicular biopsy
Jeffries et al (1958)	6	2.5–5.0 mg cortisone q.d.s. for 6 months	Improved sperm density and motility in 2 men. One pregnancy, by man in whom no improvement in semen parameters was found
Mancini et al (1966)	8 normal 8 oligozoospermia 3 azoospermia 4 hypophysectomised	Prednisolone 30 mg or 10 mg daily for one month	30 mg—depressed semen parameters and spermatogenic arrest on testicular biopsy 10 mg—no significant effect

Psychotropic Drugs (Table 13.7)

In 1962 Blair et al. reported an unexpected improvement in semen analysis in a patient who was being treated for depression. The depressed man was in fact a control subject in a trial which was being carried out to assess the effects of psychotropic drugs on the endocrine system in schizophrenic patients. Because of this unexpected result two more depressed patients were started on treatment and again improved semen parameters were noted. Phenelzine sulphate is an inhibitor of monoamine oxidase (MAO), the enzyme that degrades neurohormonal substances such as serotonin, noradrenaline and dopamine. MAO is widely distributed through the body and the effect of phenelzine treatment could be due to either central nervous system or peripheral action. Following Blair's report there have been other reports of psychotropic drugs and their effect on fertility and some have reported astonishing improvement (Padron and Nodarse 1980) but before these results can be generally accepted controlled trials are needed.

Table 13.7. Psychotropic drugs

Reference	No. in sample	Regime	Observation
Blair *et al.* (1962)	3	Phenelsine sulphate 15 mg b.d. or q.d.s for 4 weeks	Improvement in density and motility This was a chance finding made during the course of another study
Davis *et al.* (1966)	24 (in 16 cases there were other factors that could influence fertility, e.g. varicocele)	Phenelsine sulphate 2.5–5.0 mg/kg for 26 weeks	Significant improvement in volume and density but not motility
Stewart (1966)	Not stated	Perphenazine 2 mg orally t.d.s.	Said to be of benefit
Padron and Nodarse (1980)	20	Amytriptyline 25 mg b.d. for 2–3 months	Significant improvement in density and motility.

Arginine

Schachter et al. (1973) reported improved sperm density and motility in 111 of 178 men, and 28 pregnancies in their wives, after the men were treated with arginine at a daily dose of 4 g. However, Jungling and Bunge (1978) reported no benefit in 18 selected infertile patients using the same regime. Pryor et al. (1978) (see Table 1.1) found no benefit from arginine in the course of a double-blind controlled trial.

Thyroxine

This treatment was once much favoured for male infertility but there is little evidence that there is any benefit in the absence of myxoedema.

Vitamins and Trace Elements

Various vitamins have been reported to be essential for spermatogenesis: vitamin A (Horne and Maddock 1952; Lutwak-Mann 1958); vitamin B (Moore and Samuels 1931); and vitamin C (Kuppermann and Epstein 1958; Harris et al. 1979). Recently there has been interest in the part that trace elements may play in fertility; chronic lead poisoning with associated decline in fertility is historically associated with the fall of the Roman Empire. Cadmium (see p. 11) and mercury are also harmful (Henkin 1976). Biologically essential trace elements are, in order of total body content, iron, zinc, copper, manganese, chromium, iodine and cobalt, and some deficiency diseases are now recognised; but the part these elements play in normal fertility is unknown.

Recently a relationship was demonstrated between ascorbic acid and various semen metals including zinc (Harris et al. 1979). These authors point out that ascorbic acid, a biologically-active reducing agent, and zinc, an oxidative agent, both affect oxidation–reduction transfer of the intracellular enzyme systems for the proper maintenance of osmotic turgor and also in connection with the membrane transport of mineral and other nutrients. In a controlled trial Harris et al. found that all the wives of 20 men treated with vitamin C became pregnant whereas none of the control group of 7 were successful. There were significant increases in the metal content of the seminal plasma in all men treated with vitamin C and spontaneous non-immunological agglutination, which was present in all 27 men, disappeared in the 20 who received vitamin C.

There is particular interest in the relationship between zinc and fertility. High zinc levels are known to occur in the seminal plasma, the only tissue with higher levels being the cornea. The effects of zinc medication are not yet clear but Danscher et al. (1978) found poor spermatozoal motility associated with high semen zinc levels; on the other hand, Hartoma et al. (1977) showed that men with oligozoospermia and a low serum zinc responded well to zinc sulphate therapy at a dose of 220 mg three times daily. There is some evidence that zinc deficiency may occur in association with an inadequate diet or in a malabsorption state. Halstead et al. (1972), in a controlled trial, treated 15 men with malnutrition with supplementary zinc capsules and reported improved sexual function. It is possible that infertility associated with coeliac disease or Crohn's disease may be accounted for by zinc deficiency. There may also be a zinc deficiency in impotent men on haemodialysis. Antoniou et al. (1977) reported improved potency and increased testosterone levels in dialysis patients given 150 mg daily; however, Brook et al. (1980) do not confirm this.

The results of work by Kvist (1980) suggest that after ejaculation human spermatozoa take up zinc from prostatic fluid and that it acts as a reversible inhibitor of nuclear chromatin decondensation. Kvist postulates that the physiological significance of this is that it prevents oxidative destruction and thereby preserves a potential capacity of a nuclear chromatin to decondense at the appropriate moment of male genome transfer.

Artificial Insemination by Husband (AIH)

Rohleder (1934) credits John Hunter as the original practitioner of human artificial insemination though the date of this event is uncertain. Sperm may be used for

insemination in several ways: the whole ejaculate, a fraction of the split ejaculate or either one of those after treatment of the ejaculate. Insemination may be intravaginal, intracervical or intra-uterine and varying amounts of semen may be used. The indications for AIH are often the same as for 'best guess' treatment and as a form of therapy it is as difficult to evaluate as any other: most attempts to carefully evaluate AIH suffer from the lack of a control group.

In table 13.8 are summarised some reported results (in terms of pregnancy) with various forms of AIH; the overall pregnancy rate of 25.8% is similar to that seen after many of the drug regimes and serves to underline the necessity for controlled evaluation before this form of help, which is time consuming for the doctor and stressful for the patient, can be widely recommended. The variety of insemination techniques and indications makes evaluation of these reported results even more difficult.

Table 13.8. Artificial insemination by husband—various techniques

	n	Pregnancies
Farris and Murphy (1960)	100	13
Amelar and Hotchkiss (1965)	39	22
Ulstein (1973)	35	10
Barwin (1974)	20	11
Steiman and Taymor (1977)	40	9
Moghissi et al. (1977)	60	9
Cohen and Delafontaine (1978)	73	13
Gernigon and Kunstmann (1980)	119	37
Usherwood (1980)	157	42
Total	643	166 (26%)

Indications for AIH

AIH may be the only form of therapy in cases where physical deformity prevents intercourse (see Chap. 14). It has been used to treat couples where the man has retrograde ejaculation, but the full bladder technique (p. 258) is probably a better first-line treatment. An attractive possibility is AIH for those couples where antisperm antibodies interfere with sperm transport. Usherwood (1980) reports five pregnancies in 12 cases where the Kibrick titre was greater than 1:32 but Hansen and Hjort (1980) report only one pregnancy using intra-uterine insemination in five couples despite at least four cycles of treatment; in these latter cases antibodies were detected in both serum and seminal plasma and poor mucus penetration was confirmed prior to treatment.

There is no evidence that AIH with defective spermatozoa is of benefit. Results of AIH treatment for oligozoospermia are generally poor; this is probably because a low density is often accompanied by other physical and metabolic deficiencies of sperm (Behrman and Kistner 1975).

Macleod and Hotchkiss (1942) found that in healthy men the initial 39% of the ejaculate contained 70% of all the spermatozoa and that the motility in this first portion was also superior. This knowledge was applied by Amelar and Hotchkiss (1965) who reported 22 pregnancies in 39 couples; however, men with semen density of less than 10 million/ml were excluded in their series. Farris and Murphy

(1960) also used the split ejaculate but only 13% of wives became pregnant. Both Amelar and Hotchkiss and Farris and Murphy noted the occasional case where the second portion of the ejaculate, which is usually relatively sperm-poor, was in fact superior to the first portion and this suggests that the normal sequence of ejaculation is disorganised in some cases.

Another possibility is the revitalisation of semen prior to insemination but animal work is necessary to ensure that this approach is safe and free from teratogenicity. Alternatively, apparently healthy sperm may be selected from the ejaculate by migration through columns of albumin and subsequent freezing. This has been shown to be effective in yielding good semen samples (Katz 1980) but whether a greater number of pregnancies will occur using these techniques than by chance has yet to be determined.

Artificial insemination with liquid-nitrogen-stored samples from the husband may be indicated: (a) Where samples have been stored prior to cancer chemotherapy and where the husband's prognosis is reasonable; (b) Where samples have been stored shortly after traumatic paraplegia and where the semen quality is expected to deteriorate with time; and (c) Where samples have been stored during a course of pergonal treatment for hypogonadotrophic hypogonadism.

Sperm Motility

Specific problems of sperm motility may be secondary to structural abnormalities of the sperm tail (Eliasson et al. 1977) immobilising antibodies (Shulman et al. 1978) or infection of the seminal plasma. In cases of infection unsustained motility rather than a lack of motility may be found. Specific treatment with antibiotics in the case of infection or with steroids in the case of antibodies may be highly effective. There is no treatment for structural abnormalities of the sperm tail. It should also be noted that the lubricants KY jelly and Vaseline are spermicidal and if lubrication is necessary saliva is more physiological.

A number of men will be encountered who have impaired motility but the cause cannot be defined by current laboratory tests; nevertheless, it may be justified to attempt treatment.

Substances which may be Added to the Semen Sample

Caffeine has been reported to stimulate sperm motility (Haesungcharern and Chulavatnatol 1973; Schoenfeld et al. 1975). The exact mechanism of action is not understood although it has been shown that intra cellular cyclic AMP levels are increased soon after the addition of caffeine to semen samples (Hoskins and Casillas 1975). Carnitine is found in high concentration in the epididymis and has been shown to stimulate sperm motility when added to the semen sample (Tanphaichitr 1977). Kinins, kallikrin or kallikreinin (a kinin-liberating enzyme) have also been shown to increase sperm motility (Schill et al. 1974). Whether any of these substances should be added to semen samples prior to AIH is questionable unless animal work first shows this type of approach to be free from teratogenicity.

Manipulation of the Semen Samples to Increase Motility

Most busy seminology laboratories will occasionally observe improvement in motility after the spermatozoa are resuspended in another medium and this may occur in antibody-negative patients. The cause of the 'hostile' seminal plasma is not known; it is reasonable in such cases to offer AIH with a separated sample. Shulman et al. (1978) reported removal of sperm antibodies by washing sperm in a 4% solution of human albumin.

Another approach is to select that part of the split ejaculate showing the greatest motility; usually the highest motility fraction is the first part. In these cases Amelar et al. (1980) report that the husband may be taught to withdraw during coitus after the intravaginal deposition of the first part of the ejaculate.

Systemic Treatment to Try to Improve Motility

Low dose androgen therapy (Brown 1975) and human chorionic gonadotrophin (Misurale et al. 1969) have been tried but the evidence for their effectiveness is thin. The evaluation of any treatment given to improve motility is hampered by the lack of objective methods for motility assessment in most laboratories; this may be remedied if the techniques of Makler (1978) are applied more widely.

Cigarette smoking and alcoholism may be associated with poor sperm motility (Amelar 1980) but there is no objective work to confirm this.

Infection

The topic of infection in relation to infertility is controversial because: (a) apparently favourable reports are published showing benefit from antibiotic therapy although laboratory evidence of infection is often lacking; (b) it is difficult to distinguish commensal growth from pathological growth; (c) some of the organisms which may be incriminated, e.g. *Ureaplasma urealyticum* and *Chlamydia* are difficult to culture; and (d) on routine semen analysis it is difficult to distinguish immature sperm cells from white cells.

Eliasson (1976) states that 40% of infertile men in Sweden have cytological or bacteriological evidence of inflammation of the accessory sex glands and that treatment should be given if two or more of the following criteria are present:

1. There is an excess of pus cells in the expressed prostatic fluid.
2. Palpation has revealed a clearly swollen and tender prostate and/or seminal vesicles.
3. A man has subjective symptoms indicating urethritis, cystitis or prostatitis.
4. The semen contains more than occasional white blood cells.
5. The bacteriological culture shows pathogenic bacteria or a massive growth of potentially pathogenic bacteria.
6. There is a rapid decline in motility and viability of spermatozoa resulting in less than 40% live, motile spermatozoa 2 h after ejaculation.
7. There is decreased secretory function of the accessory genital glands.
8. The wife has a urogenital infection.

Antibiotic treatment should be given if symptoms suggestive of infection are present and a heavy growth is cultured from the semen. Common pathogenic bacteria include *Neisseria gonorrhoeae, Staphylococcus aureas, Escherichia coli, Proteus vulgaris, Klebsiella and Pseudomonas aeruginosa.* It should also be remembered that common commensal organisms such as enterococci and *Staphylococcus albus* may also be pathogenic if growth is sufficiently heavy. Most infertility patients, however, have no obvious symptoms referable to infection and the findings on semen analysis are equivocal. Before the role of infection in infertility can be fully defined there is a need for clearer evidence about which organisms are commensal and which are pathogenic and for better knowledge of the bacterial flora and white cell count of seminal fluid in patients and in a fertile control population. Newer staining techniques such as that proposed by Couture et al. (1976) should help discriminate white cells more easily. The significance of *Ureaplasma urealyticum* infections has not yet been clarified. De Louvois et al. (1974) found no difference in the frequency or *Ureaplasma urealyticum* between a fertile and an infertile group of patients. However, Idriss et al. (1978) found that 44% of patients with unexplained infertility had *U. urealyticum* infections as against 32% in a control population and that this difference was statistically significant. Fowlkes et al. (1975) have demonstrated association between spermatozoa and *Ureaplasma* using the scanning electron microscope and postulate that this may contribute to decreased motility. Genital *Mycoplasma* infections have also been incriminated by Gnarpe and Friberg (1972) in a carefully selected group of patients with unexplained infertility of at least 5 years' duration.

There is also a need to know how infection may influence fertility. Teague et al. (1971) showed interference with sperm motility by *E. coli* infections and Derrick and Dahlberg (1976) have also demonstrated decreased motility consequent on several different infections. Another possible way that infection may influence fertility is by inducing autoimmune infertility. Quesada et al. (1968) found an association between genital infections and sperm-agglutinating antibodies. In our own laboratory we have found no association between antisperm antibodies and the presence of organisms as detected by aerobic culture, anaerobic culture, *Ureaplasma* culture and serological tests for *Chlamydia.* (Hargreave et al. 1982).

In Vitro Fertilisation

This treatment is logical where anatomical problems in the wife prevent the egg from reaching the uterine cavity and where these problems cannot be surgically corrected. It may also be logical if deficiencies in sperm prevent enough sperm from reaching the site of fertilisation, provided that these deficiencies do not extend to the genetic material. It seems possible that in vitro fertilisation can be achieved with semen samples that would by conventional tests be regarded as having very poor quality. There is not yet enough evidence with these techniques to know whether this approach will result in a higher than normal fetal abnormality rate when poor semen samples are used. The same rules will apply when assessing the results of this form of treatment as have been given for any of the other non-specific treatments—only more so because of the expense involved.

Conclusions

In spite of the vast amount of published work there is little evidence that any non-specific treatments are effective. My own practice is either to use vitamin C treatment or alternatively to prescribe treatments according to a clinical trial protocol. There is a need for proper evaluation of AIH and in vitro fertilisation as both these treatments require a great deal of effort and expense.

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Chapter 14

Erectile and Ejaculatory Problems in Infertility

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Introduction

Difficulties with coitus or ejaculatory failure can be a cause of infertility, although impotence is usually unrelated to fertility, occurring at an age when fertility has already been determined. Psychological problems with coitus are seldom so severe as to prevent conception and indeed, if they are, many physicians would question the advisability of parenthood.

Mechanical problems of coitus are discussed in this chapter under the following headings: physical deformities, deformities of the erect penis, impotence and ejaculatory failure. It is not the intention here to discuss the detailed treatment of impotence or the detailed management of psychosexual problems, but rather to outline the management of such cases with regard to fertility. Further details of the current management of impotence can be found in the 1981 volume of Urological Clinics of North America entitled 'Male sexual dysfunction'.

Physical Deformities Making Coitus Difficult

Coitus is often possible even with the most severe of physical deformities, provided that both patient and partner have a strong enough desire to succeed and are prepared to experiment and adapt to the circumstances. It is possible for men with spinal injuries to achieve coitus and useful advice is to be found in the monograph of Mooney et al. (1975). Greater difficulty is encountered in men with fixed flexion deformities of their hips and in these it is often impossible to achieve penetration; in such cases AIH may be necessary. If possible a technique of self-insemination should be taught to the wife. This is best done in the privacy of the patient's home where the wife uses a cervical cup or alternatively a syringe. This form of help is worthwhile and in fact is more often successful in terms of pregnancy than many other treatments for infertility.

Deformities of the Erect Penis

Deformities of the penis are seldom associated with infertility but those men with gross erectile deformity may benefit from surgical correction. One of the more common problems is persistent chordee associated with an inadequately corrected hypospadias. Occasionally men are encountered with a suspensory ligament problem but in these it is possible to obtain penetration even though satisfactory intercourse is not possible without surgical correction (Pryor and Hill 1979). There is an additional group of men with either a micro-penis or congenital, traumatic or surgical loss of the penis which makes penetration impossible. Phimosis can cause painful erections and avoidance of intercourse, but fortunately diagnosis is easy and circumcision is an effective treatment.

Lesions of the erect penis are by their nature difficult to assess. One helpful outpatient procedure is to ask the patient or his partner to take polaroid photographs of the erect penis at home, from at least two different planes. Often the extent of the deformity revealed by this technique is surprisingly great.

Patients with chordee or Peyronie's disease can be managed by surgical correction of the deformity; in gross cases, if the initial correction is unsuccessful penile implants may became necessary. Cases of micro-penis are rare and phalloplasty may enable intercourse to be achieved but is unlikely to result in fertility. The couple's desire for an early pregnancy may dictate that artificial insemination techniques take precedence to definitive surgical correction, which is carried out electively.

Impotence

Causes of impotence are shown in Table 14.1. Reversible causes due to androgen or thyroid deficiency are uncommon but unsuspected diabetes mellitus and hyperprolactinaemia should always be excluded. In many cases the cause of impotence is readily apparent from the history, for example a injury, operation or priapism—but difficulty may be encountered when such factors are absent. These patients require careful evaluation and assessment.

History

It is important to obtain an exact history of the onset of impotence so that precipitating factors such as previous surgery, trauma, alcoholism or drug abuse can be recognised. Particular note is made of any other illness or any medications the patient is taking (p. 15). Organic impotence is usually of insidious onset, in marked contrast to psychogenic impotence. The persistence of nocturnal or morning erections indicates that the impotence is probably psychogenic in origin whereas when the patient maintains the ability to ejaculate it is more likely that the erectile dysfunction is organic in nature.

Table 14.1. Some causes of impotence

Congenital		
Extrophy (epispadias)		
Hypospadias		
Agenesis of the corpora		
Microphallus		
Spina bifida		
Acquired		
1. Psychiatric:	Alcoholism	
	Religious proscriptions	
	Homosexuality	
	Marital disharmony	
	Dominant parents	
	Personal devaluation	
2. Neurological:	Central: Head injury, Tumour	
	Peripheral: Traumatic	
		Side effects of treatment
		Spinal cord injury or tumour
3. Vascular:	Large vessel disease (internal iliac atherosclerosis)	
	Small vessel disease	
	Priapism	
	Angina	
4. Endocrine:	Lack of androgens	
	Exogenous oestrogens	
	Hyperprolactinaemia	
	Diabetes	
	Hypothyroidism	
5. Drug abuse:	Alcohol	
	Most addictive drugs	
6. Side effects of treatment:	Antihypertensives	
	Psychotropic drugs	
	Anticonvulsants	
7. Traumatic	Perineal prostatectomy	
	Aorto-iliac surgery	
	Fracture of pelvis	
	Amputation of penis	
8. Inflammatory	Acute urethritis (gonorrhoea)	
	? Prostatitis	
9. Idiopathic	Peyronie's disease	
10. Associated with debilitating illness		

Examination

Clinical examination of the patient is essential and deficiency of androgens or thyroid hormones may be readily appreciated. Galactorrhoea may suggest that there is hyperprolactinaemia. Evidence of other systemic diseases should always be sought and in all cases the blood pressure is noted. In patients with a deficiency of androgens, examination of the penis may show some atrophy and the volume of the testes may be reduced. Rectal examination is carried out to exclude the presence of prostatic cancer as this will preclude any subsequent androgen therapy. The femoral pulses should be palpated for evidence of vascular deficiency.

Doppler Examination

With the Doppler probe it is possible to locate six pulses within the penis and to measure penile blood pressure (Abelson 1975); any difference between brachial and penile blood pressure may thus be detected. A difference of 40 mm Hg or greater suggests that an arterial perfusion defect is the likely cause of the impotence.

Bulbocavernosus Reflex

This is a relatively simple technique for assessing the neurological component of impotence. A stimulatory electrical current is applied to the glans penis and measurement is made of the time taken for the bulbocavernosus muscle to contract. The latency period increases with the severity of disease until in severe cases the response may disappear altogether.

Investigation of Impotence

Diabetes is excluded by examination of the urine, but if any doubt persists fasting blood sugar and glucose tolerance test are performed. Measurement of plasma testosterone and prolactin levels are helpful in excluding androgen deficiency or hyperprolactinaemia but it is rare to find such hormonal abnormalities. If there is clinical evidence of hypothyroidism circulating thyroid-stimulating hormone (TSH) should be assayed.

Nocturnal Tumescent Studies

If any doubt remains as to whether impotence is organic or psychogenic in origin, the patient may be admitted for nocturnal tumescence studies (Karacan et al. 1978). During normal sleep there are periods of cerebral activity which are characterised by the occurrence of rapid eye movements and during these episodes penile tumescence occurs. This may be monitored by placing a simple mercury gauge around the penis and connecting it to a recording apparatus. The absence of an erection on three consecutive nights is considered to be indicative of organic impotence.

Radiological Studies

Radiological studies of the penis are occasionally of value in the assessment of erectile dysfunction. Cavernosography or phallo-arteriography may prove useful when surgical correction of organic erectile deformities is being considered.

