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Cancer and Fertility

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To my son, Ned, who is on a path to be an incredible man; my daughter, Emily, who is a bright star for the world; and my beautiful wife, Amy, who has stood by me for over 30 years, I dedicate this book to you. Without your love and support, this would not have been possible.

Preface

We are fortunate to live in a time where, despite a growing incidence of cancer in patients in their reproductive years, medical advances have allowed for long-term survival in the majority of such individuals. While cancer cure must remain our paramount goal, a growing number of patients face quality of life sequela from both the effects of the malignancy and the subsequent treatment. Numerous surveys of pre- and postpubertal cancer survivors reveal disappointment and regret over failure to consider fertility preservation during the period of cancer treatment.

This text is unique in its consideration of reproductive options for both male and female cancer survivors. Drawing on the experience of international experts in the field, this textbook is designed to provide a summary of state-of-the-art developments in fertility and its association with cancer for both new and experienced practitioners. The text provides a comprehensive review of normal female and male reproductive physiology as well as the impact of oncologic treatments on orderly germ cell development. In addition, focus is placed on the management of cancer diagnoses during pregnancy. Finally, future fertility preservation options including stem cell preservation as well as surgical germ cell harvest techniques are reviewed in depth. It is intended to be clear, concise, and readable to allow the reader to obtain rapid answers to this challenging medical issue. Special emphasis is placed on diagnostic and treatment algorithms to aid in standardized evaluations and management of these patients. The text is designed for urologists, gynecologists, medical and surgical oncologists, primary care providers, and allied health providers who have the privilege of assisting with fertility in both men and women.

It is indeed an amazing time to treat cancer patients as we move beyond a sole focus of cancer survival to all components of survivorship. In young patients who dream of a future family, no component of future quality of life is as important as future fertility. It is incumbent upon providers to understand the impact of various medical, surgical, and radiation treatments on a patient's reproductive potential. We hope this book stimulates your interest in this issue as we partner to assist these patients toward a fulfilling post-cancer life.

Cleveland, OH, USA — Edmund S. Sabanegh Jr.

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Demographics of Cancer in the Reproductive Age Male

1

Kiranpreet K. Khurana and Joseph P. Alukal

Fertility preservation for men undergoing treatment for malignancy is a problem of increasing importance for a number of reasons. First, as treatments for varying types of cancers in young men are increasingly successful, more men are given the opportunity to survive their malignancy and pursue family building. Of equal importance are the changing demographics of men pursuing family building; between 1980 and 2008, live birth rates amongst men ages 30–34, 35–39, and 40–44 years increased [1]. Concurrently, live birth rates for men ages 25–29 years decreased. Finally, 13 % of cancer diagnoses worldwide were made in patients under the age of 44 years [2]. As men delay fatherhood, the opportunity for them to have first developed cancer increases; as well the possibility of developing cancers such as prostate or bladder, which are not typically considered cancers of young men but do impact fertility, becomes greater. The changing demographics of men pursuing family building makes understanding the fertility risk of treating malignancy in this population even more important.

We outline in this chapter the demographics of cancer in the reproductive age male. Again, we will focus on those cancers typically thought of as common in this demographic population: leukemia, lymphoma, and testis cancer. We will consider as well the demographics of rare cancers such as central nervous system (CNS) malignancies, and we will also review the incidences of prostate, bladder, and colon cancer in men pursuing fertility. Although the topic is covered in greater detail in the rest of this textbook, we will also review briefly the fertility impact of treatments commonly utilized in this age group for management as well as a broad review of fertility preservation strategies that can be undertaken with these patients. Statistics regarding the prevalence or incidence of these diseases are given for men on an annual basis in the United States; this is in the context of 52 million American men between the ages of 20 and 45 years (the age demographic within which men are most likely to pursue family building), according to the 2010 National Census Bureau Report [3].

Thus, the comprehensive care of males of reproductive age with cancer involves minimizing the effect of treatment on fertility potential, and a focus on improving quality of life. This includes a thorough discussion of impact on fer-

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tility and possible consultation with an infertility expert prior to initiating treatment for cancer.

Cancers of Young Men: Overview

Pediatric Cancers

Approximately 10,380 children ages 0–14 years will be diagnosed with childhood cancer in 2015 [4]; the incidence of cancer in children has been increasing 0.6 % annually for the last 35 years [5]. A child born in the United States in 2015 has a 0.24 % chance of developing cancer from age 0 to 14 years [6]. Cancers are more common in Caucasian and Hispanic children than children from other races [5]. Due to the advancement in treatment of childhood cancers, over 80 % of children diagnosed with cancer will survive more than 5 years, which is a vast improvement compared to 1970s, when the 5-year survival rate was 58 % [4]. Cancer is the second most common cause of childhood death [4], but the leading cause of death after infancy [5]. About 1250 US children with cancer ages 0–14 years are expected to die in 2015 [4].