Management of Organic Erectile Dysfunction

Patients with a reversible cause for erectile dysfunction—androgen deficiency, thyroid deficiency—are easily identified and respond to the appropriate therapy.

Table 14.2. Aetiological factors in 573 men receiving penile implants

Aetiology	Total	%
Priapism	35	6
Arterial vascular insufficiency	98	17
Diabetes	98	17
Neuropathy	23	4
Traumatic paraplegia	20	4
Radical surgery[a]	92	16
Fracture of pelvis	52	9
Peyronie's disease	47	8
Psychological	85	15
Miscellaneous	23	4

[a] Following radical prostatectomy, cystourethrectomy or prostatectomy (Pryor 1981)

Those patients with irreversible erectile impotence may benefit from the insertion of a penile implant. Table 14.2 summarises the aetiological factors of 501 men who received penile implants in five different centres. A wide choice of penile implants is now available and the prostheses fall into three main groups. The semi-rigid implant designed by Small and Carrion has been well tested and gives good results (Small et al. 1975). The theoretical disadvantage of the penis being relatively stiff all the time has led to the development of more flexible prostheses of the type described by Finney (1977), Jonas and Jacobi (1980) and Subrini (1980). These prostheses certainly are more flexible but the disadvantage of the Small–Carrion prosthesis seems to be theoretical rather than practical. However, there is one area in which the inflatable prosthesis described by Brantley Scott comes into its own and that is in patients who require subsequent endoscopic instrumentation (Scott et al. 1973). This is particularly relevant in paraplegics but may also be so in older patients.

The restoration of blood flow to the cavernous tissue by surgery to the common and internal iliac arteries may improve potency (Michal et al. 1974), particularly when care is taken to preserve the autonomic nerve plexus (Sabri and Cotton 1971). The results of revascularisation procedures using microsurgical techniques (Kedia 1981) are extremely variable and many centres prefer to implant a penile prosthesis in these patients.

Ejaculatory Disorders

Ejaculatory disorders account for 10% of male problems in couples attending the infertility clinic at the Western General Hospital, Edinburgh. These problems are usually evident from history taking or because the husband is unable to provide a semen sample or produces a sample with low volume. When absent or low volume ejaculate is found, a different investigation policy is indicated (Fig. 14.1).

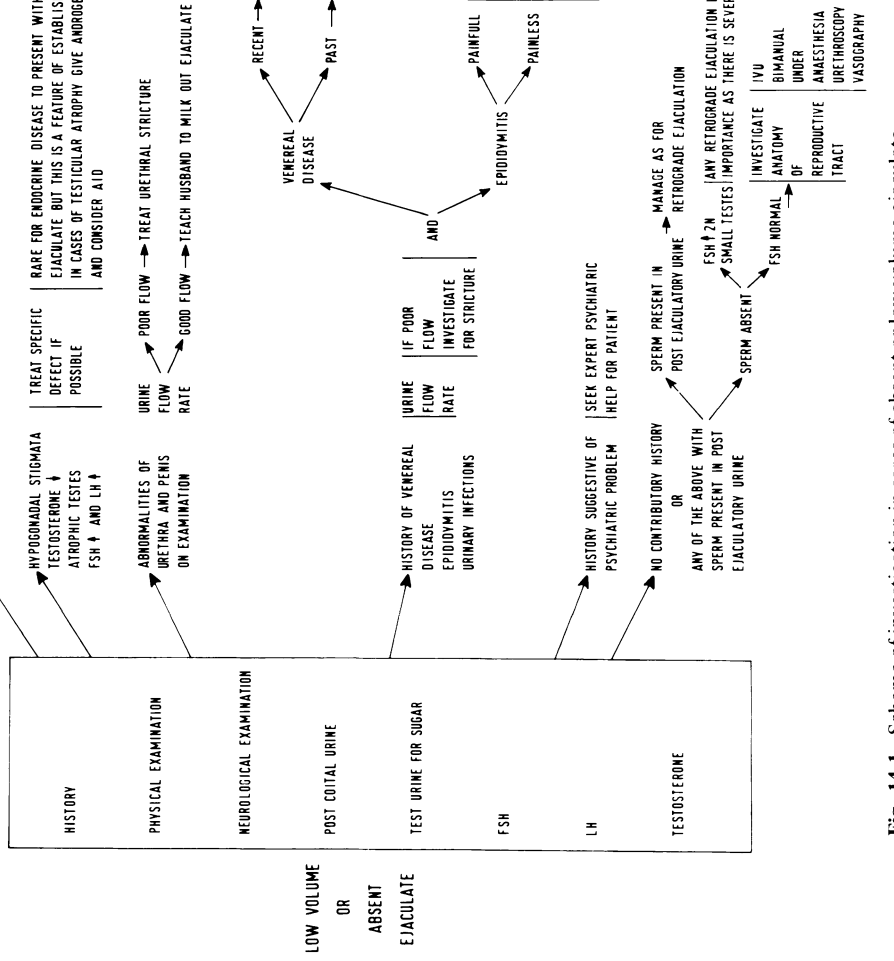

Fig. 14.1. Scheme of investigation in cases of absent or low volume ejaculate (Infertility Clinic, Western General Hospital, Edinburgh)

Psychogenic Ejaculatory Failure

Ejaculation may occur normally but at inappropriate times. Men have noticed inappropriate ejaculation at times of great excitement, for example an oilman striking oil or during fierce combat. Of concern to the infertility clinician, however, are those cases where the man is unable to have intercourse with his wife but may ejaculate during erotic dreams or when masturbating. This history may not be forthcoming at the first interview but indicates a possible psychiatric problem. The management of psychogenic ejaculatory failure is beyond the scope of this chapter: in practice however, it is rare for such cases to present to the infertility clinic and further management consists of psychosexual counselling. Likewise premature ejaculation is rarely a cause of infertility and usually responds to simple advice or pyschosexual counselling.

Neurological Problems with Ejaculation

Ejaculatory failure may be the result of neurological disorders causing interference with sympathetic nerve fibres. This may occur in multiple sclerosis, in diabetic peripheral neuropathy, after sympathectomy or accidental damage to nerves during aorto-iliac operations or abdomino-perineal resection, or as a result of anti-hypertensive drugs. Treatment is in most cases unsatisfactory and AID may be the only solution to the couple's infertility; in cases of severe neurological disease the husband's life expectancy needs to be carefully considered by the couple before they embark on AID.

Failure of the Accessory Glands

Ejaculatory failure may occur because the seminal vesicles and prostate produce inadequate secretion. This may be a primary failure, i.e. agenesis or failure of androgenic drive, or a secondary failure, i.e. consequent upon an acquired failure of testicular androgens or upon inflammatory damage.

Absence of either the seminal vesicles or the prostate is extremely rare and may be difficult to detect without endoscopic examination and bimanual examination under anaesthesia. If there is adequate semen volume (at least 0.1 ml) fructose estimation is an indicator of seminal vesicle function. This is best estimated by resorcinol colourimetry (Mann 1964). Acid phosphatase is a marker of prostatic function and may be measured by enzymatic assay (Roy et al. 1971) or radioimmunoassay (Beynon et al. 1981).

The vas deferens, epididymis and seminal vesicles, all derivatives of the mesonephric duct, are stimulated by testosterone, whereas the prostate, a derivative of the urogenital sinus, is responsive to dihydrotestosterone. Failure of the testes to produce these androgens will not only result in failure of spermatogenesis but also in a lack of ejaculatory volume. In cases where a specific endocrinopathy can be identified, prognosis is good (Chap. 6). In cases secondary to bilateral testicular damage, treatment is with androgen replacement therapy or AID.

Damage to the epididymis, seminal vesicles or prostate may result from chronic inflammation secondary to tuberculosis, venereal disease or chronic infection.

There may be a history of epididymitis, urethritis, or urinary tract infection. Tuberculous epididymitis is usually painless and there is often associated tuberculosis in the urinary tract, resulting in symptoms of urinary tract infection with the laboratory findings of a sterile pyuria. It may also be worth culturing semen for acid-fast bacilli. A diagnosis of prostatitis may be confirmed by prostatic massage and examination of the wet film of expressed prostatic secretion. Treatment involves eradication of the underlying infection. There may be some recovery of function following recent infection, although scarring of the seminal vesicles and prostate usually occur after long-standing infection

Obstruction Delaying Semen Flow

Urethral stricture is usually evident because of associated urinary symptoms, but minor strictures may not be apparent. If a patient has a poor semen volume with a past history of venereal disease it is worth doing a urinary flow estimation and urethroscopy before assuming that the poor volume is consequent upon inflammatory damage. A more common situation is the failure of ejaculation because of a previous urethroplasty. In the Denis Browne repair for hypospadias the urethra is constructed from penile skin and since this has no muscle coat, semen pools at the point where the reconstruction begins. In this case it is possible to teach the patient to milk the semen out of the urethra and for the couple to carry out AIH in their own home.

Retrograde Ejaculation

Retrograde ejaculation is the commonest cause of an absent ejaculate (Girgis et al. 1968), and may cause infertility. Many ingenious treatments have been invented and the multiplicity of techniques underlines the difficult problem this poses for the clinician. However, diagnosis can be made with simple tests and sometimes, contrary to popular belief, simple measures are effective.

In this condition the semen, instead of being propelled down the penile urethra, passes backwards through the bladder neck into the bladder. Although retrograde ejaculation is often described as a form of aspermia or lack of ejaculate, it is better defined as cryptospermia, for in uncomplicated retrograde ejaculation the semen is of normal volume and is also entirely normal in its constituents. However, it should be remembered that the secretions of the bulbo-urethral glands, which form a small part of normal semen, may be ejaculated in an antegrade manner separate from the rest of the ejaculate and thus patient may notice volumes of 0.1–0.2 ml of antegrade ejaculation which are shown by microscopic examination to be azoospermic.

Aetiology of Retrograde Ejaculation

Retrograde ejaculation is due to an abnormality of the bladder neck mechanism (Table 14.3).

Congenital Causes. Congenital causes of retrograde ejaculation are suspected when a patient complains that he has never produced an antegrade ejaculate.

Table 14.3. Some causes of retrograde ejaculation

Neurogenic
Spinal cord + cauda equina lesions
Neuropathies
Multiple sclerosis
Diabetes
Surgical injury
Retroperitoneal node dissection
Sympathectomy
Abdomino-perineal resection
Adrenergic blocking agents
Bladder neck incompetence
Congenital defects
Exstrophy
Hemitrigone
Congenital dysfuction
Bladder neck resection
Prostatectomy
Obstruction
Ectopic ureterocele
Urethral stricture
Urethral valves

There may be failure of normal development of the mesonephric duct or its derivatives (vas deferens, seminal vesicles, trigone of the bladder, prostatic urethra and ureteric buds). Examples are:

1. Ectopic ejaculatory ducts opening near the bladder neck or into the ureters (Redman and Sulieman 1976). It should also be remembered that ectopic ejaculatory ducts at the bladder neck may allow reflux of urine and thus may present as an epididymitis, especially in the presence of a urinary tract infection (Reisman 1977).
2. Ectopic ureters, causing problems either because their presence near the venumontanum obstructs the normal antegrade flow of semen (Fischer and Coats 1954) or because there is an associated malformation of the bladder neck.

Retrograde ejaculation may also occur after surgical correction of congenital abnormalities of the bladder neck and of bladder neck malfunction (Von Vogt 1971; Ochsner et al. 1970).

Urethral valves which have either been inadequately resected, or in which the bladder neck has been made incompetent by such a resection (Fuselier et al. 1976), may be associated with retrograde ejaculation.

Gross abnormalities of bladder development such as exstrophy or cloacal exstrophy may be found. Abramovici et al. (1972) describe a patient born with anal atresia and later found to have fistula between the ejaculatory ducts and rectum.

Lastly, certain congenital neurological abnormalities, such as spina bifida (Rieser 1961), may give rise to retrograde ejaculation

Acquired Causes. These are much more common, but in a proportion of patients no cause is found even after extensive investigation. The most frequently described acquired cause is surgery to the bladder neck and prostate (Reiser 1961; Girgis et al. 1968; Schirren et al. 1973). Although enucleation prostatectomy often causes retrograde ejaculation, the problem is now more commonly associated with

transurethral resection (Rieser 1961)—even minor resections may result in ejaculatory disturbance (Schirren et al. 1973). Trauma to the bladder neck after pelvic fracture may also cause retrograde ejaculation (Glazermaan et al. 1976). A common neurological cause is diabetic neuropathy especially when the diabetes is severe and is associated with peripheral neuropathy (Ellenberg and Weber 1966). In such diabetic patients it may be possible to demonstrate areas of skin anhydrosis in the distribution of L-4 to S-2 or S-3 and abnormal bladder cystometry (Green and Kelalis 1967). Retrograde ejaculation may be seen in a variety of other neurological problems, e.g. multiple sclerosis with spinal cord and cauda equina lesions, and after paralytic poliomyelitis (Schirren et al. 1973). Neurological damage has been described after the following surgical procedures: abdominoperineal resection of the rectum for both benign and malignant lesions (Schellen 1960; Williams et al. 1951), retroperitoneal lymphadenectomy (Stockamp et al. 1974), lumbar ganglionectomy (Rose 1953) and aorto-iliac surgery (Weinstein and Machleder 1974).

Drug therapy, in particular the use of adrenergic blocking agents, may also produce retrograde ejaculation (Kedia and Markland 1975). The drugs guanethidine and thioridazine hydrochloride (Melleril) are exampes (Lagrue 1973; Shader 1964).

Lastly, post-inflammatory urethral stricture is a known cause of retrograde ejaculation (Girgis et al. 1968); such strictures are now much less common and are relatively slow to develop and thus rarely present with infertility.

Effect of Urine on Sperm Motility

Normal urine has a rapid deleterious effect on the motility of spermatozoa. In the experiment described here, two fresh specimens of semen were obtained from two men with proven fertility. From these two semen specimens, 50 μl aliquots of semen were mixed with 200 μl aliquots from five urine samples taken from men attending the hospital with non-urological problems. Sperm motility was assessed visually at 5, 10, 15 and 20 min. after mixing. The results were compared with the motility of sperm suspended in 200 μl aliquots of Baker's buffer (Table 14.4), a glucose-containing buffer known to maintain sperm viability. Contact of the sperm with urine samples resulted in a marked reduction in motility, in some cases total ablation of all sperm movement within 5 min. of mixing. The results are summarised in Table 14.5. It was also seen from this experiment that even if the pH of the urine was above 7.0, this reduction in motility still occurred; in only one sample was motility maintained and the pH of this particular sample was as low as 4.6 (Crich and Jequier 1978).

Thus, if the viability and the fertilising potential of spermatozoa are to be preserved, the semen must not come into contact with urine even for a very short period of time.

Table 14.4. The formulation of Baker's buffer (pH8.1)

Glucose	3.0 g
$Na_2HPO_47H_2O$	0.46 g
$Na_2HPO_412H_2O$	0.60 g
NaCl	0.2 g
KH_2PO_4	0.01 g
Water to volume of 100 ml	

Table 14.5. The effect of male urines and the concentrations of their constituents on the motility of spermatozoa in 2 normal fertile semen specimens (50 μl semen + 200 μl urine)

Urine no.	Osmolality mosmol/ kg.H_2O	pH	Na^+	K^+	Cl^-	Urea	Donor I 5 min	10 min	15 min	20 min
1	548	7.1	136	40	170	120	10%	10%	5%–10%	Nil
2	479	6.3	42	11	63	207	20%	10%	5%	Nil
3	315	4.6	87	34	113	65	15%	5%	Nil	–
4	155	7.3	15	21	18	72.5	Nil	–	–	
5	136	6.3	19	10	17	65	Nil	–	–	
Baker's buffer	276	8.0	15.4	0.21	21	Nil	30%–40%	30%–40%	30%–40%	90%

Table 14.5. (*continued*)

Donor II 5 min	10 min	15 min	20 min
20%–30%	10%	Nil	–
20%	Nil	–	–
90%	60%	50%	60%
10%	10%	Nil	–
10%	5%	Nil	–
90%	70%–80%	70%–80%	

Na^+, K^+, Cl^-, Urea } expressed as millimoles/litre

Motility % of spermatozoa showing active forward movement.

Diagnosis of Retrograde Ejaculation

The husband usually complains of a lack of ejaculate volume and sometimes may notice that after intercourse the urine looks turbid. The diagnosis may be unsuspected until semen samples are examined in the infertility clinic and low volumes (<1.0 ml) are consistently found. History may provide an indication of the cause for retrograde ejaculation. A full neurological assessment is often necessary and in the absence of a definitive diagnosis further investigations by videocystometry, cystourethroscopy and vasography may be indicated.

Post-Coital Urine. The diagnosis of retrograde ejaculation is confirmed by the finding of large numbers of spermatozoa in a urine specimen taken soon after intercourse. The patient is provided with a 500-ml container and requested to void urine following coitus. After thorough mixing two 10-ml aliquots of the urine specimen are centrifuged and the supernatant discarded. Wet preparations of the sediment are then examined microscopically. The number of spermatozoa per ml of urine are then assessed visually or, more accurately, by counting in a Neubauer

haemocytometer. In this way the total sperm content of 10 ml of urine as well as the total sperm content of the whole specimen can be calculated.

It should be remembered that if a postcoital urine specimen is taken from men who ejaculate normally, the urine will also contain some sperm which have remained in the urethra after ejaculation. If urine specimens from normal men are examined in the way described above only the occasional spermatozoon will be seen in the urine aliquots, even after centrifugation, while in men with retrograde ejaculation, upwards of 100 million sperm are usually present in the whole urine specimen (Crich, unpublished data).

The diagnosis of retrograde ejaculation is very difficult when it is associated with azoospermia or severe oligozoospermia.

Treatment of Retrograde Ejaculation

1. *Drug Therapy.* Being simple and non-invasive, drug therapy would seem to have many advantages in the treatment of retrograde ejaculation. Theoretically, sympathomimetic agents would seem to be the most useful for, as has been stated above, it is the adrenergic blocking agents that may cause retrograde ejaculation. However, the use of sympathomimetic agents, although occasionally successful (Stewart and Bargant 1974; Sandler 1979; Stockamp et al. 1974), has produced poor results. This may depend on the cause of the retrograde ejaculation in each individual patient—for example, one would not expect these drugs to work in a patient with ectopically sited ejaculatory ducts, while a favourable response might be seen in a patient with retrograde ejaculation secondary to a neurological lesion where the sympathetic nerve supply has been damaged.

Some success has been achieved by drug therapy in diabetes by the use of the antihistaminic and anticholinergic agent brompheniramine maleate (Andaloro and Dube 1975; Budd 1975). This drug is given in doses of 8 mg twice daily and may be of value in diabetic patients with retrograde ejaculation although anticholinergic side-effects such a dry mouth and visual symptoms can be a problem.

2. *Surgery.* Surgery may be indicated where there is gross anatomical abnormality following previous surgery or trauma, in cases where both ureteric orifices open ectopically into the prostatic urethra or where congenital deformity of the tissues makes it unlikely that any conservative management will work. Good results may be achieved (Abrahams et al. 1975). The Young–Dees operation converts the funnel-shaped urethra into a tube, thereby lengthening the prostatic urethra. In cases where there is not enough tissue to give adequate urethral length the Leadbetter technique may be used; both ureters are reimplanted and a neourethra is created from the trigone. This operation is improved if an omental flap is created and used to fill the retropubic space thus keeping the neourethra and the bladder apart (Johnston 1977).

3. *Retrieval of Semen from the Bladder by Post-Ejaculatory Catheterisation.* This method of treatment of infertility due to retrograde ejaculation is the most commonly used and several pregnancies have been described. The patient is asked to restrict fluids for 6 h prior to ejaculation (Hotchkiss et al. 1955) and a dose of sodium bicarbonate may be given in order to raise the pH of the urine (Glazermaan et al. 1976). Hotchkiss et al. (1955) described the use of a pre-ejaculatory bladder washout using 180 ml of 5% dextrose–Ringer solution aftter which 2 ml is left inside the bladder. A larger volume of Ringer lactate may also be used (Fuselier

et al. 1976; Walters and Kaufman 1959). Immediately after masturbatory ejaculation, the semen is retrieved either by catheterisation and a further bladder washout or by post-ejaculatory voiding of the semen (Hotchkiss et al. 1955). The semen is then used for AIH. However, these measures are invasive and can easily lead to urinary tract infection and in a patient with ectopically sited ejaculatory ducts epididymitis may result. Also such procedures must be very unpleasant for the patient. Most important of all, even momentary contact with urine can immobilise motile sperm and the procedures may have to be repeated many times before a pregnancy is achieved (Bourne et al. 1971). Nevertheless, pregnancies have resulted from such treatment and even simple postcoital intravaginal voiding of semen has reportedly once resulted in conception (Schram 1976).

4. *Ejaculation on a Full Bladder.* A simple method of achieving antegrade ejaculation now described has proved most successful. Antegrade ejaculation may result when patients try to ejaculate with a full bladder (Crich and Jequier 1978). This may seem paradoxical after all that has been said about the effect of urine on sperm motility, but the method is simple, non-invasive and effective. It is believed that if ejaculation takes place with the bladder full the weight of the urine, especially when the patient is standing up, prevents the semen from refluxing through the bladder neck; it is also possible that the bladder neck shuts more tightly when the bladder is full rather than empty.

The patients are asked not to pass urine, if possible, for 3 hours prior to ejaculation and preferably to have a drink of water, tea or coffee during that time. No other preparation of the patient is required. If, as is often the case, a reasonable volume of semen (1 ml or more) is obtained by antegrade ejaculation, this is washed immediately in Baker's buffer, and used for AIH. If very little or no antegrade ejaculation results, the patient is instructed to pass immediately the first 5 ml of urine into a suitable container. The remainder of the bladder content can then be discarded. The semen obtained by either antegrade ejaculation or this method of collection of retrograde ejaculate is usually minimally contaminated by urine, and the motility of the spermatozoa is unaffected. With reassurance and 'training' this technique, where an antegrade ejaculate is obtained, can be applied to normal intercourse. Patients can also be instructed in AIH at home using collected retrograde ejaculate (Crich and Jequier 1979).

5. *Stimulation of Ejaculation.* Ejaculatory failure in paraplegic patients may be stimulated by electro-ejaculation or by the use of a vibrator. The latter technique may also be used in those patients with psychogenic ejaculatory failure. The management of the paraplegic patient is the subject of the next chapter.

Conclusions

Retrograde ejaculation is the commonest cause of ejaculatory failure and should be considered in all cases where there is an absence of ejaculation or low semen volume. The diagnosis is confirmed by the finding of large numbers of sperm in the post-ejaculatory urine. A simple technique of training the husband to have intercourse with a full bladder will result in antegrade ejaculation and a 50% pregnancy rate. Some cases, however, remain very difficult to treat.

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Chapter 15

Physiology of Erection and Management of Paraplegic Infertility

G. S. Brindley

Normal Physiology

Vascular Mechanism of Erection

The penis contains two erectile spaces, the cavernosal and the spongiosal, and erection depends on the filling of these spaces with blood. The cavernosal space consists of the right and left corpora cavernosa, which communicate freely with each other; the spongiosal space consists of the corpus spongiosum and the erectile tissue of the glans, which communicate freely with each other but not with the corpora cavernosa.

The principal mechanism of erection is an increased inflow of arterial blood into the erectile spaces. For the dog this was directly demonstrated by Eckhardt (1863): if the flaccid penis is cut open, the erectile spaces bleed only slowly, but bright red blood pours out from them as soon as erection is induced by stimulation of the appropriate nerves. For man, the evidence for increased inflow during erection has until recently been indirect, but this indirect evidence has been good enough to convince every writer on the subject: the erect penis is always warm and pulsates with the heart-beat; the flaccid penis is often cold and does not pulsate. Fig. 15.1 confirms the familiar experience that a cold penis becomes warm when it erects. The changes in temperature are far too rapid to be explained by changes in penile metabolism; they must depend on heat carried to the penis by the blood. If the inflowing blood is at the same temperature in the erecting as in the flaccid penis (and anatomically no variable heat-exchanger exists that could make this assumption substantially false), the rapid transition from steady to rising temperature seen in Fig. 15.1 at the beginning of each erection can only imply that more blood per second flows into the erecting penis than through the flaccid penis. When sexual stimulation ceases, Fig. 15.1 shows for the glans a rapid transition from rising to falling temperature. For the dorsolateral shaft this transition is much less clear, perhaps (but not certainly) because the thermistor is then further from the erectile tissue. If we again assume constant inflow temperature, we can conclude that less blood per second enters the glans when the penis is shrinking than when it is stably erect. A corresponding conclusion for the cavernosal space would be insecure.

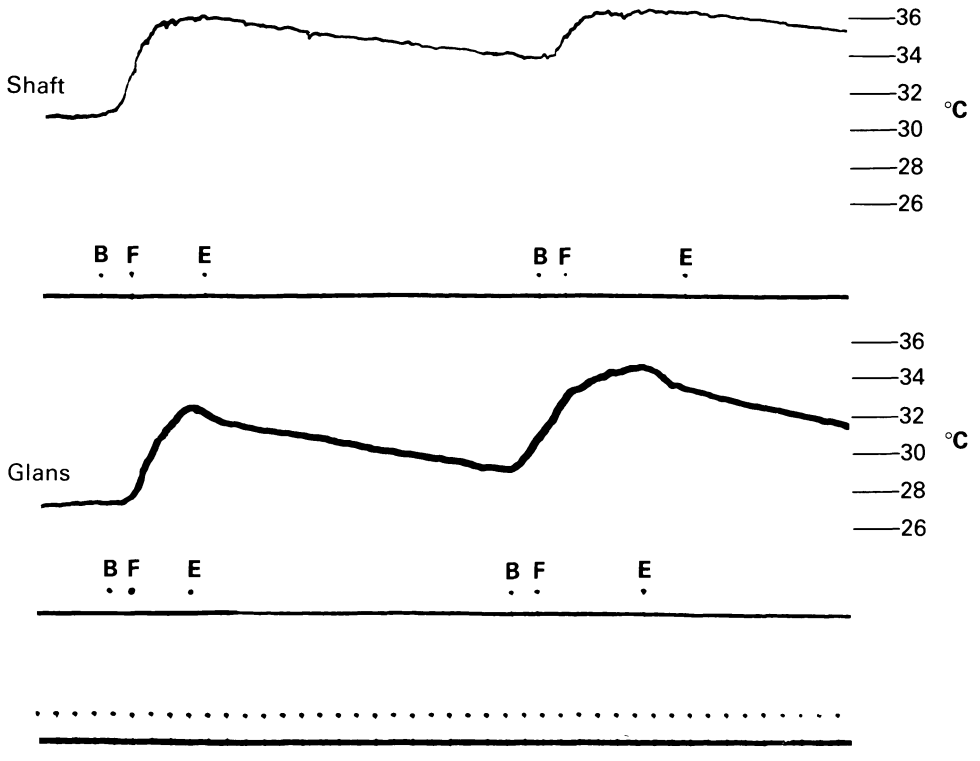

Fig. 15.1. Surface temperature of the flaccid, swelling, stably erect and shrinking penis. The measuring thermistor was glued to the skin of the dorsolateral shaft for the upper trace, and to the glans for the lower trace. In each group of three event-marks, *B* represents the beginning of sexual stimulation (fantasy and application of vibrator). *F*, coming about 90 s later, represents the attainment of full erection. *E* represents withdrawal of sexual stimulation, after which visible shrinkage of the penis began within about 5 s. The time-marks indicate minutes

The blood outflow from the flaccid penis has been measured by xenon washout (Wagner and Uhrenholdt 1980), and found to be 2.5–8 ml/min. Since the erecting penis can swell by over 100 ml/min, this establishes the first of the two points argued above from indirect evidence: more blood per second flows into the erecting penis then through the flaccid.

In the erect penis the corpora cavernosa are hard, and squeezing them between finger and thumb will not collapse them. The spongiosal space, on the contrary, is soft and easily compressed. By milking the corpus spongiosum of the erect penis from the tip towards the base, one can make the glans and the distal part of the corpus spongiosum flaccid while the corpora cavernosa remain fully erect. These simple observations show that in the erect penis the spongiosal space has very much freer venous drainage than the cavernosal space. The hardness of the cavernosal space of the erect penis suggests that its venous drainage is obstructed by some mechanism that does not operate in the shrinking or flaccid penis. This guess has indeed often been made (Conti 1952 and many earlier writers), but almost equally often doubted (e.g. Newman et al. 1964).

Until recently, only very weak arguments have been given either for or against regulated venous closure, but Fig. 15.2 shows clearly that the venous channels that drain the cavernosal space can be greatly influenced by the presence or absence of sexual stimulation. A sphygmomanometer cuff is applied around the erect penis, and inflated to 220 mm Hg, i.e. well above the systolic blood pressure. The pressure in the cavernosal space must then rise to very near this pressure; therefore no arterial blood can flow into it. The cross-sectional area of the penis, measured by its longitudinal electrical conductance, is found to fall only very slowly if vibratory stimulation is applied to the glans, but rapidly if there is no vibratory stimulation and the subject's attention is concentrated on non-sexual matters.

An extension of the experiment of Fig. 15.2 proves that contraction of the somatic muscles innervated by the pudendal nerves plays no important part in the regulated venous closure: if, while the cuff is inflated and the subject's attention directed to non-sexual matters, a maximal pelvic floor contraction is induced by stimulation of the pudendal nerves by the technique of Brindley et al. (1974), the shrinkage of the penis continues unchecked.

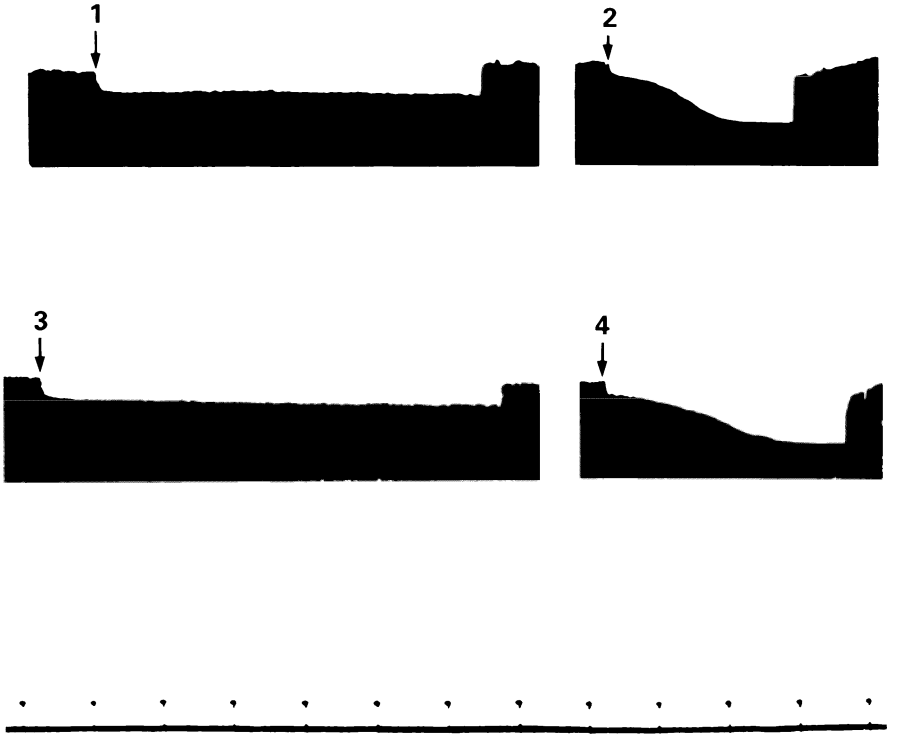

Fig. 15.2. Longitudinal electrical conductance of the initially erect penis, constrained by an enveloping sphygmomanometer cuff to have an intracavenosal pressure of 220 mm Hg. In all four records the cuff was inflated at the arrow. Even the erect penis is sufficiently compressible for the inflation to cause an immediate fall in conductance. In records 1 and 3 the sexual stimulus (fantasy and application of vibrator to glans) that caused the erection was continued until the cuff was deflated $2\frac{3}{4}$ or $3\frac{1}{4}$ min later. The conductance remains nearly stable. In records 2 and 4 sexual stimulation was withdrawn as soon as the cuff was inflated. The conductance falls rapidly. The time-marks indicate minutes

The blood outflow from the shrinking penis has been found by xenon washout to be several times higher than that from the stably erect penis (Wagner and Uhrenholdt 1980). This provides an alternative clear proof that the venous drainage is regulated.

The vessels supplying and draining the human penis have been described in detail, from their own and earlier observations, by Stieve (1930) and by Conti (1952). It is generally agreed that the arteries to erectile tissue contain specialised 'cushions', consisting of longitudinal muscle lying between the inner and outer layers of the internal elastic lamina. These cushions look as though they could occlude the arteries. Conti described similar structures in certain of the veins draining erectile tissue, but he found them in those veins that, according to him, mainly drain the glans, and not in those that he believed to drain the corpora cavernosa. There is thus an obscurity about the anatomical basis of regulated venous drainage, although there can be no doubt from the experiment shown in Fig. 15.2 and from the observations of Wagner and Uhrenholdt that the venous drainage of the corpora cavernosa is regulated, and that at constant intracavernosal pressure the outflow can vary as much as by a factor of 10. Even on the arterial side, the traditional opinion that the 'cushions' are the structures that control flow depends solely on their histological appearance. Newman and Tchertkoff (1980) have argued that they play no important part in erection. Their arguments are not compelling; but neither are those for the traditional view.

Psychogenic and Reflex Erection

Nearly all men have erections when they think sexual thoughts, and in nearly all men the penis thus 'psychogenically' erected can, without other aid, be stiff enough for intromission.

In most men if the penis is stimulated mechanically by friction or by a vibrator it tends to become more erect. But this 'reflex' erection is quantitatively very variable. In some men, mechanical stimulation will produce full erection while the whole attention is directed to a non-sexual matter, e.g. mental arithmetic. In others, equally sexually potent, mechanical stimulation has no measurable effect on the penis while the attention is directed to non-sexual matters, and 'reflex' erection is detectable only in that the penis erects faster and more fully when sexual fantasy is supplemented by mechanical stimulation than when it is unsupplemented. Among eight healthy men between 25 and 45 years of age, unselected except for willingness to be experimental subjects, Dr. P. W. Gillan and I found two of the first kind, three of the second, and three intermediate. Even in the two men in whom vibratory stimulation at 80 Hz caused full erection while they were doing mental arithmetic, this reflex response could be inhibited almost completely by painful electrical stimulation of the teeth.

Ejaculation and Orgasm

In reaching orgasm, as in achieving erection, both psychological and reflex factors can contribute. But whereas for erection the psychological factors are usually the more important, for orgasm the reflex factors predominate. Only a tiny minority of

men, three or four out of 5000 according to Kinsey et al. (1948), can reach orgasm without mechanical stimulation. Many men say that they can do so only by combining mechanical stimulation with a real or imagined sexual situation, but many others can masturbate to orgasm without the aid of any sexual fantasy. Ejaculation and orgasm do not necessarily require erection. Erection can be prevented by compression of the pudendal arteries by a mechanical device. If this is done, ejaculation and orgasm can still occur, the penis remaining entirely flaccid.

The reproductively essential parts of ejaculation are the contraction of the cauda epididymidis, vas deferens and seminal vesicle of each side, of the prostate, and of the bladder neck. If they alone occur, the process is called 'emission'. Semen thus emitted will appear at the external urinary meatus if it is plentiful, but may remain in the upper urethra if its volume is small. Normally emission is supplemented by a series of from four to ten or more contractions of the external sphincter of the urethra and the bulbospongiosus muscle, which expel the semen from the urethra in spurts. The contractions follow each other at increasing intervals, the second being about 0.8 s after the first, but the last two separated by 2 s or more. These contractions, though they are of 'voluntary' muscle, are strictly reflex and not inhibitable by any effort of will. They are often preceded by a state in which there is a strong *tendency* to make various more or less rhythmic pelvic and other movements; but this state is distinguishable in that the movements involve many more muscles, are less stereotyped, and can be inhibited by effort of will.

'Orgasm' is the name given to the subjective experience that accompanies ejaculation in men, and to a very similar experience of women. Pelvic floor contractions occur at female orgasm, and have practically the same temporal pattern as in men. In both sexes there is often a sudden decrease in heart-rate at the time of the pelvic floor contractions.

The discharge of semen into the urethra can be prevented by α-adrenergic blocking agents (phenoxybenzamine, phentolamine). Under the influence of these drugs the subjective experience of orgasm remains almost unchanged, and the characteristic rhythmic contractions of the pelvic floor occur normally. These contractions are thus not reflex responses to filling of the upper urethra with semen.

Orgasm is followed in most men and many women by a refractory period lasting many minutes, sometimes many hours, in which a second orgasm cannot occur. There is often also a refractory period for erection, shorter than that for orgasm. These refractory periods must be properties of the spinal cord or the brain, for there is no corresponding refractoriness in any of the peripheral afferent or efferent pathways or end-organs concerned.

Peripheral Nervous Pathway Concerned in Erection and Ejaculation

Sacral Parasympathetic Outflow

It was shown first for the dog (Eckhardt 1863), and later for many other mammalian species, that on the medial surface of the levator ani muscle there is a pair of nerves or plexuses (nervi erigentes, pelvic nerves, pelvic splanchnic nerves), stimulation of which causes erection. The fibres of these nerves belong anatomically to the parasympathetic system, and they originate in sacral roots. In man erection can be produced by stimulation of the S-2, S-3 or S-4 roots (Habib 1967; Brindley et al. 1982). Only two of the roots are effective in any one subject. In both

of Habib's patients, S-3 was the major erectile root and S-4 the minor. In three of our five patients, S-3 is the major erectile root and S-2 the minor; in the other two, S-2 is the major erectile root and S-3 the minor.

The parasympathetic erectile pathway in the baboon is very resistant to atropine, and also to α- and β-adrenergic blocking agents (Brindley and Craggs, 1976). Reflex and psychogenic erection in man are similarly resistant (Wagner and Brindley, 1980). The peripheral transmitter therefore is probably not acetylcholine or noradrenaline. There is no evidence to suggest what it may be. Ganglion-blocking agents readily produce erectile impotence in man, so probably the ganglionic relays, both in this pathway and in the sympathetic erectile pathway (see below), are cholinergic.

Sympathetic Outflow

In all mammals studied so far there lies just in front of the bifurcation of the aorta and the left common iliac vein a nerve or plexus (hypogastric nerve, hypogastric plexus, presacral nerve), stimulation of which causes contraction of the vasa deferentia, seminal vesicles, prostate and bladder neck. Fibres of this plexus arise from the upper lumbar and perhaps lowest thoracic roots. They belong to the sympathetic system, and are largely preganglionic and myelinated. Reports of the effects of stimulation of the hypogastric plexus on the penis have been very conflicting, some investigators describing shrinkage and some erection, even in the same species (rabbit, cat, dog). The matter was much clarified by Bessou and Laporte (1961), who found that in the majority of anaesthetised cats stimulation of the hypogastric plexus caused shrinkage of the penis at first, but then erection if it was continued for 30 s or more. The α-adrenergic blocking agent dihydroergotamine abolished the shrinking response, leaving a pure erectile one. In baboons anaesthetised with pentobarbitone, I have seen only shrinkage of the penis in response to stimulation of the hypogastric nerve, except under the influence of the α-adrenergic blocking agent phentolamine; then the response becomes erectile. It seems that in the cat and baboon the hypogastric plexus contains both erectile and anti-erectile fibres, and that the anti-erectile ones can be partly or wholly blocked by α-adrenergic blocking agents. In the rabbit also there are erectile and anti-erectile sympathetic pathways, but they are anatomically separate at the level of the bifurcation of the aorta (Sjöstrand and Klinge 1979). In one human patient with a radio-linked implant to stimulate the hypogastric plexus, such stimulation under general anaesthesia consistently causes erection, together with seminal emission (Hendry and Brindley, unpublished observations). It seems probable that the presence of both erectile and anti-erectile sympathetic pathways is general for mammals, including man.

Lumbar sympathectomy (Whitelaw and Smithwick 1951) or operations on or near the bifurcation of the aorta that endanger the hypogastric plexus (Weinstein and Machleder 1975) cause erectile impotence in some men, and ejaculatory failure without disorder of erection in others. The observations on sympathectomies make it likely that the segmental levels needed for ejaculation in man are lower than the thoraco-lumbar ones concerned in erection.

Psychogenic erection can still occur when the sacral parasympathetic outflow is destroyed (see next section). Whether it then depends on activation of sympathetic erectile fibres, inhibition of sympathetic anti-erectile fibres or both of these together is unknown.

The human sympathetic anti-erectile pathway appears to be α-adrenergic and to possess tone in non-sexual mental states, on the evidence that 2 mg/kg phenoxybenzamine (which does not prevent psychogenic or reflex erection) causes subpriapism in normal men. In one man who lacked both psychogenic and reflex erection because of a complete T-11 spinal cord lesion and a concurrent sacral root lesion, I have seen phenoxybenzamine cause full priapism, lasting about 1 h.

Somatic Motor and Afferent Pathways

Though the ischiocavernosus and bulbospongiosus muscles play no important part in the regulated venous closure that occurs during erection, they can raise the pressure in the cavernosal space by squeezing it, and thus make an incomplete erection more complete for as long as they can maintain a powerful contraction; but they cannot do this for more than a few seconds, because of fatigue. This temporary effect on erection, and the important part played by the external sphincter of the urethra and bulbospongiosus muscle in ejaculation, depend on the pudendal nerve, which is also the main afferent pathway (and in intact men perhaps the sole afferent pathway) for reflex erection and ejaculation. In men with complete spinal cord lesions the receptive field for reflex erection may spread outside the area supplied by the pudendal nerve.

The roots carrying sensory fibres from the penis are S-2 and S-3, mainly the former (Bohm et al. 1956; Brindley and Cardozo, unpublished observations). Textbook statements and diagrams showing S-5 (e.g. Brügger and Rhonheimer 1980) or L-1 (e.g. Brocklehurst 1976) as the principal supply are certainly wrong. The reflex afferent fibres from the penis are probably a subset of the sensory fibres, or at least travel in the same roots.

Central Nervous Structures Concerned in Erection and Ejaculation

Mechanisms for reflex erection exist in the sacral segments of the cord, as is proved by the preservation of the reflex in the majority of patients in whom these segments are isolated from the rest of the central nervous system. In intact men there must be inhibitory descending fibres that can act on these cord mechanisms, since reflex erection can be inhibited by pain. The spinal erection reflex can certainly (on the above evidence) use the parasympathetic efferent pathway. Whether it can also work through the erectile or anti-erectile sympathetic efferent pathway is unknown.

Reflex ejaculation in men with complete transections of the spinal cord was formerly thought to be rare, but is now known to be common if the correct stimulus is applied. The mechanisms must involve a number of segments of the cord, from S-3 up to L-1 or higher.

The course of the spinal tracts concerned in sexual responses can in principle be investigated by studying patients with incomplete cord lesions. The best studied lesions are bilateral anterolateral cordotomies, done, usually at an upper thoracic level, for the relief of pain. After these operations many patients have said that they have lost erection and ejaculation. Some, however, have retained erection and ejaculation but lost the orgasmic sensations that should accompany ejaculation, and probably also the lesser erotic sensations that ordinarily accompany erection (Foerster and Gagel 1932—Case 3; White and Sweet 1969). The tactile threshold of the penis is unchanged, and so, probably, are two-point discrimination and the ability to detect vibration.

From these observations and from analogous ones on women there seems to be little doubt that the afferent pathway for erotically coloured genital sensations in both sexes runs with or close to the spinothalamic pathways for pain and temperature, at least at upper thoracic level. It is unknown whether the downgoing pathways for psychogenic erection and for the messages from the brain that assist the achievement of ejaculation are interrupted by anterolateral cordotomy. We can suppose them uninterrupted, and explain the common reports of lost erection and ejaculation as consequences of the loss of erotic sensations; or we can suppose them interrupted, and interpret the preserved erection and ejaculation less commonly reported as spinal reflexes.

The sexual responses of men with incomplete spinal injuries have been described and tabulated in several publications. But in no such source known to me have the responses been correlated with the kind of incompleteness of the lesion. Our ignorance of the course of the tracts involved remains deep.