The types of cancers in childhood from most common to least common and their respective prevalences are: leukemia (30 %), brain and other CNS cancers (26 %), neuroblastoma (6 %), Non-Hodgkin lymphoma (5 %), Wilms tumor (5 %), soft tissue sarcomas (3 %), bone cancer (3 %), and retinoblastoma (2 %) [4]. Brain and CNS cancers remain the most common cause of cancer deaths in childhood [5].

Acute lymphocytic leukemia (ALL) accounts for 26 % of cancers diagnosed in children ages 0–14 years and 8 % of cancers diagnosed in children ages 15–19. ALL is more common in boys than girls. The 5-year survival in children with ALL has increased from 57 % in mid-1970s to 90 % in mid-2000s [6], and presently, greater than 75 % of pediatric patients with ALL reach adulthood [7]. Brain and CNS tumors are the second most common type of cancers in children below age 20, accounting for about 26 % of all cancers below that age. About one in five childhood cancers are CNS tumors. Three out of four children with CNS tumors will survive at least 5 years [8]. Non-Hodgkin's lymphoma accounts for approximately 5 % of all childhood cancers. In children ages 0–14 years, about 500 cases of Non-Hodgkin's lymphoma are diagnosed each year. In adolescents ages 15–19 years, another 400 cases of Non-Hodgkin's lymphoma are diagnosed [9].

Adolescent Cancers

About 5330 adolescents ages 15–19 years will be diagnosed with childhood cancer in 2015 [6]. An adolescent between ages 15–19 years, who is born in the United States, has a 0.35 % chance of developing cancer [6]. Of these, approximately 610 adolescents are expected to die in 2015 [6]. The types of cancers from most common to least common and their respective prevalence in this age group are: Hodgkin lymphoma (15 %), thyroid (11 %), brain and other CNS (10 %), and testicular germ cell cancers (8 %) [6, 10].

Data up to January 1, 2010 suggests that there are about 380,000 survivors of childhood cancers ages 0–19 years living in the United States [6]. One in 530 young adults between the ages of 20 and 39 years is a childhood cancer survivor [6]. The adolescent cancer incidence has been steadily increasing along with survival from cancer, resulting in greater number of population with history of cancer and cancer-related treatment in the reproductive aged cohort. The most common cancer diagnoses amongst the survivors are acute lymphoblastic leukemia, brain and CNS tumors, and Hodgkin lymphoma [6].

Treatment of Adolescent and Childhood Cancers in Males and Impact on Fertility

Treatment for ALL consists of 4–6 weeks of induction chemotherapy followed by consolidation chemotherapy for several months and 2–3 years of maintenance chemotherapy. There is also a role for allogeneic bone marrow transplant in children with high-risk features at time of diagnosis, recurrence after remission, and the

inability to go into remission after induction chemotherapy. Children with high risk of CNS recurrence may be treated with cranial irradiation, which has largely been replaced with intrathecal chemotherapy in recent treatment protocols. Acute myeloid leukemia has a lower incidence (5 %) in children ages 0–14 as compared to ALL and accounts for 4 % of cancers in adolescents ages 15–19 [6].

A common regimen used in leukemia, namely cyclophosphamide or melphalan in addition to total body irradiation, resulted in permanent sterility in approximately 83 % of patients [11]. Cyclophosphamide has a dose-dependent negative effect on gonadal function, and greater than 10 g/m^2 cumulative dose has a high risk of permanent damage to gonadal function [12]. This detrimental effect exists even in prepubertal testes showing that there is some germ cell proliferative activity in infancy [13].

The most spermatotoxic chemotherapy drugs are nitrogen mustard derivatives, i.e., busulphan and melphalan, and alkylating drugs, i.e., cyclophosphamide and procarbazine [14]. Table 1.1 classifies chemotherapy drugs into high, medium, and low risk of impairment on spermatogenesis. When comparing two regimens used for Hodgkin's disease, namely nitrogen mustard, vincristine (oncovin), procarbazine, and prednisone (MOPP) and adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD), MOPP has a higher risk of infertility than ABVD [14]. Males who received >3 courses of MOPP were found to have azoospermia in 85–90 % after >1 year of follow-up. Comparatively, 90 % of patients had normal sperm counts a year after therapy with ABVD. MOPP is also more detrimental to fertility than bleomycin, etoposide, and cisplatin (BEP) for testicular germ cell tumors (TGCT). In one study, TGCT treated with a cisplatin-based therapeutic regimen resulted in normal sperm parameters in 63 % of patients after 1 year, which increased to 80 % at 5 years [14].