Within the brain, many sites have been found at which electrical stimulation causes erection in the unanaesthetised squirrel monkey (MacLean and Ploog 1962) and rhesus monkey (Robinson and Mishkin 1968). Regions that are often effective in both species include the anterior part of the cingulate gyrus, preoptic region, lateral hypothalamus, and tegmentum. In these regions ineffective sites are found, mixed up among the effective ones. Regions of the brain that are consistently ineffective include the hippocampus, fornix, mammillary bodies, posterior cingulate gyrus, caudate nucleus, ansa lenticularis, and the genital receiving area of the postcentral gyrus. In men, among the many published accounts of effects of stimulation in the course of stereotaxic operations under local anaesthesia, I can find no report of erection being produced. Reference 24 of Weiss (1972) is said to be such a report, but is not. Sem-Jacobsen (1966) states definitely that in 429 electrode placements near the third ventricle in 82 patients, the responses to electrical stimulation, though they included movements, changes in tremor or rigidity, speech arrest, sensations of many kinds, mood changes, loss of consciousness and cardiovascular effects, were never sexual.

Genital sensations produced by electrical stimulation of the upper end of the postcentral gyrus are not erotically coloured, according to Penfield and Jasper (1954).

There is one clear and convincing report of lasting impotence produced, similarly in two patients, by a small destructive surgical procedure in the brain (Meyers 1962). The purpose of the operation (done bilaterally in two stages) was to cut the ansa lenticularis by an open procedure from the third ventricle. The approach was through the corpus callosum, and a piece of the fornix was sacrificed bilaterally to gain access to the third ventricle. Lesions of the corpus callosum and of the fornix have not caused impotence in other patients, nor have stereotaxic lesions that were intended to interrupt the ansa lenticularis bilaterally. Probably the impotence in the two patients of Meyers depended on damage to some neighbouring structure, but we have little knowledge to help us speculate on what structure it was.

Tricyclic antidepressants and, less frequently, monoamine oxidase inhibitors can cause failure of erection and ejaculation. For the tricyclics the effect is often attributed to their peripheral anticholinergic action, but this hypothesis is certainly false, since large doses of atropine leave erection and ejaculation unaffected. For both groups of drugs the depression of sexual responses probably depends on an action on the brain or spinal cord; where or how is unknown.

The above are the only facts, other than of pure anatomy, known to me, that

bear on the physiology of the mechanisms within the brain concerned in sexual responses. They do not suffice to build a theory that inspires any confidence, and I think it would be premature to attempt such a theory until more facts have been collected.

Sexual Function in Men with Spinal Injuries

Reflex Erection

Men with complete spinal injuries of more than a few weeks' duration usually have some reflex erection unless the sacral segments of the cord are destroyed. The completeness of the erection and the length of time for which it can be sustained vary greatly. Part of this variation doubtless depends on ischaemic or other damage to the sacral cord short of complete destruction. But even with long-standing cervical lesions where there is no clinical evidence of ischaemic or cavitational damage to the lumbosacral cord, reflex erections may be slight and ill-sustained, or may be absent. This is not surprising in view of the variability of reflex erections among normal men in a neutral non-sexual mental state; much of the variability among paraplegic men may reflect variations that existed before injury.

Psychogenic Erection

The papers of Bors and Comarr (1960) and Comarr (1970) record 4 out of 77 patients with complete cervical lesions and 42 out of 251 patients with complete lesions below T-8 who said that they had psychogenic erections. The four patients with cervical lesions are very surprising if their statements are true. But it is possible they could be honest but mistaken, since all had reflex erections, and the distinction is not always easy to make. One man with a complete cord lesion at T-6 who had excellent reflex erections told me he also had psychogenic erections. I asked him to observe himself closely and reconsider whether his 'psychogenic' erections really were so. Six weeks later he told me that he had come to the opinion that none were really psychogenic; they resulted from cuddling his wife in a way that was not intended to stimulate the penis mechanically, but accidentally did so.

The psychogenic erections reported by patients with complete lesions below T-8 cannot be dismissed. It must be accepted that psychogenic erection can be mediated by roots between the 9th thoracic and the 2nd lumbar. It seems reasonable to doubt the few reports that, taken at their face value, seem to show that it can be mediated by roots above the 9th thoracic. It is not known whether preserved psychogenic erection in a man with a complete lesion of the cauda equina or conus medullaris depends on excitation of erectile sympathetic fibres or inhibition of anti-erectile ones; but animal experiments suggest that both may be involved.

Preserved psychogenic erections are reported more often by men with incomplete than with complete cord or cauda equina lesions. This remains true for each region of the cord taken separately.

Coitus

Many men with spinal injuries achieve coitus, using either reflex or psychogenic erection. Combining the data of Bors and Comarr and of Comarr on complete non-cervical lesions, 63% of men with reflex but not psychogenic erections and 89% of men with psychogenic erections said they had coitus. For complete cervical lesions, those reporting coitus were 48% of all patients and 49% of those with reflex erections.

Advice on sexual technique for men with spinal injuries is given in a thorough and well-illustrated book by Mooney et al. (1975). Some of the problems are not obvious to the outsider, but can be solved, with or without expert advice, by the paraplegic man and his wife. For example, it seems theoretically likely, and is often the case, that the best position for intercourse if the man has no voluntary hip or knee movement is with the woman above. But I know a couple where the T-5 paraplegic husband's reflex erections are poorly sustained when he is supine, but well sustained when he is prone above his wife, she wrapping her legs around him and causing him to make thrusting movements by pressing the backs of his upper thighs with her heels.

Ejaculation

Like psychogenic erection (and unlike reflex erection), ejaculation is not often witnessed except by the patient and his sexual partner. Therefore we ought not to place absolute trust in all reported instances of it in men with complete cord lesions. However, it had been reported often enough already before 1980 to leave little doubt about its existence. More recently (Francois et al. 1980; Brindley 1981b), experience with vibratory stimulation of the penis has made it clear that the ejaculation reflex is preserved in a much larger proportion of paraplegics and tetraplegics than had been supposed.

Combining the data of Zeitlin et al. (1957), Bors and Comarr (1960) and Comarr (1970) for complete lesions, ejaculation without the aid of a vibrator has been reported in 4 of 110 cervical, 3 of 78 upper thoracic and 41 of 416 lower thoracic and lumbar cases. Of the 41 patients with lower thoracic or lumbar lesions who reported ejaculation, 25 were classified as having 'lower motor neurone' lesions, and all of these lacked reflex erection. Presumably the ejaculations of most of these patients were produced psychogenically. A few of them may have retained some fibres ascending from the sacral to the upper lumbar segments, despite the lack of sacral cord reflexes, but surely not more than a few.

The prevalence of purely psychogenic ejaculation in men with complete spinal injuries at low level is surprising in view of the supposed rarity of purely psychogenic ejaculation in normal men. Perhaps the latter should be re-investigated; I know of no survey since that of Kinsey et al. (1948).

The use of vibrators increases greatly the fraction of paraplegic men who can ejaculate. Combining the data of Francois et al. (1980) and Brindley (1981b) and

my subsequent experience up to March 1982 for complete lesions, ejaculation was obtained in 10 of 11 cervical, 27 of 40 T-1 to T-10, and 2 of 9 T-11 to L-1 cases.

To provoke a reflex ejaculation, a vibrator needs to be fairly powerful. A peak-to-peak amplitude of 2 mm when loaded by the penis is sufficient. The best frequency is probably about 80 Hz, but 100 Hz is nearly as effective. The glans is much more effective as receptive field than the shaft, and the ventral surface of the glans more effective than the dorsal or lateral surfaces.

Reflex ejaculation induced by vibrator can be accompanied by a large and long-sustained rise in blood pressure in patients with lesions above T-5. In such patients, electroejaculation is probably safer.

According to Comarr (1970), all of 11 patients with complete lesions and 10 with incomplete lesions who ejaculated also reported 'orgasms'. But these 'orgasms' may have been very different from those of normal men. Among the 14 men with complete cord lesions in whom I have produced ejaculation by vibrator and the three others who have described to me their experience of reflex ejaculation in private, none has claimed to have had sensations resembling those of normal orgasm. However, one of these seventeen, a man with a T-9 complete lesion of 12 years' duration, says that when achieved in his wife's company as part of loving play, reflex ejaculation with its accompanying sensations (which involve the pelvis little and the genitalia not at all) is very enjoyable.

Obtaining Semen Artificially

Intrathecal Neostigmine

Intrathecal injection of 0.3 mg neostigmine causes ejaculation in the majority of paraplegic men (Guttmann and Walsh, 1971). Often several ejaculations occur during a period of up to 2 h, the first commonly occurring 1–3 h after the injection. Intrathecal neostigmine also causes depression of reflexes acting on skeletal muscle, and erection. It can cause a large rise in blood pressure, with headache and vomiting. One death from cerebral haemorrhage has been reported. The procedure is obsolete, since electroejaculation and the use of a vibrator are safer.

Application of a Vibrator to the Penis

This valuable technique is considered in the previous section (see p. 270); it hardly counts as artificial.

Electroejaculation

This is the word generally used to denote the obtaining of semen by electrical stimulation with electrodes in the rectum. The result of it, when it is successful in man, is trickling emission, not true ejaculation. The technique has been used on farm animals since 1936 and was first applied to paraplegic men by Horne et al. (1948). There is no published reference to its use on man between then and 1966, and only in the last 4 years has there been any attempt to make rational choices for

the stimulus parameters, the sizes and shapes of the electrodes, and the sites of stimulation. I describe below my practice; the grounds for the choices and the evidence for its safety and efficacy are given elsewhere (Brindley 1981a).

I use the electrode mount shown in Fig. 15.3. It fits on the right index finger, and extends beyond the tip of the finger by about 20 mm. On its palmar surface is a single cathode, and on its dorsal surface two anodes connected in parallel. Stimulation is largely limited to nerve fibres near the cathode, in part because a cathode is more effective at stimulating than an anode, but mainly because the smaller total area of cathode ensures higher current densities there.

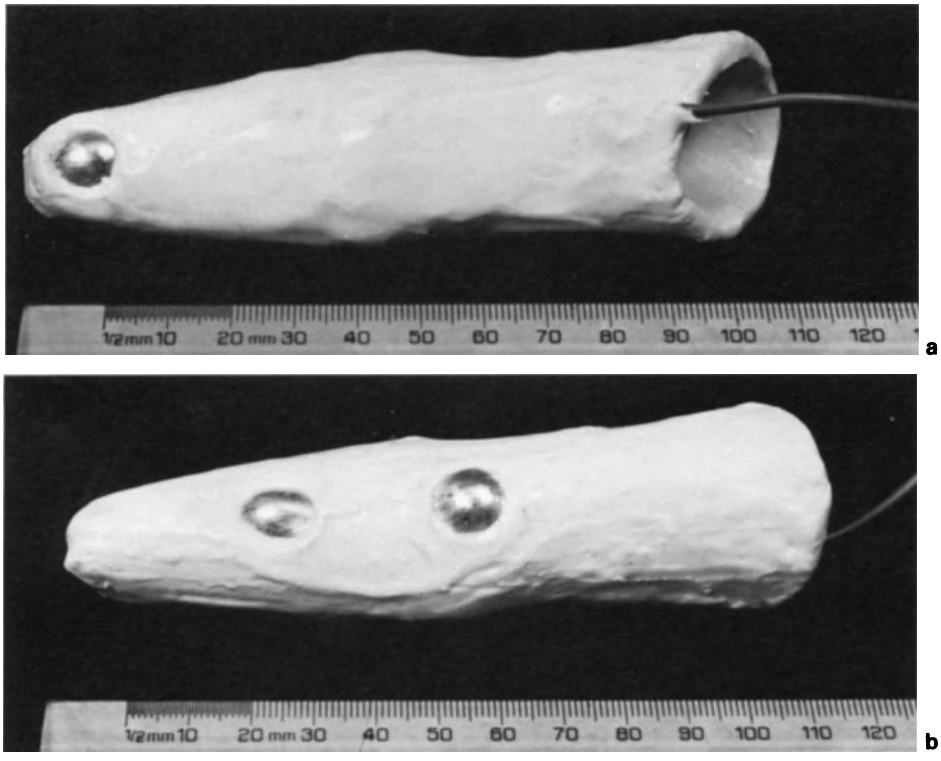

Fig. 15.3a, b. Electrode-mount for electroejaculation. **a** shows the palmar surface, with one cathode, and **b** the dorsal surface, with two anodes. The mount is made of silicone rubber reinforced with nylon mesh, and the electrodes of silver

The battery-driven stimulator, designed by and purchasable from Mr. C. M. Andrew of 43 Landcroft Road, London SE22, measures 19 × 11 × 8 cm and gives the following stimulus parameters: pulse duration 100 μs; peak voltage of pulse up to 108 V into 1 MΩ, up to 80 V into 250 Ω (nine equally-spaced steps); time-constant of sag of pulse 800 μsec; peak current (into 250 Ω) up to 316 mA; nett current less than 0.1 μA; output impedance 60–90 Ω (highest at the highest voltage-setting); time-constant of decay of return current between pulses 10 ms; frequency of pulses 30 or 15 per second (switchable).

My usual procedure is to have the patient supine with knees bent. I stand on the patient's right, and put the electrode-mount on the right index finger over a plastic or rubber glove. I put some aqueous jelly on the tip of the electrode-mount and over the electrodes, and insert the electrode-mount into the anal canal until the middle of the proximal anode is at the anal margin. Then stimulation at 9 or 18 V should cause contraction of the anal sphincter and the right ischiocavernosus muscle. By rotating the finger the left ischiocavernosus can be substituted for the right.

I next turn my hand so that the palm faces down and, pressing gently downwards with the finger-tip, advance the electrode-mount as far as it goes easily with very gentle pressure. The cathode is now near the best position for stimulating components of the sacral plexus. Using 36- to 54-V pulses, I explore the posterior wall of the pelvis for sites giving flexion of the toes, plantarflexion at the ankles, visible contraction of the hamstrings, abduction or external rotation of the thighs, visible contraction of the buttocks, or palpable contraction of the obturator internus muscles. It should be possible to obtain all of these, separately or in various combinations. Obtaining them (or failing to) may provide useful diagnostic information; but when the procedure is being used solely to get semen, it suffices to see any one of the somatic motor effects. If I see none at 54 V I try at 63 V. If still none can be seen, then either no L-5 to S-2 anterior horn cells survive (which implies, but is not implied by, flaccid legs and wasted muscles), or the stimulator is not working, or there is much gas in the rectum, preventing the anodes from making good contact. In the last case the gas can be expelled by pressing on the abdomen, pulling laterally with the finger to assist its release.

I next turn the palmar surface of the fingers so that it faces directly to the patient's right, and advance the finger as far as possible pushing hard. It is then appropriately placed for exploring the right lateral wall of the pelvis for the obturator point, i.e. the site where stimulation causes powerful adduction of the right thigh. Stimulation at this point usually yields semen (if semen can be obtained at all) after 5–20 s. If there is none in 40 s, or not as much as is needed for insemination, I try the left obturator point. 54-V stimulation often suffices, but in a first attempt I habitually use 80 V, since it is harmless and may avoid occasional disappointments.

If no semen is obtained externally, the next urine passed should be examined for spermatozoa.

From ninety-two patients who were electroejaculated (18 of them five or more times each) semen was obtained externally in 42, retrogradely in 14, and not at all in 36. Of the 36 failures, seven, all early in the series, were due to pain, and it would very probably have been possible to obtain semen under general anaesthesia. Another seven, mostly early in the series, were patients who were seen only once and failed to produce a specimen of urine, so that it is not known whether there was any retrograde ejaculation. For another eight of the failures there is independent clinical evidence that the patients lacked the preganglionic sympathetic fibres that the procedure is designed to stimulate, and for another eight the procedure probably produced external (in five) or retrograde (in three) ejaculation of azoospermic semen. There remain only a few unexplained failures.

A general anaesthetic is never needed if the patient cannot recognise pinprick in any lumbar or sacral dermatome. It is always needed if he can recognise pinprick in the L-4 dermatome or below. If he can recognise pinprick at L-1 to L-3 level but not below, the procedure will cause some pain, which may or may not be tolerable.

In patients with lesions above T-5, electroejaculation nearly always raises the blood pressure, and this could be dangerous. The blood pressure must be measured either continuously or repeatedly during the procedure, and the patient asked to report any headache. It is wise to stop if the systolic pressure reaches 200 mm Hg or if headache is reported. Stimulation can be resumed when the pressure has fallen, if semen has not yet been obtained. With this practice, the proportion of successes is higher in tetraplegic than in paraplegic patients (as it should be, since tetraplegics have very rarely lost the intermediate horn cells of their lower thoracic and upper lumbar segments).

Retrograde ejaculation is common (but not universal) in patients who have had transurethral resection of the bladder neck and in patients with lesions at T-11 to L-1 level. In other patients it is unusual. It should not be assumed that if a patient ejaculates retrogradely once he will always do so; among the 18 patients electroejaculated five or more times there are seven who have produced semen sometimes externally but sometimes retrogradely.

The prospects for pregnancy are improved if a wife learns to electroejaculate her husband at home, and inseminate herself. When offered the opportunity, 14 of 16 wives of paraplegic men wished to try. All those who wished to have now learned and have used the technique at home, though eight subsequently changed to using vibrators. Six now have babies and one is pregnant for the second time (December 1982).

A Radio-Linked Implant to Stimulate the Hypogastric Plexus

A radio-linked device has been implanted into a monkey. Bringing an appropriate radio transmitter up to the subcutaneously implanted receiver (the monkey sitting in a chair) causes immediate emission of semen, and the monkey gives no indication of feeling any pain.

Use of such an implant might be justified in a spinally injured patient for whom anaesthesia was needed for each electroejaculation, or in whom electroejaculation caused an unacceptable rise in blood pressure.

The Quality of Semen from Men with Spinal Injuries

Semen obtained by electroejaculation from men with spinal injuries is usually liquid; only about one specimen in ten is gelatinous. The volume can be from 0.2 ml to 10.5 ml, the average being about 2 ml. The count can exceed 500 million/ml, but in the majority of specimens is less than 40 million/ml. The motility has been under 26% in all but eight of 176 external ejaculates. The four patients who have (once each) produced ejaculates of volume over 1 ml *and* count over 40 million/ml *and* motility over 40% include one who has a T-5 complete lesion for 17 years—so long duration of paraplegia is not necessarily very harmful.

There is a tendency for the number of motile spermatozoa per ejaculate to increase with successive electroejaculations at monthly or more frequent intervals. Four men who at their first few electroejaculations produced ejaculates that were azoospermic (one case) or contained only non-motile spermatozoa (three cases) now usually produce ejaculates containing motile spermatozoa, though never more

than 5 million of them. Three men who started with some motile spermatozoa have made progress as shown in Fig. 15.4. For each of them the regression of log count on date has a significantly positive gradient, though the scatter of points is large.

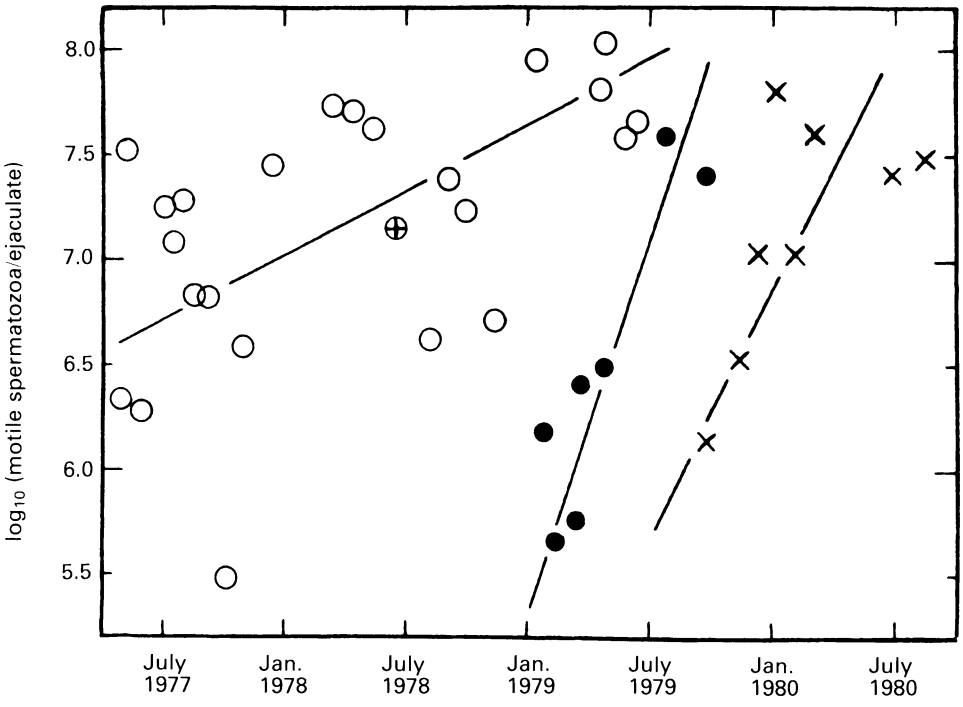

Fig. 15.4. The log of the number of motile spermatozoa per ejaculate, plotted against time, for three paraplegic patients. ○: T-6 complete lesion. First electroejaculation $2\frac{1}{2}$ years after injury. ⊕: Insemination with this specimen from the above patient resulted in pregnancy. Healthy daughter born 28 February 1979. ●: T-10 complete lesion. First electroejaculation $1\frac{1}{2}$ years after injury. X: T-5 complete lesion. First electroejaculation 16 years after injury

From these observations it seems that non-drainage is one factor in the poor quality of paraplegic semen. Almost certainly chronic infection in the genital tract is another, and treating it may improve the semen; though preventing reinfection is likely to be difficult, and much of the damage may be irreversible. A third potentially remediable factor is raised scrotal temperature, which will be the subject of the next section. Occasionally there may be testosterone deficiency, and if so it ought to be remedied, but it does not seem to be common. In a few cases, the accident that damaged the spinal cord also injured the genital tract, and some men who suffer spinal injuries were already subfertile. There may perhaps be a neurological factor, but there is no firm evidence for this. A reasonable working hypothesis is that in most men with spinal injuries the deficiencies of the semen result wholly from a combination of non-drainage, chronic infection and raised scrotal temperature.

The Scrotal Temperature in Paraplegia

The surface temperature of scrotal skin can be measured by thermography or other forms of infra-red thermometry, but it is a poor guide to deep scrotal or testicular temperature. The deep scrotal temperature is best measured by invagination thermometry or by radio-linked implant (Brindley 1982; and see Figs. 15.5 and 15.6). A thermistor mounted in a hypodermic needle provides a possible alternative technique, provided that great care is taken to avoid errors arising from conduction of heat along the needle.

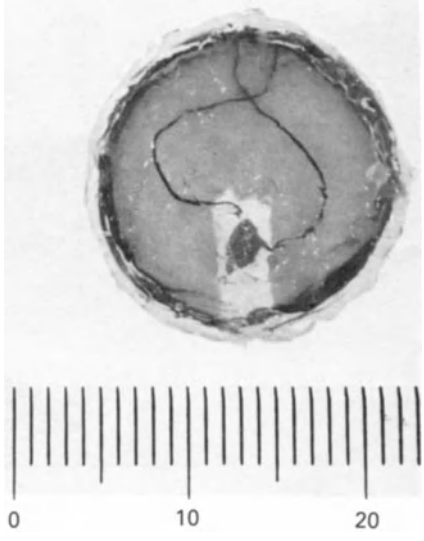

Fig. 15.5. Temperature-sensitive radio-frequency tuned circuit, designed for use as a sensor of deep scrotal temperature. It consists merely of a coil and a temperature-sensitive capacitor, mounted on an alumina plate 0.5 mm thick, and covered in silicone rubber. A slot in the alumina plate accommodates the capacitor, and both surfaces of the implant are made slightly concave. It is implanted so that, though mobile within the scrotum, it tends when undisturbed to sit between the two testes

Fig. 15.6. The implant in place. For the photograph it is pushed forward with one finger to make it easily visible

In 29 rehabilitated men with spinal injuries the mean deep scrotal temperature, measured by invagination thermometry while the patient had been sitting in a wheelchair for at least 20 min, was 36.24°C. The mean for 13 normal men of similar age sitting on ordinary chairs in similar environments for 20 min was 35.27°C. The difference is statistically highly significant ($P < 0.001$). Over a whole waking day, the mean difference is likely to be more than the 0.97°C found for sitting, because in normal men walking lowers the scrotal temperature substantially. No difference was found between paraplegic and non-paraplegic deep scrotal temperatures in bed at night; the difference measured during the day seems to be due to the manner and conditions of sitting, the long intervals between changes of position and the long total duration of sitting.

Eighteen of the men whose deep scrotal temperature was measured also had successful electroejaculations. Dividing these into the nine with highest and the nine with lowest deep scrotal temperature (boundary 36.35°C), motile spermatozoa were present in the semen of eight of the nine with low deep scrotal temperature but only two of the nine with high. This is a statistically significant difference ($0.02 > P > 0.01$), but more work will be needed before a causal relationship between scrotal hyperthermia and low motility is established. Nevertheless, it gives enough ground for suggesting that paraplegic men who wish to be fertile should adopt any simple measures directed at scrotal cooling that are harmless and easy. These include props between the knees, loose trousers, scrotal slit underpants such as those of Fig. 15.7, and thermally conducting seat cushions. The underpants of Fig. 15.7 have been proved by radio-linked implant to cause substantial reductions in deep scrotal temperature in one normal and one paraplegic man.

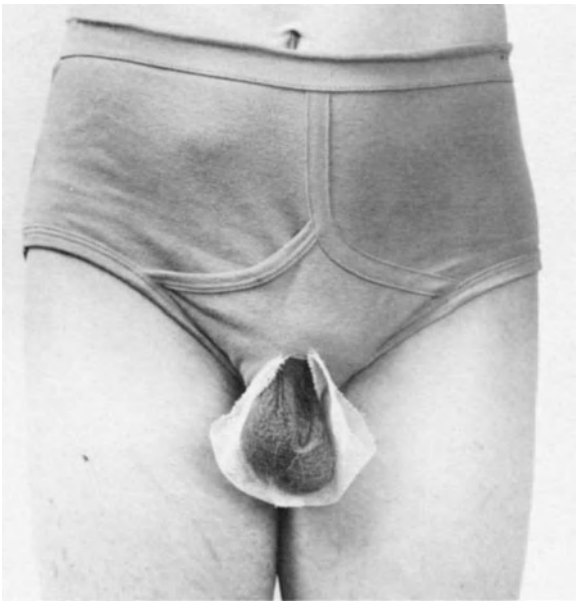

Fig. 15.7. Y-front underpants modified so that the scrotum sits outside them, and is separated from the thighs by a double layer of terylene net. Same subject as Fig. 15.6. The scrotal temperature sensor is invisible because it sits, as it should, between the testes

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Chapter 16

Treatment of Antisperm Antibodies

W. F. Hendry

Antisperm antibodies can be detected by a number of different tests in serum, seminal plasma or cervical mucus, but a positive result does not necessarily imply that the antibodies are causing infertility (see Chap. 9). The decision to treat such antibodies, therefore, must be based upon confirmatory evidence that they are impeding the process of fertilisation.

Diagnosis

Certain clinical findings in subfertile males should raise suspicions about the possible existence of antisperm antibodies. Obviously a past history of vasectomy is relevant, but the doctor should be ready to recognise other more subtle forms of obstruction, e.g. obstruction associated with a previous hernia repair or orchiopexy. A past history of injury with testicular swelling, mumps orchitis, or of epididymitis, especially if associated with gonorrhoea, may be relevant. There is a well known association between prostatitis and antisperm antibodies (Quesada et al. 1968; Fjallbrant and Obrant 1968). Coexisting medical illness should be considered—for example, a patient with antisperm antibodies and diabetes can also have autoantibodies against pancreatic islet cells and gastric parietal cells.

Marked disparity in size between the testes suggests previous unilateral disease and the possibility should be considered of unilateral obstruction with secondary depressed spermatogenesis or impaired sperm transport in the contralateral testis (Hendry et al. 1982). Epididymal obstruction on one side may be detected by palpation, and it should be remembered that this may be caused by tropical diseases such as sandfly fever or malaria or by smallpox. Unilateral absence of the vas should be noted. If the prostate is tender, expressed secretion should be examined bacteriologically. Finally, the foreskin should be checked, since a tight phimosis may impair ejaculation. A possible predisposing cause was found in 20 of 50 patients with antisperm antibodies using the serum gelatin agglutination test (GAT) (Kibrick et al. 1952, titre more than 32) compared to only 3 of 50 control patients without antibodies (Hendry et al. 1978).

Ideally, every male and every female partner in an infertile marriage should be tested for antisperm antibodies, but this would put a great strain on laboratory services and inevitably produce problems with false positive results. It is probably not necessary, provided that the mode of action of antisperm antibodies is remembered.

Seminal Analysis and Postcoital Test

Taken together, these two basic tests exclude immunological infertility, provided *both* are normal. A normal sperm count should exceed 20 million/ml, with more than 40% moving actively within $5\frac{1}{2}$ h of production in a volume of at least 1.5 ml (Macleod 1951; Macleod and Gold 1951) and it should be demonstrated that sperm can produce an adequate postcoital test (PCT) in normal peovulatory cervical mucus—usually defined as more than five *motile* sperms per high power field (Santomauro et al. 1972).

The co-existence of a normal sperm count and a persistently poor PCT strongly suggests the existence of antisperm antibodies, and these most commonly occur in the husband. Kremer et al. (1978a) investigated 30 couples with persistently poor PCT due to antisperm antibodies and found that the antibodies were present in the husband in 25 cases and in the wife in only five cases. The partner with the antibodies can be identified by direct testing for antisperm antibodies, and their significance should be confirmed by sperm cervical mucus contact (SCMC) testing using donor sperm and mucus as controls (Kremer and Jager 1976, and see below). The presence of spontaneous agglutination of sperm in a semen specimen does *not* by itself indicate the presence of antibodies (Table 16.1).

Table 16.1. Degree of sperm clumping related to results of serum gelatin agglutination test for antisperm antibodies

Serum antisperm antibody test	Sperm clumping			
	None	Slight	Moderate	Severe
Negative (179 patients)	88	29	48	14
Positive (34 patients)	4	2	6	22

Mixed Erythrocyte–Spermatozoa Antiglobulin Reaction (MAR test)

This simple and rapid test was introduced by Jager et al. (1978) for screening spermatozoa in fresh human semen for antisperm antibodies. It is based on the formation of motile mixed agglutinates between erythrocytes sensitised with incomplete anti-Rh antibodies and fresh swimming spermatozoa with surface antisperm antibodies, after mixing one drop of sensitised red cells with one drop of semen and one drop of anti-human IgG antiserum (Behring). The result is read within 10 min as *negative, doubtful* (less than 10% of motile spermatozoa attached

to red cells); *positive* (10%–90% of motile spermatozoa incorporated in mixed agglutinates); and *strongly positive* (over 90% of motile sperms caught in clumps). Agglutination of the red cells serves as an internal control (Fig. 16.1).

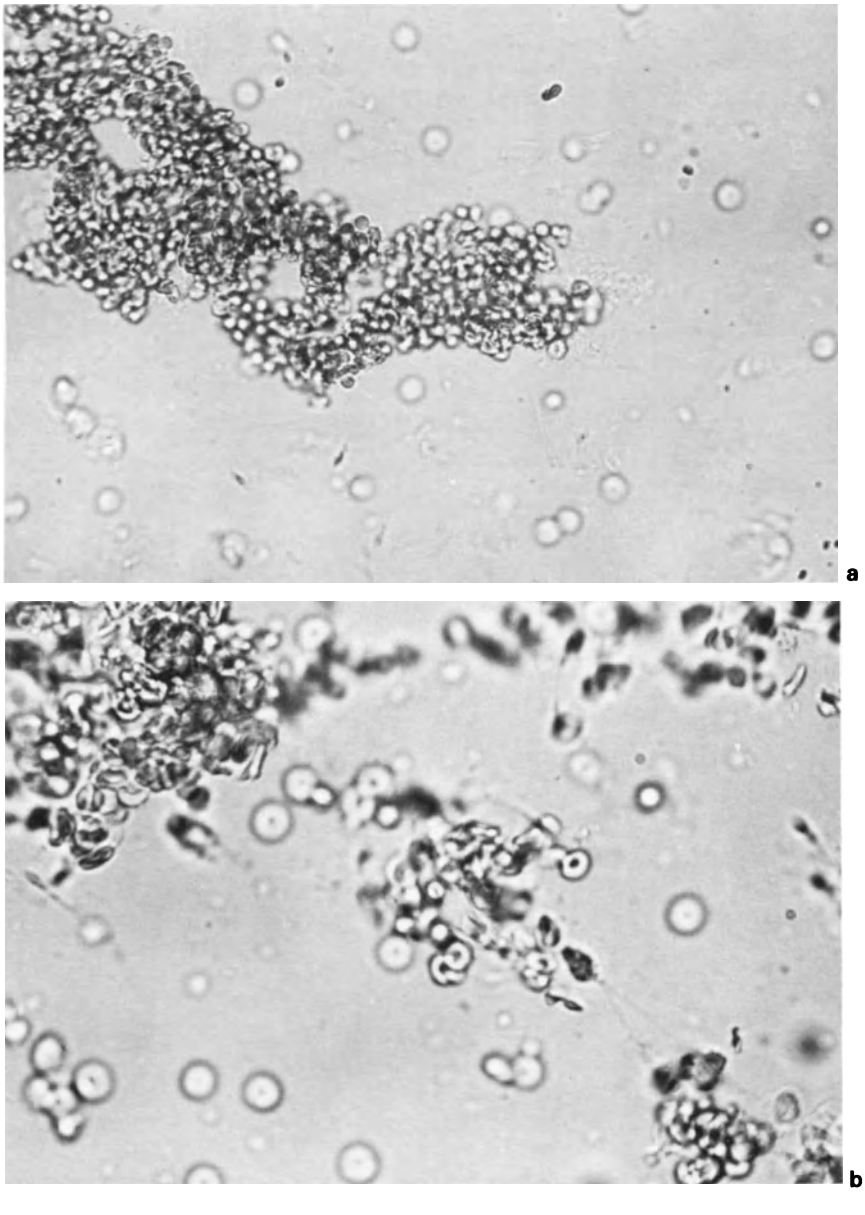

Fig. 16.1. Mixed antiglobulin reaction (MAR) test: **a** red blood cells are clumped, with motile spermatozoa swimming freely in between—a negative result. **b** motile mixed agglutinates—a positive result

We evaluated this test with 775 semen samples from the male partners of infertile marriages, obtaining satisfactory reactions with 664 specimens (86%). The test was unsatisfactory in the remainder because of a severely abnormal sperm count or very low motility. Reproducible results were found in 172 (97%) of 178 patients who were tested more than once. The MAR test was negative in 256 (95%) of 268 samples from 251 patients without serum antisperm antibodies, and strongly positive in 27 of 29 men with significant titres (more than 32) of agglutinating antibodies. Two of five specimens from men with agglutinating titres of less than 32 were strongly positive, and both men produced pregnancies without treatment (Table 16.2). It was concluded that the MAR test is a very useful addition to routine seminal analysis, but that positive results should be supplemented by seminal plasma or serum antibody measurements (see p. 169) and SCMC testing before recommending treatment (Hendry and Stedronska 1980).

Table 16.2. Results of MAR test related to serum spermagglutination titre on gelatine agglutination test in 37 patients with positive results in one or other test. (A titre of 32 or more is generally accepted as significant in males)

Serum spermagglutination titre	Result of MAR test			
	Negative	Doubtful	Positive	Strongly positive
Negative			2	3
4	1			1[a]
8		1		
16	1			1[a]
32				3
64	1			3
128				7
256	1			9
512			1	3
1024				1

[a] Wife became pregnant without treatment

Antisperm Antibody Tests for Serum or Seminal Plasma

The technical details of the various tests have been described in Chap 9.

Sperm–Cervical Mucus Contact (SCMC) Test

By studying directly the behaviour of spermatozoa in the new environment in which they must survive and make forward progression, this test provides the reference point against which all other observations should be checked. Done with fertile donor sperm and cervical mucus as controls, it gives positive evidence as to which partner is likely to have the antibodies and provides fundamental confirmation that the results obtained by other tests are reflected by significant interference with sperm activity. In 21 of 22 couples tested (Table 16.3) sperm from husbands with antisperm antibodies (GAT titre more than 32) could not penetrate cervical mucus—neither their wives' nor fertile donors'—whereas fertile donor sperm

Table 16.3. Comparison of results of husbands' antisperm antibody tests and impaired sperm penetration of cervical mucus (Morgan et al. 1977)

	Husbands' antibody status	Impaired sperm penetration			
		Husband + wife	Husband + donor	Donor + wife	Donor + wife
Group I (22 couples)	GAT[a] + SIT[b] positive	21[d]	21[d]	2	0
Group II (10 couples)	IFT[c] positive	2	1	2	0
Group III (12 couples)	Negative	2	1	2	0

[a] GAT = gelatin agglutination test
[b] SIT = sperm immobilisation test
[c] IFT = immunofluorescent test
[d] With 'shaking' of sperms; see text for description of the 'odd-man-out'

showed good penetration of both specimens of mucus (Morgan et al. 1977). The one exception out of the 22 couples was a man with serum antisperm antibodies following reversal of vasectomy, whose sperm showed good mucus penetration; he subsequently impregnated his wife without further treatment.

The SCMC test has been described by Kremer and Jager (1976); 0.1 ml of semen is withdrawn from approximately 0.5 cm below the fluid surface of semen that has been standing in an upright test-tube (internal diameter 8 mm) for 30 min at room temperature to allow agglutinated spermatozoa to settle to the bottom. A drop of preovulatory cervical mucus with signs of good oestrogenic stimulation and a pH greater than 7.0 is placed on a glass slide next to a drop of the pretreated semen. Both drops are thoroughly mixed with the coverslip before it is placed on the mixture. A second drop of the pretreated semen is put on the same slide for comparison and covered with a second coverslip. The spermatozoa are examined immediately and again after being placed in a Petri dish for half an hour at room temperature.

A positive reaction is shown by the change from progressive movement to a characteristic non-progressive shaking or jerking movement (Fig. 16.2). Kremer and Jager (1980) have shown that a positive shaking phenomenon is dependent upon the presence of immunoglobulin A antibodies in the genital secretion. The

Table 16.4. Immunoglobulin class of antisperm antibodies, agglutination titre and results of SCMC test in 18 infertile couples with serum antisperm antibodies in the husband (from Kremer and Jager 1980)

Number of men	Percentage of motile sperms coated with		Sperm agglutination titre (TAT)[a]		SCMC test	
	IgG	IgA	Serum	SP[b]	Positive	Negative
14	⩾90	⩾70	32–512	4–128	14	0
4	⩾90	0	128–512	0	0	4

[a] Tray agglutination test
[b] Seminal plasma

presence of IgG on the sperm as shown by a positive MAR test (see above) is not sufficient to produce the shaking phenomenon in the absence of IgA antibodies in the genital secretion (Table 16.4).

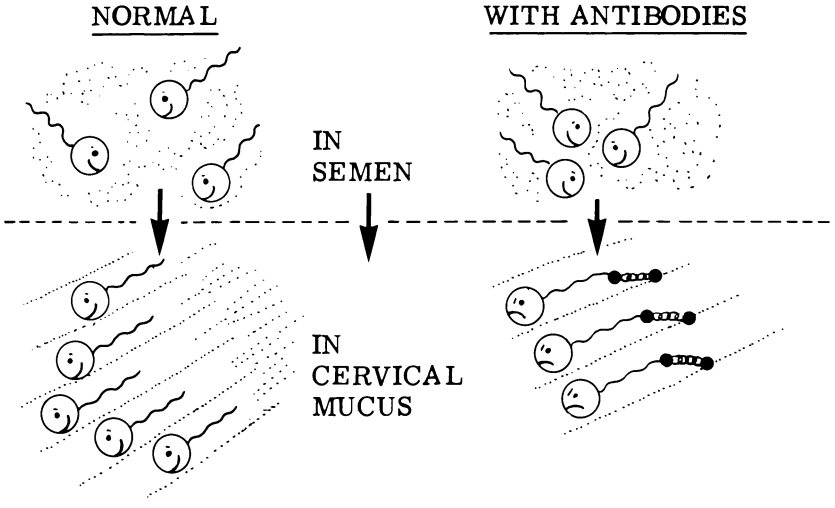

Fig. 16.2. The sperm–cervical mucus contact (SCMC) test of Kremer and Jager (1976). Normally, sperm form phalanges and quickly penetrate preovulatory cervical mucus. In the presence of antisperm antibodies in either husband or wife, the sperm become attached to glycoprotein micelles in the cervical mucus and exhibit a characteristic non-progressive shaking movement

Critical Path

Several points should be noted about the critical paths through the various tests that have been described, leading eventually to treatment (Table 16.5). The MAR

Table 16.5. Clinical and laboratory diagnosis of antisperm antibodies prior to treatment

Clinical features	There may be no abnormality in either partner shown by standard investigations. Previous vasectomy or epididymitis may be significant.
Suspicious features	Poor postcoital test in association with normal semen analysis. Massive auto-agglutination in fresh semen sample. Positive MAR test on fresh semen sample.
Confirmatory tests that antibodies are present	Serum and seminal plasma are tested for agglutinating and immobilising antibodies[a]. The two are often associated but antibodies in the seminal plasma are usually the most significant. Likewise a positive MAR (IgA) test is probably of more significance than the MAR (IgG).
Confirmatory test that antibodies are clinically significant	Observation of the shaking phenomenon in the sperm–cervical mucus contact test.

[a] It is wise to repeat serological tests before starting treatment

N.B. Steroid treatment should not be started until it has been confirmed that there is no other abnormality in either partner

and postcoital tests should be regarded as screening tests, which simply serve to arouse suspicion. These must be supplemented by serum testing for antisperm antibodies, which will provide a baseline titre. But since serum sperm agglutinins are not necessarily accompanied by seminal plasma antibodies (Fig. 16.3) their presence must be established by testing the seminal plasma, and their significance established by obtaining a positive shaking phenomenon on SCMC testing. Treatment should *not* be commenced until this has been done.

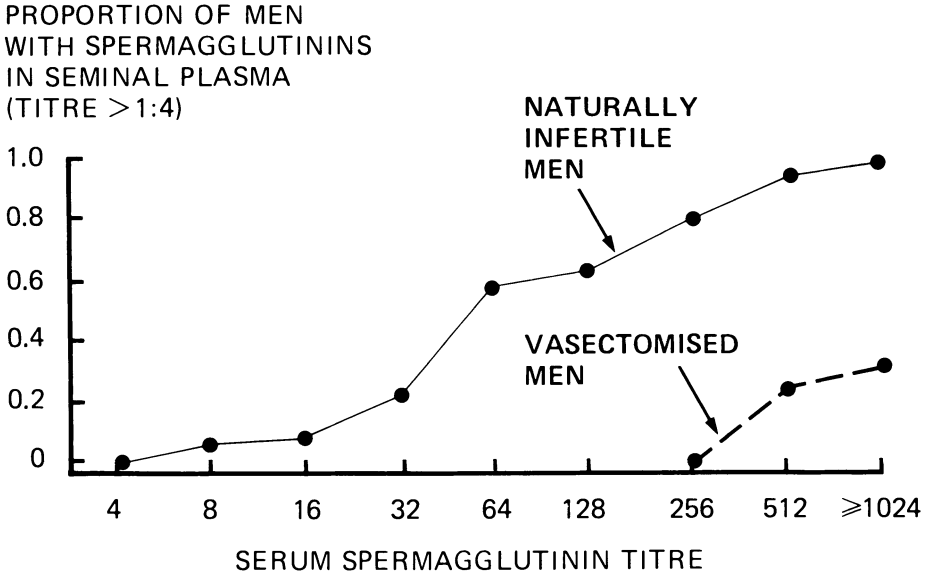

Fig. 16.3. Incidence of sperm agglutinins in seminal plasma in relation to the serum titre in naturally infertile and vasectomised men. Note the much smaller proportion of vasectomised men with antibodies in seminal plasma (Rumke 1978)

Treatment

Any therapy for antisperm antibodies will be demanding on the time of the clinical and laboratory staff, and will require great patience and perseverence from the couple concerned. It could also produce serious side effects. It is therefore essential to make quite sure before starting treatment, (a) that the antisperm antibodies are really interfering with fertility, (b) that the husband's sperm count is as good as possible, and (c) that the wife has patent tubes and that the presence and timing of ovulation has been established. Close liaison between the staff looking after husband and wife is clearly desirable.

There are at least three possible lines of treatment, none of which is completely satisfactory at present.

Antibiotics

Prostatitis was noted in 16 of 43 men with antisperm antibodies in Sweden (Fjallbrant and Obrant 1968). Similarly, we found that 17 of 31 patients with antisperm antibodies (serum GAT titre more than 32) had positive semen cultures (*Staphylococcus* coagulase positive 8, *Escherichia coli* 8, *Streptococcus* group A1) compared with only five of 32 unselected patients without antisperm antibodies. Fjallbrant and Nilsson (1977) reported eight cases with more than 20 white blood cells per high power field in expressed prostatic secretion; these were treated with long-term antibiotics. In five patients, the serum antibody titre dropped (128–1024 down to 4–128 over 1–5 years); improved penetration of cervical mucus was observed and the wives became pregnant.

In our experience with 290 patients, who had semen cultured and serum antibody status defined, pregnancies were produced with roughly equal frequency with or without positive semen cultures (Table 16.6), irrespective of whether the organisms were treated with antibiotics or not (unpublished observation). Furthermore,

Table 16.6. Incidence of pregnancy in wives of 290 infertile men related to results of semen cultures and presence of antisperm antibodies (treated as described in the text)

Semen culture	Serum antisperm antibodies	
	Negative	Positive (GAT titre > 32)
	No. of pregnancies/No. of men	
Negative	15/157	3/23
Positive	9/80	5/30

electron microscopic studies in 57 patients with large numbers of cells in the ejaculate showed that in 34 cases the leucocytes contained spermatozoa, and in only 16 were bacteria seen within the cells (Hughes et al. 1981). These findings suggest that an excess of leucocytes in semen in patients with antisperm antibodies may be a result of local cell-mediated antibody reaction provoked by spermatozoa in the ejaculatory system rather than a subclinical prostatitis. Further work is required to clarify the matter.

Artificial Insemination—Sperm Washing

Kremer et al. (1978b) have used intrauterine insemination to get beyond the barrier created by the cervical mucus, and obtained three pregnancies (including one abortion) in 15 women whose husbands had spermagglutinin titres of more than 1 in 32.

Artificial insemination was coupled with sperm washing by Halim et al. (1974). After giving the patient prednisolone 5 mg three times daily for 2 weeks, freshly ejaculated sperm were washed with phosphate buffered saline (PBS) at pH 7.2, centrifuged at 300 r/min for 30 min, resuspended in 1 ml of PBS and inseminated. Eleven pregnancies were obtained in 26 couples, in 10 of which the husband had

the antibodies. Shulman et al. (1978a) have modified the washing technique by using a sterile 4% solution of human serum albumin and then centrifuging at 2000 r/min for 5 min, repeating the process to wash the sperm three times. One success was recorded in seven couples treated. Comparative studies in the seminology laboratory at Chelsea Hospital for Women indicated that sperm motility was better preserved in Shulman's medium.

We have treated 30 couples in whom the husband had an antisperm antibody titre of more than 32 by this technique for 1–13 months (average 4.5 months) achieving only three pregnancies. Analysis showed that in all cases where the wife became pregnant, the husband had previously received steroid therapy and no pregnancies occurred with otherwise untreated couples. There are good grounds for doubting whether antibodies can be removed from spermatozoa by simply washing them, and also it is unlikely that the immunological defence mechanisms in the female are confined to the cervical canal. In view of our disappointing results, the theorectical objections outlined above, and the great logistical difficulties for patients and staff, we have now abandoned this method of treatment in favour of corticosteroid therapy.

Steroids

Before giving steroids a family history of diabetes or glycosuria is noted, and the fasting blood sugar, liver function and chest x-ray are checked. Dyspepsia is fully investigated and treated prior to therapy. Patients are told of the risks involved, and advised to abstain from alcohol whilst on treatment. The two regimes that have been used are described below.

Long-Term Low-Dose Prednisolone

Fifteen patients with average pretreatment sperm counts of less than 20 million/ml received prednisolone 5 mg three times daily for 3–12 (average 6.1) months. The sperm counts rose to consistently normal levels in 10 of the 15 patients and two wives became pregnant (Fig. 16.4). In one patient with a high titre of antibodies (GAT titre 1024), the sperm count, which was less than 1 million/ml before treatment, rose to near normal on long-term prednisolone, and then declined to less than 1 million/ml again when prednisolone was stopped (Fig. 16.5). It was concluded that these patients probably had a steroid-responsive immune epididymo-orchitis (see above). Fourteen patients with normal sperm counts also received prednisolone 5 mg t.d.s. for a similar period: but only two pregnancies were produced (Hendry et al. 1979).

Intermittent High Dose Methylprednisolone

Introduction. Shulman et al. (1978b) recommended methylprednisolone (MP) 96 mg/day for 7 days, given to the man on days 21–28 of his wife's menstrual cycle. This was based on the observation by Butler and Rossen (1973) that in 17 normal men it produced a significant reduction in circulating IgG (in 86%) and IgA (in 43%) which reached a miximum depression in the third week after therapy. Shulman et al. (1978b) claimed five pregnancies in 15 couples after treatment of the

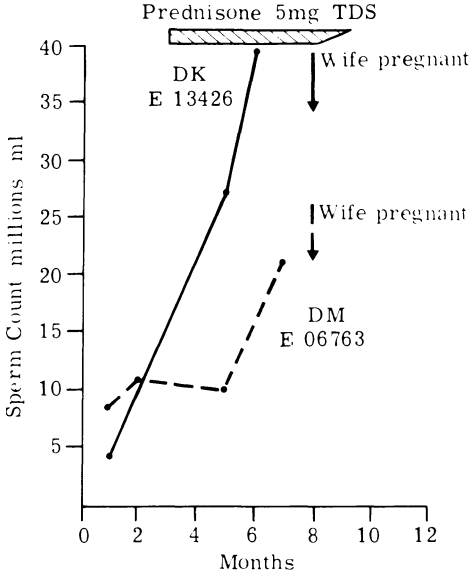

Fig. 16.4. In 2 of 15 men with oligozoospermia treated with prednisolone 5 mg three times daily, sperm counts rose to normal and the wives become pregnant

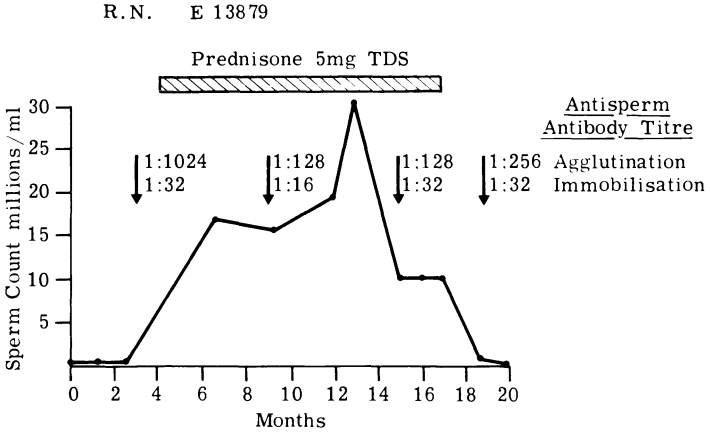

Fig. 16.5. In one patient with less than 1 million sperm per ml, sperm count rose to normal during treatment with prednisolone 5 mg three times daily, and deteriorated to the initial level when treatment was stopped

husband for up to three successive cycles and Hargreave and Elton (1982) claimed 11 live births in 34 couples using Shulman's regime for up to six successive cycles. In a pilot study there was only one pregnancy in nine couples treated with MP which was not synchronised with the wife's menstrual cycle, but much better results (five pregnancies in nine couples treated) were obtained when the MP was given on days 21–28 of the wife's cycle, and repeated in alternate months (Hendry et al. 1979).

Pregnancies. A further 45 men have been treated: 28 completed three or more courses of MP, 11 received two courses and six had only one course. Two men received up to three additional monthly courses of MP at times ranging from days 1–8 to days 5–12 of successive cycles, depending on the results of additional serial antibody tests (every two or three days for 10 days) which were made following the third course of MP to allow fine adjustment of the timing of therapy. In two patients the primary treatment was given on days 1–7 of successive cycles (for example, see Table 16.7). Fifteen wives (33%) became pregnant in the cycle

Table 16.7. Serial sperm counts, MAR tests and antisperm antibody titres following three courses of MP given in successive months on days 1–7 of wife's menstrual cycle. Patient NY, aged 31

Treatment	Date	Sperm count		MAR test		Antisperm antibodies			
						Serum		Seminal plasma	
		m/ml	% motility	IgG	IgA	GAT	SIT	GAT	SIT
	30/10/79	4	25	+++	–	–	–	–	–
	29/11/79	11	25						
	8/1/80	3	50	+++	–	128	8	16	–
M.P.[a] ①									
	14/3/80	54	40	+++	+	256	8	8	–
M.P. ②									
	10/4/80	66	40	+++	–	32	1	–	–
M.P. ③									
	20/5/80	145	40	+++	–	16	–	–	–

[a] M.P. = methylprednisolone 32 mg t.d.s. for 7 days

following treatment of the husband: two after one course, six after the second course and seven after the third or subsequent courses. Eleven pregnancies occurred after treatment was given on days 21–28 and four after treatment was given to ten men on days 1–7 or later. One miscarriage and one ectopic pregnancy occurred.

Effect on Sperm Density. Sperm counts before and after treatment are shown in Table 16.8. Counts of 20 million/ml or more were obtained by 39 of the 45 men, compared with 29 before treatment.

Table 16.8. Distribution of average sperm counts of 45 patients before and after treatment with methylprednisolone

	Sperm count (million/ml)				
	0–10	11–20	21–40	>41	Unknown
Before treatment	14	4	4	24	
After treatment	5		9	30	1

Effect on Antibody Titres. The GAT results before and after treatment are shown in Fig. 16.6; there is no evidence that the titres fell more profoundly in 14 men whose wives became pregnant than in those whose wives did not. The sperm immobilisation test (SIT) titres in 33 men tested in one laboratory before and after

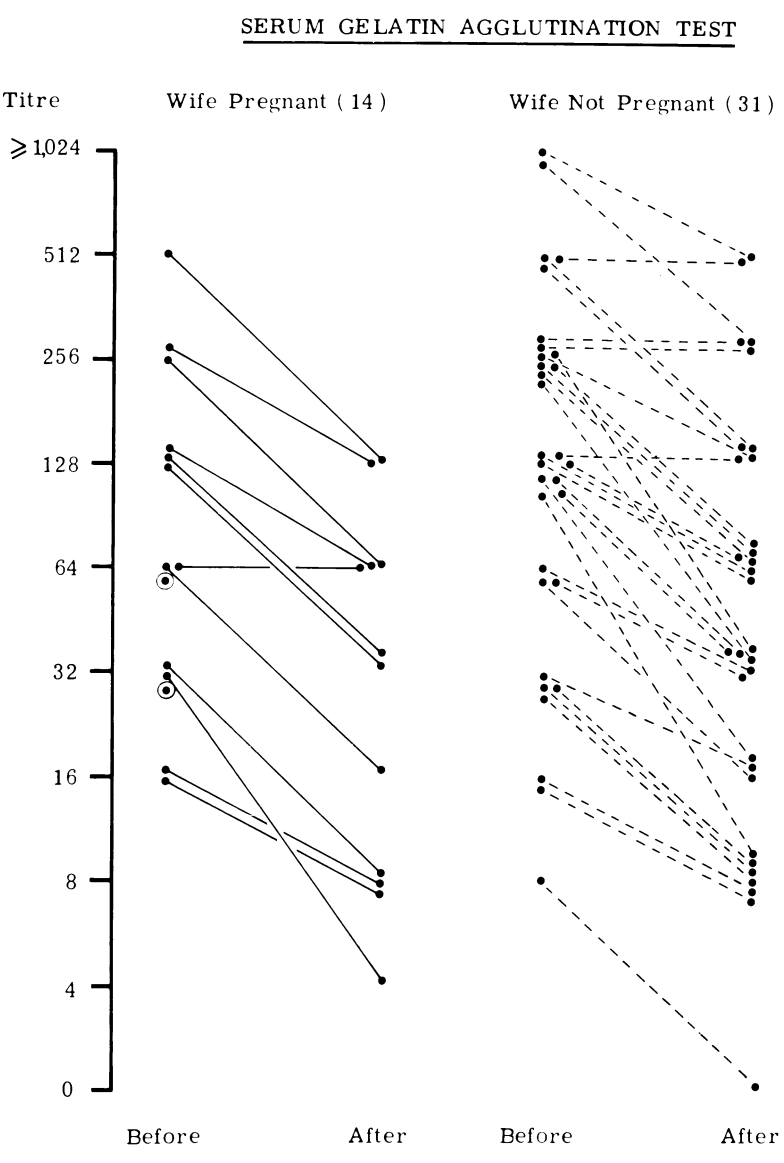

Fig. 16.6. Serum gelatin agglutination test titres before and after steroid therapy, comparing men whose wives did and did not become pregnant: there is little difference between the two groups

treatment are shown in Fig. 16.7: production of pregnancy appeared to be associated with a fall in sperm immobilisation titres to zero or near zero; however, a similar fall was obtained in many men whose wives did not become pregnant. Seminal plasma antibody testing was introduced in the latter part of this study and it is difficult to draw firm conclusions as yet; however, serial testing showed that the steroid treatment caused the antisperm antibodies to disappear from seminal plasma in six patients, two of whose wives became pregnant (see Table 16.9).

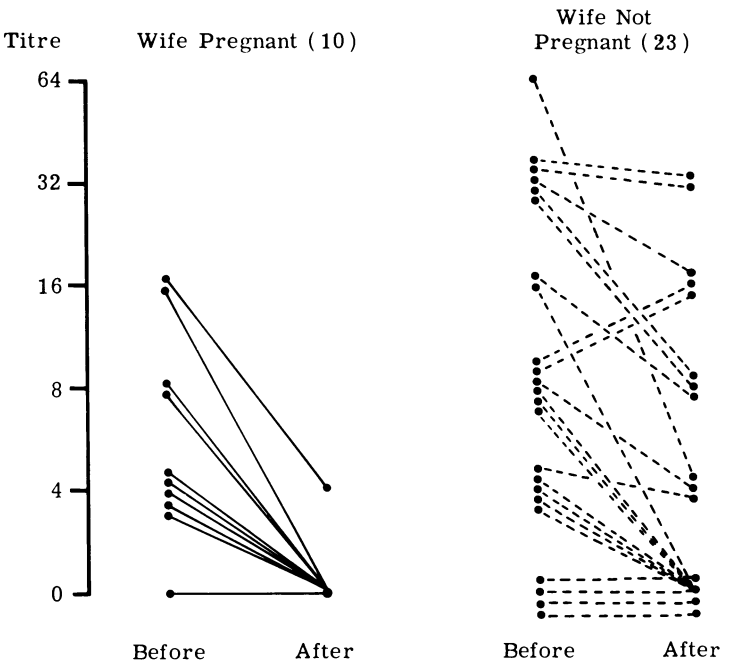

Fig. 16.7. Serum sperm immobilisation test titres before and after steroid therapy: production of pregnancy appears to be associated with a fall in sperm immobilisation titre to near zero

An example of serial sperm counts, MAR test results and antibody levels in serum and seminal plasma is shown in Table 16.7 in one patient who received MP on days 1–7 of three successive cycles. The rise in sperm counts and fall in serum GAT and SIT titres that accompanied MP therapy can be observed; the MAR test for IgA became negative as the seminal plasma antibodies disappeared; however, the MAR test for IgG stayed positive. Table 16.10 shows serial sperm counts and MAR test (IgG) results for a period of three weeks following the third course of MP in a man whose inital GAT titre was 256, and which had fallen to 16 prior to

Table 16.9. Relationship between presence or absence of seminal plasma antibodies (determined by GAT testing) before and after treatment, and occurrence of pregnancy in the spouse[a]

	Antibodies present (GAT test)	
	Before treatment	After treatment
Wife pregnant	4/7	2/7
Wife not pregnant	16/24	12/25

[a] Seminal plasma antibody testing was introduced late in this study and not all patients were tested

this course of treatment. The MAR test became negative on the third post-treatment day, at which time good sperm penetration of donor mucus was observed. The timing of subsequent courses of MP in this patient was adjusted as a result of these observations.

Side Effects Out of the nine patients treated in the pilot study and 45 patients treated subsequently, three patients suffered such severe side effects that treatment was discontinued. One had marked dyspepsia, and discontinued therapy until he had had a course of cimetidine; he subsequently tolerated two further courses of MP, taking one tablet of cimetidine 1 h before each dose. One patient had a small haematemesis and no further steroid treatment was given. One patient developed pain in the hips and refused further treatment. This patient subsequently developed aseptic necrosis of both hip joints after an interval of about one year, and is now awaiting bilateral hip replacement. This complication has also been reported after the use of high dose steroids in neurosurgery (McCluskey and Gutteridge 1982). In addition 14 other patients have experienced less severe side-effects: transient pain in the hips (3), dyspepsia (1), headaches (1) flashing lights (2), tinnitus (1), aggressive behaviour usually directly against the wife (3) or marked blotchy face and acne (3). In general men who were fit, working in physically demanding occupations, suffered less side-effects and responded less well, as compared with men who were unfit and had sedentary occupations (Hendry et al. 1981).

Which Steroid Regime to Use?

Continuous therapy may be preferable initially for patients with oligozoospermia and high titres of antibodies, since the sperm count may rise to normal, thus improving the patient's fertility potential. However, intermittent higher dose treatment is probably better if the sperm count is normal, although care should be taken to synchronise treatment with the wife's cycle. In suitable cases the two regimes can be combined. The use of testosterone to depress spermatogenesis temporarily before steroid therapy has also been reported: however, the results (12 pregnancies in the wives of 48 men) were not significantly better than those reported in other studies with steroids alone (Dondero et al. 1979).

As a result of the case of bilateral hip necrosis the dose of intermittent steroid therapy has been reduced to prednisolone 20 mg twice daily with meals, taken on days 1–10 of the wife's cycle for 3 months followed by remeasurement of antibody levels. The subsequent steroid dosages are either maintained at a similar level or incrementally increased according to the patient's tolerance and the antibody response. So far, no side-effects have occurred with this modified regime and several pregnancies have been produced. The final results of this regime will be reported in due course.

Conclusions

The previous long history of infertility (average 5.3 years), (see Fig. 4.6) and the production of pregnancies which coincided with profound drops in sperm immobilisation titres and seminal plasma antibody levels after intermittent high-dose steroid therapy, make it reasonable to ascribe the pregnancies to the results of this therapy. The success rate (33%) is comparable with other reports (44%, Shulman

and Shulman 1982; and 33%, Hargreave and Elton 1982) and is certainly better than that obtained by long-term low-dose steroids (14%) or a single dose of MP not synchronised with the wife's cycle (11%). It appears that successive courses of MP produce a stepwise decrease in antisperm antibody levels (see Table 16.7), and this may be why most pregnancies occurred after the second or third courses of MP. The optimum time and optimum dose of steroid therapy remain to be defined, and they may vary from patient to patient. The MAR test may be useful to defined the exact timing of therapy (Table 16.10). The present dose of steroids appears to

Table 16.10. Serial sperm counts and MAR tests (IgG) before and after a third course of MP. Serum GAT titre was 256 initially and fell to 16 prior to this course

Time in relation to 7-day course of MP	Sperm count		MAR (IgG)
	m/ml	% Motility	
Before	16	40	+++
After:			
Day 1	171	50	+
3	43	50	−
7	33	50	+
9	24	40	+
15	36	50	+
20	65	50	+

cause side-effects in some patients, and to be ineffective in others and there may be a case for adjusting the dose according to each individual patient's response. The risk of serious side-effects make it essential that the patient and his wife should be made fully aware of all the possible consequences of this form of therapy.

Repeated checking of postcoital tests and antisperm antibody titres should give a useful indication of the degree of response shown by the patient. The serum GAT titre is found to bear no relation to the occurrence of pregnancy in the spouse, and the best indicator from serum testing appeared to be the complement-dependent sperm immobilisation test. However, it seems clear that the presence or absence of seminal plasma antibodies is probably the critical factor in determining whether or not pregnancy occurs (Linnet et al. 1981), and this can be measured directly in a semen specimen or assessed indirectly by observation of the percentage of sperm shaking on SCMC testing. For practical purposes, one monthly semen sample can be expected to provide all the information necessary to monitor the response to treatment, provided it is analysed for sperm count and motility, antibody titre and SCMC results; obviously it should not be provided for the laboratory until after the wife's expected date of ovulation has passed. Following intermittent high-dose steroid therapy, seminal plasma antibodies may disappear—sometimes transiently, sometimes more permanently—even though serum antibodies persist (see Tables 16.7 and 16.10). However, many of these wives did not become pregnant, and on further detailed investigation disorders of ovulation were detected in some women who had previously been considered normal. Close liaison between staff looking after husband and wife is clearly essential in the management of these couples.

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Chapter 17

Vasectomy

T. B. Hargreave

Bilateral vasectomy for contraceptive purposes is now one of the most frequently performed minor operations in the United Kingdom. It has been estimated that 10% of husbands of women aged 25–34 will be vasectomised by the time the wife is 35 (Bone 1978). In the United States one million male sterilisations are done each year (Hackett and Waterhouse 1973), and in India six million were sterilised in 1976 (Nortman and Hofstattere 1978). In the United Kingdom the increase in vasectomies has resulted in part from disquiet over the long term use of oral contraceptives for women and also from the lack of other aesthetically acceptable methods. Thc perfect vasectomy would be 100% effective in a very short time, have no complications or side effects and be easily reversible. There are various methods but none perfect, and patients should be warned that minor complications do occur. It is unwise with any type of vasectomy operation to give absolute guarantee that recanalisation can never happen.

Indications and Contra-indications to Vasectomy

There are few indications for vasectomy although hereditary disease in the husband is a possible one. In those cases where there are marked contra-indications to further pregnancy in the wife and where the wife has reasonable life expectancy the couple may decide that vasectomy for the husband is the simplest option. Vasectomy is contra-indicated if the wife has gynaecological pathology likely to result in imminent hysterectomy. The results of vasectomy reversal are not certain and thus vasectomy is contra-indicated in those situations where the marriage might break up. Vasectomy is also contra-indicated in the psychologically unstable unless expert psychiatric advice is available. Vasectomy should not be performed in cases of mental deficiency unless the man is able to understand the implications of the operation. In view of the animal evidence (see below) and in spite of the lack of human evidence careful thought should be given before agreeing to vasectomy in cases where there is known increased risk of cardiovascular disease, e.g. familial hypercholesterolaemia.

Operative Technique

Two approaches to vasectomy have been adopted. One is to perform a wrecking operation where as much vas as possible is removed making any chance of spontaneous recanalisation remote; the second is to interrupt the continuity of the vas in a simple way that is effective. The problem with the former approach is that unless long lengths of vas are removed cremasteric contraction will approximate the cut ends to a surprising degree and recanalisation may occur. Another reason for advocating a conservative approach is that increasing numbers of men request vasectomy reversal because their personal circumstances have unexpectedly changed and this occurs even when these men have been most thoroughly counselled by independent experienced counsellors prior to the original operation.

A method that is widely used whereby the cut ends are separated in different tissue planes is illustrated in Fig. 17.1. The complications using this method are compared with those of the method where a length of vas is excised and the ends doubled back (Table 17.1). Although the above technique is effective the wisdom of ligating the testicular end has recently been questioned by Silber (1979) who finds that those cases who have the sperm granuloma at the end of the vas have a lesser chance of epididymal granulomata and a greater chance of having a successful vasectomy reversal. Shapiro and Silber (1979) report a technique where the testicular end is left open in an attempt to reduce dilatation and damage to the epididymis. This technique has been used in 500 patients by Shapiro, who applied a haemoclip to the abdominal end and separated the ends by a fascia plane. In all but 3% of cases sperm granulomata formed at the end of the vas but, contrary to general expectation and to other reports (Schmidt 1966), these granulomata were not painful and no reoperations were required. In Shapiro's series there were no cases of recanalisation. Whether epididymal damage secondary to vasectomy is a cause of failure of a significant number of cases of vasectomy reversal is not yet established. However in view of the youth of many men requesting vasectomy and the frequency with which reversal is requested any technique that improves chances of subsequent reversal while remaining effective is worthy of evaluation.

Complications

The immediate complication of serious concern to the patient is scrotal haematoma. Minor degrees of bruising are common and patients should be warned about this. If, however, a large haematoma develops this is best managed by admission of the patient to hospital and evacuation of the haematoma with scrotal drainage with the patient under general anaesthetic. In the Margaret Pyke series 0.7% of patients had haematomas and required further treatment (Blandy 1973) and in our own series this figure is 0.4% (Table 17.1).

Infection is also a risk following vasectomy and can in some cases develop into the dangerous Fournier's gangrene (Pryor et al. 1971). In our own series using strict aseptic techniques and absorbable sutures the rate of infection was 0.3% (Table 17.1).

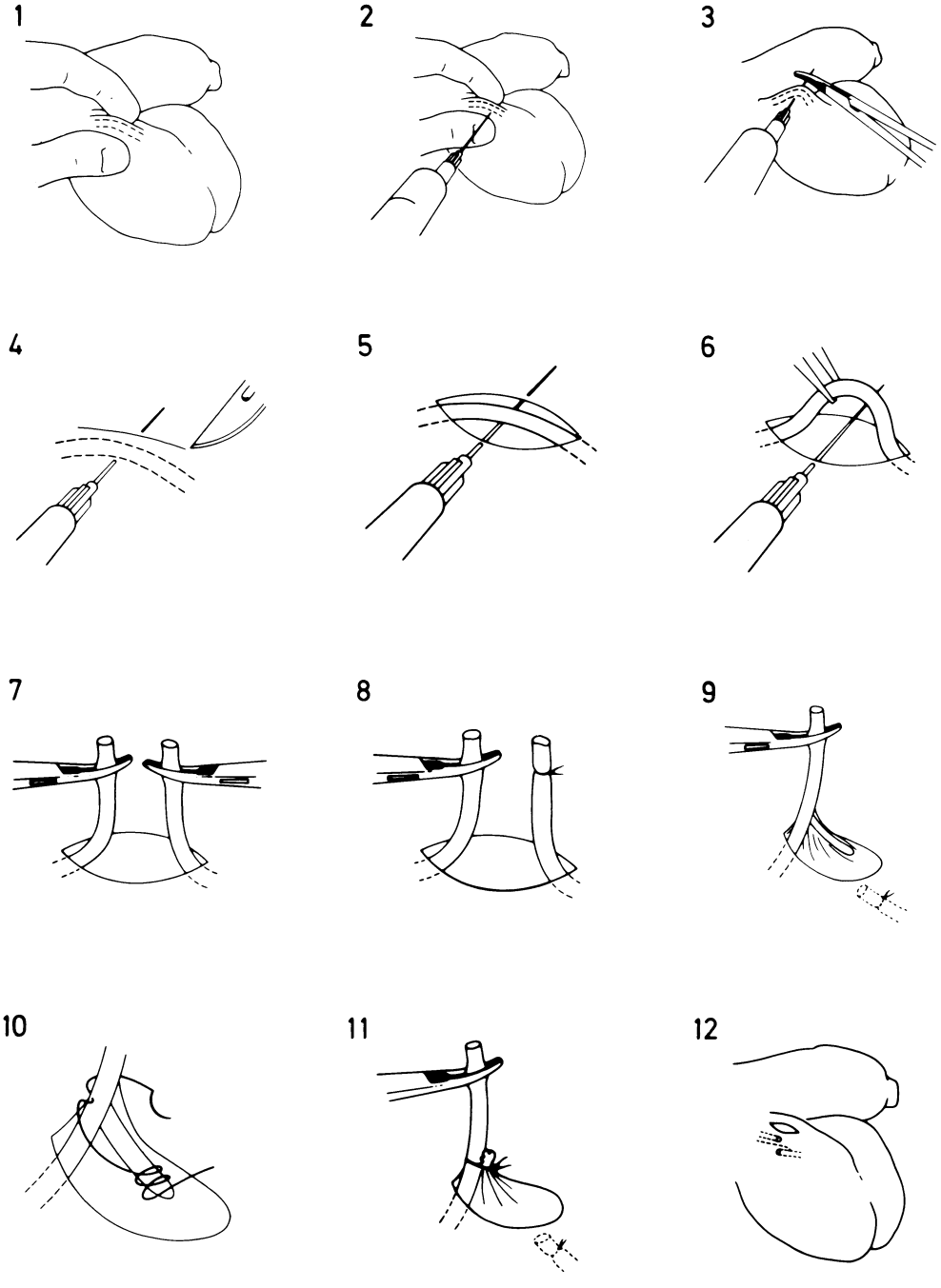

Fig. 17.1. A simple local anaesthetic technique for vasectomy

Table 17.1. Comparison of complications between two different vasectomy techniques: (a) Tissue plane technique (Fig. 17.1); (b) Excision of a length of vas and each end doubled back

a) Tissue plane technique	
2343 vasectomies between 1970 and 1976	
5 Reoperation because of persistent positive sperm count	0.2%
10 haematoma } requiring medical	0.4%
4 bleeding from skin edges } advice	0.17%
4 infection } requiring medical	0.17%
3 epididymitis } advice	0.12%
2 sperm granuloma—requiring reoperation	0.08%
1 impotence	0.04%
2 pregnancy[a]	0.08%
Total complications = 31 (1.3%)	
b) Doubling-back technique	
437 vasectomies between 1970 and 1976	
11 haematomas—requiring medical attention	2.5%
4 infection	0.9%
1 testicular swelling	0.2%
1 caught upper end of vas requiring re-operation	0.2%
Total complications = 17 (3.9%)	

[a] Two patients reported pregnancy following vasectomy. In one case the patient had intercourse without contraception soon after vasectomy and later sperm counts were all negative. It seems this case may be regarded as a failure of counselling rather than a failure of vasectomy. In the second case we have been unable to explain the pregnancy as subsequent sperm counts have been persistently negative

Sperm Granuloma

A later complication is the development of painful granulomata at the site of vasectomy or in the epididymis. Extravasation of the sperm from the vas gives rise to a characteristic foreign body giant cell reaction thought to occur because of reaction to acid fat material coating the sperm head (Friedmann and Garske 1949). These granulomata may occur up to 6 years after operation (Schmidt 1966). Usually they settle down with conservative measures, e.g. hot baths and a scrotal support. If symptoms persist religation is sometimes necessary; in such cases it is usually better to divide the vas or the tail of the epididymis at a site distal to the granuloma. Silber (1979) reported that 28 men undergoing vasectomy reversal who were found to have no sperm in the testicular end of the cut vas had on epidiymal histological examination extensive interstitial sperm granulomata resulting from rupture of the epididymal duct. The need for a tight seal at the testicular end of the cut vas at the time of the original vasectomy in order to avoid painful sperm granuloma at that site is now being questioned in view of the low morbidity reported by Shapiro and Silber (1979) using a vasectomy technique where the testicular end was not ligated or sealed with diathermy. A further argument for reconsideration about the morbidity from sperm granulomata at the cut end of the vas is that some men who have had effective ligation of the testicular end appeared to develop a painful epididymitis.

Recanalisation

In most cases this occurs within the first 3 months after operation. Recanalisation may occur after vasectomy irrespective of whether the ends have been separated in different tissue planes or not (Rhodes et al. 1980). There is often a history of scrotal haematoma or sperm granuloma into which epithelial channels grow and then unite (Pugh and Hanley 1969). The presence of motile sperm in the ejaculate at 3 months should make one suspect this complication. Another explanation for the persistence of motile sperm is failure to ligate at the original operation a duplicate vas, but this is extremely rare because most cases of duplicated vas are in fact intra-abdominal.

Clearance of Sperm

In the majority of cases azoospermia will be achieved by 3 months after vasectomy. Dodds (1972) found that 10.5% of 1600 cases still had positive analysis 3 months after operation. One patient in his series continued to produce sperm until 17 months after vasectomy. In some cases these long delays seem to be related to infrequent ejaculation. Marwood and Beral (1979) reported the rate of disappearance of sperm from the ejaculate after vasectomy in relation to age and coital frequency. They noted that coital frequency of less than once per week in men aged over 40 was associated with significantly prolonged periods before azoospermia was achieved; only 54% of men aged 50 became azoospermic at 12 months. Whether these non-motile sperm can fertilise is not known. Dodds (1972) states that if sperm are found to be dead on vital staining and numbers are not increasing then assurance of sterility can be given. Yeates (1976) states that he has had no cause to regret accepting a few non-motile sperm in the fresh (not more than 2 h old) specimen after 8 weeks, as being good enough to declare the patient safe. Unfortunately the occasional pregnancy is reported (Whittaker 1979), and although this is likely to be due to temporary recanalisation as described by Marshall and Lyon (1972) this cannot be proved. Surprisingly live but immotile sperm may appear in the ejaculate for some weeks after vasectomy and in view of the fact that motile sperm can sometimes be activated by changing seminal environment it is wise to confirm that sperm are dead before giving the all-clear to a patient with persisting occasional immotile spermatozoa. It is also worth noting that two azoospermic semen analyses with a week in between taken 3 months after operation are considered acceptable evidence of sterility in the United Kingdom. In some centres it is practice to send portions of vas for histological examination to ensure that the correct structure has been divided. This costly practice will not protect the patient against recanalisation (Table 17.1) and has not caused us to reconsider the operation in any of our patients. It seems reasonable to omit histological examination of vasa accepting that in very occasional cases the wrong structure may be divided but that postoperative semen analysis will quickly make this evident.

Psychiatric Aspects

Rodgers and Ziegler (1968) suggest that after vasectomy there is a tendency for the man to adopt a more masculine role. This does not seem to cause harm or marital break-up. If, however, the patient is psychologically unstable then vasectomy can precipitate psychiatric illness (Johnson 1964). In one study three couples with no pre-existing sexual problems suffered postoperatively from impotence, vaginismus and persistent premature ejaculation (Wolfers 1970), and recently a case was reported where vasectomy precipitated homosexual behaviour in a previously heterosexual man (Bass and Rees 1980). Such reactions are rare and can often be prevented by effective preoperative counselling (Lear 1972).

Post-Vasectomy Antibodies

A high incidence of circulating antibodies following vasectomy has been reported by many workers (Table 17.2). These antibodies do not cross-react with other somatic antigens so there is no risk to other cells but there may be a risk of immune complex disease. In spite of the presence of antisperm antibodies following vasectomy other tests for immune competence are normal. In mice there was no difference in mitogenic response, lymphocyte response, in vivo challenge with picral-chloride or humoral response to foreign protein between vasectomised and sham operated animals (Anderson and Alexander 1981). The mode of induction of antibodies following vasectomy would appear to be different from that in cases of infertility secondary to seminal plasma antibodies because after vasectomy seminal plasma antibodies do not appear so readily (Hellema et al. 1979). This is thought to be the reason why the presence of antibodies will not necessarily preclude fertility following vasovasostomy. Antibodies do sometimes appear in high concentrations in the seminal plasma after vasectomy (Linnet and Fogh-Anderson 1979). These cases often have a rather poor sperm density and this raises the possibility that there may be secondary auto-immune orchitis or epididymitis and that steroid therapy could possibly help. Jenkins et al. (1979), however, did not find any evidence of cell mediated immunity as judged by lymphocyte transformation tests in patients tested between one and eight years after vasectomy.

Immune Complex Disease

The normal antibody response to circulating antigen results in a rapid removal of the antigen and continuing circulating antibody. Occasionally immune complexes may form at the time when the initial surge of antibodies is being produced while there is still a large circulation load of antigen. In normal circumstances this risk is small because there is only a short time when both antibodies and antigen are present and also because the antigenic stimulus does not usually continue. The production of antisperm antibodies in response to vasectomy provides a situation

Table 17.2. Post-vasectomy antibodies

Reference	Patients	Method	Observation
Phadke and Padukone (1963)	25 after vasectomy	GAT[a] TAT[a]	32% positive on both tests
Shulman et al. (1972)	22 before and after vasectomy	GAT Passive haemagglutination test	55% Kibrick positive after operation. Two peaks of activity, one approx. 2–6 months postoperatively and one after 6 months The passive haemagglutination test was not helpful
Ansbacher (1972)	27 after vasectomy 37 before and after vasectomy	GAT SIT[a]	15 out of 27 +ve (55%) agglutinating 11 out of 27 +ve (41%) immobilising 19 out of 37 +ve (51%) agglutinating 14 out of 37 +ve (38%) immobilising
Coombs et al. (1973)	15 after vasectomy	Antiglobulin[a] reaction	8 out of 15 IgG antibodies in serum
Van Lis et al. (1974)	52 before and after vasectomy	GAT	60% kibrick positive The onset of spermagglutinin production was generally between 6 weeks and 6 months
Tung (1975)	114 before 112 two months after vasectomy 71 six months after vasectomy	Immunofluorescence	61% +ve before vasectomy[b] 77% +ve at 2 months[c] 90% +ve at 6 months[d] *Acrosome* *Tail Nucleus* 61%[b] 3%[b] 73%[c] 25%[c] 80%[d] 55%[d]
Hellema et al. (1979)	34 at 1 year and 5 years after vasectomy	TAT Microimmobilisation Immunofluorescence of swollen sperm heads Circulating immune complex	*1 year* *5 years* Agglutination 24/34 26/34 Immobilisation 11/34 17/34 Nuclear Immunofluorescence 9/34 8/34 Immune Complex Formation 5/29

[a] See Chap. 9

where both the antigenic challenge and the antibody response may be present for a long time and conditions may be suitable for the formation of immune complexes. In such circumstances immune complex disease may result, with the deposition of immune complex in the affected organ, in this case the testes, and also in other organs, usually the renal glomeruli, arterial walls, joints and choroid plexus.

Patchy orchitis has been reported in cats, mice and rats (Cunningham 1928). There is no direct proof that all these lesions are a result of antibody action. In guinea pigs lesions typical of allergic orchitis were reported by Tung et al. (1970) both on the vasectomised side and in the contralateral testes. Testicular changes have been reported following vasectomy in man but whether these are secondary to antibody formation has not been established (Gupta et al. 1975; Jenkins et al. 1979). Bigazzi et al. (1976) demonstrated that in rabbits the orchitis was associated with immune complex and also found immune complex glomerulonephritis.

Glomerulonephritis associated with immune complex has also been found in the glomeruli of monkeys following vasectomy but not in sham operated animals (Alexander 1982).

Vasectomy has been reported to increase the incidence of atherosclerosis in monkeys. In the Cynomolgous monkey (*Macca fascicularis*) Alexander and Clarkson (1978) found an increase in atherosclerosis affecting the great vessels, cardiac and cerebral vessels in vasectomised monkeys when compared with sham operated animals. In this experiment the monkeys had been fed on a diet containing about twice as much cholesterol as that consumed by the average North American man and plasma cholesterol levels in the monkey of 500 mg/100 ml were at least twice as high as that found in the average North American man. A second study compared vasectomised and sham operated rhesus monkeys but with a normal monkey chow (low cholesterol diet) and again there was increase in atherosclerosis affecting the aorta in the vasectomised animals (Clarkson and Alexander 1980). A third study examined the effect of response to cholesterol in the diet (Alexander 1982). Monkeys were fed a diet containing the equivalent cholesterol to the average North American diet for 2 months. At the end of this period the animals were divided into two groups according to serum cholesterol, one group with higher than normal circulating cholesterol and one group with normal levels. The monkeys in each group were then randomised to either vasectomy or sham operating and fed for a further 18 months on the same diet. At postmortem all vasectomised animals were found to have more atherosclerosis than non-vasectomised animals and the degree of atherosclerosis was worse in the group of monkeys who had responded to the diet with higher serum cholesterol levels.

Circulating immune complexes following vasectomy have been detected in man (Hellema et al. 1979). There is no evidence of increased risk of cardiac infarction in men following vasectomy but the present studies are not necessarily sensitive enough or of sufficient duration to detect small increases (Goldacre et al. 1978; Walker et al. 1981a, b). In a study of men attending a blood donation clinic an increased systolic blood pressure was found in men who had had vasectomy compared with other men (Alexander et al. 1981). There is also report of a possible association between peripheral arterial disease and vasectomy (Campbell et al. 1983). Further long term studies are needed; however, in view of the current decline in the rate of death from ischaemic heart disease in the UK (Heller et al. 1983) and in the USA (Levy 1981), there is no need for an overreaction to the possible adverse effects of vasectomy.

Endocrine Effects

In most experimental animals there is temporary depression of spermatogenesis immediately after vasectomy followed by a gradual return to normal. Biochemical changes are more profound, with a marked reduction in free amino acids and an increase in fructose and citric acid (Mann 1964). This might suggest increased androgen activity but no endocrine consequences following vasectomy have been reported despite careful search (Smith et al. 1975; Naik et al. 1976).

Resection of Nerve Fibres During Vasectomy

Pabst et al. (1979) report a study where cross-sections were made of cadaver human spermatic cords and proportions of vas removed during vasectomy. The specimens were stained for nerve fibres and the total cross-sectional area of the nerves in the vasectomy specimens amounted to one half of the total area in the whole spermatic cord. The authors postulate that following vasovasostomy the reason for poor fertility could be lack of contraction of the vas and epididymis secondary to interrupted nerves.

Vasectomy Reversal

In view of the widespread use of vasectomy as a method of contraception there is bound to be an increasing demand for vasectomy reversal. It is wise to counsel patients undergoing vasectomy that the operation should be considered irreversible because in that individual's case that may be true; nevertheless, efforts should be made to improve technique so that the original vasectomy operation is potentially reversible.

The two main approaches to vasectomy reversal are: (a) an end-to-end anastomosis using 3–4 approximating stitches with or without an intraluminal splint; (b) an end-to-end two-layer anastomosis using microsurgical techniques.

Silber (1977a) reports excellent results (Table 17.3) using microsurgical techniques for vasovasostomy and he also reports excellent results for microsurgical vasoepididymostomy following previous failed vasovasostomy (Silber 1978). In 42 patients, using a two-layer microsurgical end-to-end vasovasostomy anastomosis, 36 (86%) had normal postoperative semen analysis and 30 (71%) wives have reported pregnancy during a $1\frac{1}{2}$-year follow-up (Silber 1977b). These results are a yardstick by which other methods must be measured. In Table 17.3 some of the best reported results of macroscopic techniques are listed. In 270 cases the pregnancy rate was 53% and this is a significiantly worse result than with expert microsurgical technique. However, the cost in time and equipment if all vasectomy reversal operations in the UK are to be done using microsurgical techniques must be borne in mind. It is probably reasonable to rely in the first instance on macroscopic technique allowing for the fact that in some cases this will fail and a second operation will be necessary using microsurgical vasoepididymostomy as described by Silber (1978). What is now clear is that accurate apposition of the vas ends will give better results and if the operating microscope is not used, consideration should be given to using some form of magnification.

The part that antisperm antibodies may play in persistent infertility following apparently successful vasectomy reversal has not yet been established. There is a need for a prospective study of the effect that the presence of seminal plasma (not serum) antibodies may play in preventing fertility in men who have had technically good vasectomy reversal operations. Undoubtedly many failures attributed to antisperm antibodies are in fact failures in technique resulting in partial anastomosis. It must be borne in mind that two-thirds of the spermatozoa are stored in the body and tail of the epididymis (Freund and Davis 1969) and in order for ejaculation of adequate numbers of spermatozoa following vasectomy reversal

Table 17.3. Results of vasovasostomy

Conventional techniques	No	Motile sperm	Pregnancies	Method
Phadke and Phadke (1967)	76	83%	55%	Splint end-to-end
Fitzpatrick (1978)	14	100%	64%	Transverse
Middleton and Henderson (1978)	72	74%	39%	End-to-end. No splint
Jenkins and Blacklock (1979)	17		65%	Splint end-to-end
Lee and McLoughlin (1980)	41	90%	46%	Splint
Urquhart-Hay (1981)	50	88%	52%	End-to-end splint. (3.5 × magnification)
Microsurgical techniques				
Owen (1977)	50	98%	62%	3 layer
Lee and McLoughlin (1980)	26	96%	54%	2 layer
Silber (1977b)	42 Unselected patients	36 Normal 6 Below normal	28 2 (71%)	2 layer. End-to-end
Silber (1981)	*Not Stated*: Patients with failed previous microsurgical vasovasostomy + patients found to have lack of sperm at testicular end of vas during attempts at vasovasostomy	80% normal		Anastomosis of abdominal end of vas to a single dilated epididymal tubule. 2 layer. Microsurgical techniques

there must be a widely patent channel. Another possible reason for failure to produce a pregnancy is that those patients requesting vasectomy reversal are often in their middle or late thirties, and the natural fertility of the man or his new partner may be on the wane.

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Chapter 18

AID and Adoption

A. A. Templeton and J. Triseliotis

Part I

AID (*A. A. Templeton*)

One purpose of this book is to encourage a rational approach to the treatment of male infertility but despite advances in the understanding of male reproductive physiology, the fact is that many will be fortunate if they ever father a child. Realisation of this truth among physicians and also the increasing public acceptance of artificial insemination by donor (AID) has led to a dramatic increase in demand for this treatment. In the Infertility Clinic, Royal Infirmary, Edinburgh, there is a waiting list of one year and only 50% of the patients accepted into the AID programme actually reside in Edinburgh. Many patients have to travel long distances for treatment and the stress involved must take its toll on their quality of life and also on their chance of conceiving. While several centres run small clinics often using fresh semen, the establishment of larger AID clinics is often thwarted by difficulties relating to the recruitment of suitable donors, the introduction of semen cryostorage and the development of precise methods of timing insemination.

Despite these problems there can be no doubt that AID is accepted as appropriate treatment among medical practitioners and the lay public. Since 1960, when the Feversham Committee reported on AID there has been increasing discussion in Britain. In 1973 the CIBA Foundation held a symposium on the 'Law and Ethics of Artificial Insemination by Donor'. One of the conclusions was that AID was an acceptable and legitimate solution to the problem of male subfertility. Since that time the Peel Committee (British Medical Journal 1973) has made specific recommendations which included the suggestion that AID should be made available within the National Health Service and this was endorsed by a meeting of the Royal College of Obstetricians and Gynaecologists in 1976. Despite these recommendations, AID is not yet readily available in most clinics in the UK. One solution is a national system of semen storage such as exists in Denmark (Brogaard-Hansen et al. 1979) and in France (Schwartz et al. 1979). In the former a Central Semen Bank, designed to serve gynaecological departments in the whole of Denmark, was established and this has allowed the accumulation of much useful information on donor recruitment and semen transport. The area-based system developed in France, fully supported by the Ministry of Health, has achieved a remarkable success rate considering the many gynaecologists involved.

Indications for AID

The chief indication for Artificial Insemination is inadequate insemination. The main categories of patients requesting AID encountered in our clinic are as follows:

1. Those with azoospermia and oligozoospermia on repeated seminal analysis, where the cause is non-remediable or where AID is preferred to surgical or medical treatment
2. Those with a consistent defect of sperm quality (motility, morphology or viability) who remain infertile despite all other investigations on husband and wife being normal
3. Those men, commonly diabetics, with retrograde ejaculation where attempts at antegrade ejaculation or retrieval of sperm from the urine have proved unsuccessful
4. Those carrying a lethal or deleterious gene

A series of 100 consecutive patients seen at the Royal Infirmary clinic in Edinburgh presented with the following problems:

Azoospermia (including 4 with Klinefelter's syndrome and 3 chromosomally normal hypogonadal men)	54
Oligozoospermia	32
Oligozoospermia + structural chromosome rearrangement	1
Oligozoospermia + morphological sperm abnormality	1
Oligozoospermia + impaired sperm motility	1
High sperm density + impaired sperm motility	1
Retrograde ejaculation	1
Paraplegia	2
Genetic disorders	7

Our indications are as yet restricted to these categories and we have not considered patients with infertility of unknown aetiology as being suitable. Similarly we do not consider any female problems, for example immunological defects affecting interaction between spermatozoa and the female tract, as indications for AID. With further research it is possible that this area will be clarified and a rational approach to therapy will evolve.

Selection of Patients

Selection of patients for AID is a problem which raises many social, ethical and logistical issues. The immediate question is whether doctors are entitled to withhold their services from patients they consider unsuitable. A practitioner's chief justification for this course is responsibility to the unborn child. The current unsatisfactory legal status of the AID child, who is illegitimate and who has no real protection in law should the parents subsequently divorce (Cusine 1976), underlines the doctor's responsibility to make some assessment of the couple being treated. These and other ethical issues are discussed more fully elsewhere (Dunstan 1976; Templeton 1979). In reality, most patients requesting AID have

selected themselves; only those couples who are determined that the treatment is appropriate will run the gauntlet of many hospital visits.

There should be a time interval between informing the couple of their problem, explaining their options and then discussing AID in more detail. It is inappropriate to mention azoospermia in one breath and AID in the next without giving the man and his partner time to come to terms with their predicament. This particular mistake is no where more painfully illustrated than in the autobiographical novel 'Blizzard and the Holy Ghost' by Joseph Blizzard (1977). This book makes salutary reading for practitioners and recipients of AID.

In practical terms, the process of selection of patients for AID should be aimed at considering the following three questions:

1. *The Man*—Does the man have a real problem of inadequate insemination, that can be best helped by artificial insemination? In one study in the UK 15% of patients were found not to need artificial insemination after careful review (Newton 1976)
2. *The Woman*—Is the woman potentially fertile and fit for pregnancy? (Chap. 8)
3. *The Couple*—Is the couple sufficiently informed about, and accepting of AID and are they suitably prepared to have an AID child?

In counselling we normally use the following guidelines. The husband must be seen to have entirely accepted his own infertility. The wife must accept her husband's infertility and not see artificial insemination as a means of reproaching her husband. The couple's motivation to have children must be examined, although in practice this is extremely difficult. It would, for example, be inappropriate to carry out artificial insemination where the couple's need for children is merely a response to pressure from outside their marriage. Finally, all moral and religious scruples must be settled.

Counselling is best undertaken by more than one member of the infertility team. To further ensure that the couple are taking the right decision for themselves, all pressures for a hasty decision should be resisted and there should be a significant time interval between acceptance into the AID programme and beginning inseminations.

Timing of Insemination

For optimal timing, insemination should coincide with the immediate preovular and ovular days of the menstrual cycle. Where the cycle is ovular but irregular, say 28–49 days, it may become necessary for logistical reasons to induce more regular cycles with clomiphene. For minor irregularities, 28 ± 7 days, ovulation induction should be eschewed if at all possible because of the recognised discrepancy between ovulation and pregnancy rates with clomiphene (Shearman and Korda 1972). Methods available for detecting ovulation have been discussed in Chap. 8.

Success Rates with Fresh and Frozen Semen

Three main factors will affect the success rate of artificial insemination. These are the woman's fertility potential, with particular regard to menstrual and ovular regularity, the timing of insemination and the quality of the semen. A recent review

(Richardson 1976) reports a 60%–75% success rate with fresh semen and a 40%–55% success rate using frozen semen. However, there are many problems in recording success rates and some of these have been outlined by Schwartz et al. (1979).

It appears that women over 30 years find it more difficult to get pregnant through AID than younger women, and that wives of azoospermic husbands will conceive more readily than those whose husbands are oligozoospermic (Van Noord-Zaadstra et al. 1980).

Although frozen semen is less effective, in large clinics where matching is attempted it may be logistically impossible to use fresh semen. Brogaard-Hansen et al. (1979) report that 22 out of 30 women eventually became pregnant using frozen semen that was highly selected in that the post-thaw motility was greater than 50%; however, other large clinics (e.g. Hopkins et al. 1979) find it difficult to achieve more than a 50% success rate with frozen sperm.

Full details of cryopreservation techniques have been published by Richardson (1976).

Selection of Donors

The selection of donors is a neglected area in the practice of AID, not so much from the medical aspect but more from social and ethical considerations. Several authors have described the medical assessment of donors (e.g. Joyce 1976; Hopkins et al. 1979) and the following minimum screening procedures are recommended:

1. Initial interview with medical and family characteristics. An exhaustive family history is mandatory
2. General examination of physical characteristics
3. Semen analysis which should include bacteriology, and in particular culture for gonococcus
4. VDRL
5. Blood group with Rhesus typing

Further tests, such as karyotyping, are not strongly indicated. Some clinics carry out this out routinely (Hopkins et al. 1979) but have failed to identify any significant abnormalities (Matthews 1980, personal communication). It has been estimated that the chance of a phenotypically normal fertile husband displaying an abnormal karyotype which would affect his offspring is 1 in 1000 (Joyce 1976). Bacteriological culture for gonorrhoea is important as the gonococcus will survive freezing and there is a report of three women being infected by the same donor (Brogaard-Hansen et al. 1979).

Our current main source of donors is through the recruitment of husbands of post-natal patients. The initial approach is by letter and this is only pursued if there is some response from the patient or her husband. This method has the advantage that the donors are fertile, are usually stable and mature and the partner has the opportunity to voice her opinion. The disadvantage is that more work can be involved in recruitment as most patients will be preoccupied by domestic arrangements for the new baby. Schoysman (1975) has estimated that as few as 10% of patients approached in this way will become donors. Medical students are usually

readily available and will be keen to help especially if a fee is offered. However, they are often unmarried and cannot yet know the attitude of their spouse-to-be. Furthermore, their fertility is unproven and there is the slight possibility that a student will be oligozoospermic or even azoospermic. This knowledge may be difficult to handle.

If the semen is to be frozen and cryopreserved there are additional limitations of quality control. In two large centres the experience is that as few as 10% of potential donors have satisfactory semen quality, after seminal analysis and test freezing has been performed (Brogaard-Hansen et al. 1979; David G. 1980, personal communication).

The use of family members as donors is best avoided, except in exceptional circumstances, because of the uncertain emotional and psychosexual problems (Schellen 1957). The question of financial imbursement is as yet unresolved. Most clinics choose to pay donors (Joyce 1976) but we have chosen not to, as our clinic is run within the National Health Service, and the patients do not pay. This way, we are also happier about the motivation of the donors.

There are of course many other medical, social and ethical considerations associated with the practice of AID and the following volumes are particularly recommended: Artificial Insemination (1976); Finegold (1976); Blizzard (1977). The next section deals with some of the social aspects of AID and considers the lessons that can be learned from the adoption experience.

Part II

Adoption (*J. Triseliotis*)

Few institutions mirror as closely as adoption the social changes taking place in society and as society is never static, adoption itself evolves to reflect these changes. In the 25 or 30 years since the end of the Second World War adoption came to be seen as a means of providing children for infertile couples. Physicians faced with distressed and desperate couples would increasingly recommend adoption as a solution to the problem. However, changes that began in the late 1960s have altered the nature of adoption in ways that take much less account of the needs of these couples.

Parenthood by Adoption

A historical examination of adoption practice from ancient to present time reveals four distinct patterns. The original custom which emerged in the ancient Eastern civilisations was the adoption of legitimate males to perform religious ceremonies or in order that a family might not die out. The first adoption legislation in Western societies is to be traced to Massachusetts (USA) where an adoption law was passed in 1851. Whilst the main aim of this law was to regulate the conditions of mostly illegitimate children who were farmed out, it also helped to provide a kind of home help in a homestead economy. Adopted children were looked upon as second class citizens and were expected to work hard to repay the kindness of the adopters. In England the first adoption law was passed in 1926 to deal with the problem of increased illegitimacy and orphanhood resulting from the First World War. Adoption was then surrounded with secrecy and stigma and seen as suitable for the lower classes who were not supposed to bother about 'bad blood' and 'transmitted immorality'. The period following the end of the Second World War marks the third stage when adoption became acceptable among the middle classes. It also marked the stage of viewing adoption as a solution to the problem of childlessness and infertility.

From the late 1960s onwards and as a result of social changes adoption became more of a child care service practised in the interests of children than those of childless couples. The appeal now was for families willing and able to offer a child care service mostly to older, handicapped or coloured children who, ten or fifteen years age, would not have been considered. The needs of infertile couples feature very little in this new type of adoption. In fact the problems of childless couples and of 'parentless' children, are seen as separate and distinct issues.

Change in Adoption Practice

From the mid-1960s changes in lifestyles, sexual behaviour, contraceptive methods and parental responsibilities have resulted in a drop in the number of available infants for adoption. As an example, the number of children adopted in England and Wales fell from a peak number of approximately 25 000 in 1968 to 10 609 in 1980. Because of the shortage of babies in the 1970s most adoption agencies closed their lists and as a result childless couples wishing to create or increase their family were looking for new ways of achieving this. In some cases market factors began to

operate and inter-country adoptions, mainly from South-East Asia and South Korea became widespread. These adoptions have now decreased because of greater controls exercised by the governments of the countries of origin.

At the same time that the number of white infants available for adoption was decreasing, studies were showing that in Britain there were thousands of children who were destined to spend most of their childhood lives in local authority homes or in unstable fostering arrangements. These were not the children traditionally seen as suitable for adoption but older children, sometimes mentally or physically handicapped, or black children or groups of siblings who could have benefited from the advantages of family life. The children's families of origin had in most cases either disappeared, lost interest in them or could not care for them. They came to be known as 'children who wait' or 'hard-to-place' children.

Children Who Wait

Until the early 1970s there was no data from the various organisations about children undergoing care. Pressure for more information came from frustrated would-be adopters, particularly in the United States. A study by Rowe and Lambert (1973) which covered 29 agencies in Britain, found 6000 children who were in impermanent situations. In this study nearly two-thirds of the children were of school age, and about a quarter were black or of mixed race. Questions were asked on how real were the expectations that these children would one day be rehabilitated with their families of origin. Some highly publicised cases of ill-treatment by parents or step-parents forced re-examination of the concept of rehabilitation. A book by Goldstein et al. (1973) reinforced this move by helping to differentiate the role of biological creation of a child and parenting in the sense of nurturing and bringing up. Studies in adoption and fostering (Triseliotis 1973, 1980) further showed that from a child's point of view the parents are the people who give day-to-day care and love. Legal constraints further complicated the position. Following the findings about children who wait, individual organisations, some local authorities and adoption agencies mounted big campaigns and set up Resource Exchanges to find homes for these children. This change has been from 'adoptable infants' to 'this child needs a family'.

It was not only necessary to convince would-be adopters about the changing nature of adoption, but also social workers, doctors and other professionals whose own attitudes were often responsible for applicants sticking to young white babies. It was assumed that adverse experiences in early childhood were irreversible and even good mothering was almost useless if delayed after the age of $2\frac{1}{2}$ (Bowlby 1952). Recent studies refute this view and have demonstrated the reversibility of early adverse psychological experiences (Kadushin 1970). Clarke and Clarke (1976) conclude that early experiences have immediate effects which if not reinforced will fade in time. Children growing up in institutions or even in group foster homes were found to be less well equipped to cope with adult life than those growing up in adoptive or permanent foster homes (Triseliotis 1983)

Who Is an Adoptable Child?

The current thinking is that every child is adoptable provided there is a family wishing to offer it a home. It is not the professionals' responsibility to declare

children 'fit' for adoption but to explain to would-be adopters the special needs of the children available. The final decision has to come from the families themselves. Sawbridge (1979), writing about the work of her agency, describes a number of cases of children like the following for whom adoptive homes were eventually found. Matthew, $4\frac{1}{2}$ years old and crippled by spina bifida, functioning at a $2\frac{1}{2}$-year-old level, cheerful and responsive but subject to fearful tantrums aroused by frustrations; Leonard, aged 11, who was partially deaf, and very confused and immature, needing hours of help with sorting out his past and mourning his dead mother; and Enid, who was 15 and mentally retarded, but able with plenty of encouragement to function fairly normally and manage most day-to-day routines. Each one of these children requires something different, but unlike babies they also bring their own history, memories and experiences of good or bad relationships. Many people were shocked when some adoption agencies started to advertise children on television or in newspapers alongside appeals for families. Obviously a lot of preparation and sensitive handling has to go into such 'advertisements' or shopping-type 'catalogues'.

Who Can Adopt?

The placement of older or handicapped children is a very different matter from placing babies with infertile couples. One of the most controversial areas of adoption practice has been the vetting or selection of adoptive parents. In the absence of any guide as to what constitutes a good parent, it was not surprising that many workers incorporated their own prejudices. Such characteristics as social class, by which most 'matching' was arranged, age of applicants, years after marriage and religion were subsequently found by a number of studies to be irrelevant to the outcome of adoption.

The current practice of the more progressive agencies is to try to prepare couples for the adoptive role, by using educative methods and discussion groups and by introducing them to people who have already adopted. Though the agency still retains final responsibility about placement, couples are generally encouraged to decide for themselves whether to continue or withdraw. Bearing in mind the problems of children who need adoptive families, adoption as it is practised now is not the kind of parenthood for all.

A Comparison of Adoption and AID

Adoption and AID have a number of things in common and some important differences and some of the experience gained through adoption practice maybe relevant to AID. Adoption may provide some illumination in the following three areas: feelings about infertility, the selection of couples and the child's need to know about its origin.

Feelings About Infertility

At first adoption was practised to meet the needs of infertile couples. Of 92 couples who adopted through three adoption agencies in 1965, in almost two out of every three there was evidence of infertility, functional or otherwise (Triseliotis 1970).

In most of the rest there was evidence of miscarriages, still-births, etc. In 17% of cases the husband and in 25% of cases the wife was reported as 'infertile or subfertile'. Adoption agencies were insistent that would-be adopters went through detailed infertility investigations because it was thought that couples would reject an adopted child if they subsequently had one of their own; however, Jaffee and Faushel (1970) found no evidence that this was so. Most adoption agencies have now abandoned the rule on infertility, preferring to concentrate on a couple's readiness and capacity to give ongoing care to a child. Of relevance to children born through AID is the fact that until recently the degree to which a couple had come to terms with their feelings of being infertile was a central issue in the assessment of their readiness to adopt. Humphrey and Ounstead (1964) found that one-fifth of the couples whose adopted children were referred for psychiatric treatment had lasting preoccupations with their failure to have children. It seems that if a sense of personal inadequacy persists in either partner, this may affect their attitude to their child (Humphrey 1969). The implication is that such feelings need to be worked through before placing a child and this might hold equally true of children born by AID. Unlike adoption, in the case of AID such preoccupations can only be entertained by the husband; the two partners do not start as equals. The possible effects on a child of preoccupations with infertility suggest the need for counselling to be offered to couples contemplating AID.

Selection of Couples

The main issue is whether there should be a selection procedure to establish 'suitability' for parenthood by AID. Any selection process would create similar problems to the methods now discarded by the more progressive adoption agencies. Listening to a physician at a conference held in 1978, it was obvious that some doctors were becoming judges of suitability for parenthood by AID on even more shaky grounds than those previously used by adoption agencies.

A helpful way to look at selection is to remember that it is only through a quirk of nature that the man in this case cannot produce a child. Otherwise, no one would have the right or power to declare a couple fit and suitable. My own preference is for self-selection, following a process of discussion, social education and exploration of the issues concerned with infertility, the meaning of parenthood by AID, parenting, a child's need to know the truths about its origins and so on. In the end it should be up to the couple in question to accept a method which leads to self-selection through increased understanding and some new awareness of what they are embarking on. The opportunity for preparation for parenthood is infinitely preferable to subjective judgements about psychological or other suitability. Those who would prefer a more rigorous investigation might like to ponder on what their position would be if do-it-yourself kits were to become available. If such kits were put on the market, their widespread use, like back-street abortion, could depend on how far fertility clinics and physicians adopted a non-rationing attitude towards those seeking to be parents by AID.

What to Tell the Child Born of AID

After many years of unnecessary secrecy, studies have shown how important it is for all children, whether adopted, fostered or brought up in step-parent rela-

tionships, to know the truth about their parentage and origins. This applies equally to children born by AID. Children need to know the truth to enable them to build their developing personality and self-concept on accurate facts. This will include the construction of an identity based on a comprehension of biological and psychological parenting. Told the truth, children seem to find no difficulty in doing this and achieving a satisfactory adjustment. We know that adopted people experience great psychological trauma if they come to know late about their adoption, especially from sources other than their parents (Triseliotis 1973). The child born by AID has similar needs to other children and the responsibility for explaining rests with the parents. Yet recently, a judge was advising a mother who gave birth to a child by AID, not to tell him about the true circumstances of his parentage, and simply to say that his father was lost at sea!

It could be argued that, unlike adoption, where other people besides the adoptive parents play a part in the process, in AID, the most likely persons to know are the mother and her husband and the medical practitioner who assisted in the process. Experience suggests, however, that there is a high probability of husband or wife under stress or provocation telling the child the truth. This is how a number of adopted or step-children came to know about their true origins. Such a sudden and often attacking form of revelation can be bewildering to a child and lead to alienation. For the 'parents' the keeping of an important secret such as this can be a continuously emotionally draining experience.

My own studies involving adopted and fostered people have shown that people want to know the truth about themselves even when the truth is unpalatable. 'With the truth', as many put it, 'you can come to terms, but with lies you never know where you stand'. The facts about an individual's roots belong to that individual and the 'parents' have no right to withhold them. In doing so they may be storing trouble for themselves and the child. What I am suggesting is that children born of AID have a right to be told at an early stage about the true circumstances surrounding their conception and birth. A good opportunity is possibly around the time when they begin to ask questions about babies and birth. Explanations will need to be repeated at intervals and additional information can then be given appropriate to the child's age. By the time children are in their early teens or mid-teens they should know everything there is to know about AID and about their own origins. A child's identity would also be strengthened if the law were to change to ascribe the same status to all children irrespective of the circumstances of their conception.

The next question concerns the nature of information to be passed on to the child. Information about the biological 'father' is something that mothers should insist on obtaining from the physician involved. These could be non-identifying particulars about such aspects as medical history, personal and physical characteristics, interests, hobbies and so on. When a couple are dependent on a doctor, they may not have the courage to ask for the information, and it should be the responsibility of the physician to provide it, preferably in writing, without having to be asked. A full concept of self is not possible for anyone aware that important links in their 'make-up' are missing. The question might be asked: 'If it is important to tell and share information about the biological father, will it not be important to give also his name and address, which will prove a disincentive to anyone volunteering to be a donor?' Not necessarily. The research studies of Triseliotis referred to earlier have shown that approximately 98% of adopted people are satisfied with non-identifying information passed on to them by their parents. For

the tiny minority who are not satisfied and set out on a quest, the reasons are often associated with secrecy and evasion pursued by the adoptive parents, or with some other serious identity crisis.

Even if the 'parents' have no background information about the biological father, it is still important that they tell the child the truth. All these and other developments point to the need for widespread social education, particularly at schools, to familiarise children and people in general about the many different ways by which families are now being built. In effect, the family constructed by two people who marry, have children and stay together till death is only one type. There is now an increasing number of people who marry and divorce, and step-parenting is becoming a very common way of reconstituting a family. So also is adoption, or becoming a parent by AID. Children need to grow up knowing there is nothing shameful about being adopted, a step-child, or a child conceived by AID. The widespread ignorance and often prejudice that exists in the community can create feelings of shame or of being different in children who are not growing up in traditional-type families.

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