The effect of chemotherapy is greater on Sertoli cells than Leydig cells, thereby having a detrimental impact on spermatogenesis. This can result in significant reduction in sperm count to the point of oligospermia or azoospermia. A Norwegian study [15] found the overall prevalence of azoospermia in childhood cancer survivors to be 18 %, with the prevalence being 19 % for leukemias, 53 % for Hodgkin lymphoma, 11 % for non-Hodgkin lymphoma, and 11 % for testicular cancer. Comparatively, the prevalence of azoospermia in normal males is 1 %. In men treated with high cumulative dose alkylating agents or cisplatin, 80 % were azoospermic, whereas this rate was significantly lower at 5.3 % if treated with below-threshold cumulative doses of alkylating agents or cisplatin [15]. See Table 1.2 for threshold cumulative doses of different chemotherapy drugs on spermatoxicity.

Other factors associated with high rates of azoospermia in childhood cancer survivors

Table 1.1 Classification of chemotherapy agents by risk of damaging spermatogenesis

Low risk	Medium risk	High risk
Bleomycin	ABVD (adriamycin, bleomycin, vinblastine, dacarbazine)	Busulfan
Dactinomycine	BEP (bleomycin, etoposide, cisplatin)	Chlorambucil
Mercaptopurine	Carboplatin	Chlormethine
Methotrexate	Cisplatin	Cyclophosphamide
Vinblastine	Doxorubicin	Dacarbazine
Vincristine		Ifosfamide
		Melphalan
		MOPP (nitrogen-mustard, vincristine, procarbazine, prednisone)

Source: Modified from Wallace WH, et al. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncology. 2005; 209–18

Table 1.2 Cumulative threshold doses with high risk of azoospermia for different chemotherapy agents

Chemotherapy agent	Cumulative threshold dose with high risk of azoospermia
Carmustine	1 g/m^2
Lomustine	500 mg/m^2
Chlorambucil	1.4 g/m^2
Cisplatin	500 mg/m^2
Cyclophosphamide	19 g/m^2
Melphalan	140 mg/m^2
Procarbazine	4 g/m^2

Source: Modified from Romerius P, et al. High risk of azoospermia in men treated for childhood cancer. International Journal of Andrology. 2010; 34(1): 69–76

included use of radiotherapy, lower inhibin B levels (≤50 ng/L), elevated follicle stimulating hormone (FSH) (≥10.9 IU/L), and decreased testicular volume (right + left testicular volume ≤ 24 mL). The prevalence of azoospermia was 66 % in men with lower inhibin B level, 50 % in men with higher FSH level, and 61 % in men with lower testicular volume. It is notable that the prevalence of azoospermia in men with normal FSH and inhibin values was only 1–2 %, implying that azoospermia may be due to alteration of these two hormonal values. This study did not find azoospermia in men treated for brain tumor or Wilms tumor, as well as for brain surgery not involving the pituitary and non-testicular radiation [15].

Leukemia, lymphoma, and CNS tumors may affect the hypothalamus and pituitary with direct cell invasion and irradiation is often the treatment option for these patients. A study of 25 males with ALL who received a median of 25 Gray (Gy) (range 15–30 Gy) radiation dosage to the cranium with a median follow-up of 19 years showed that there was no difference in luteinizing hormone (LH), FSH, inhibin B, and testosterone levels between those who received cranial irradiation and those who did not. This study also evaluated seven patients who received total body irradiation and testicular irradiation and showed high levels of LH, FSH, and low levels of inhibin B indicating testicular damage. Age at the time of diagnosis was not a risk factor for alteration of these hormones [7]. Therefore, this data showed that irradiation to the cranium did not impact the hypothalamus pituitary axis in the long term. However, radiation to the testes and whole-body radiation prior to bone marrow transplantation was shown to be damaging to the testicular components. Furthermore, germ cells are more sensitive to radiation than Leydig cells, with a dose more than 4 Gy capable of causing permanent damage, whereas a dose greater than 20 Gy is needed to cause damage to Leydig cells and produce hypogonadism [11].

Similar treatment regimens are employed in young adults diagnosed with these malignancies. Alternate fertility preservation strategies may be employed given the possibility of sperm banking in these patients. Negative impacts on spermatogenesis are generally less in postpubertal males.

Testis Cancer

Males of reproductive age are often affected by TGCT as it is the most common cancer in males ages 15–44 years, accounting for over 60 % of cancer diagnoses in this cohort. An estimated 8400 new cases of testicular cancer will be diagnosed in 2015 [16]. A recent study showed that the rates of TGCT increased significantly during 2007–2011 versus 1992–1997 time period, especially for non-seminoma GCT. However, seminomas remained more common overall. The median age at diagnosis of seminoma was 36 years, while it was 28 years for non-seminomas. About 20 % of seminomas were diagnosed at non-localized stages compared to 40 % for non-seminomas [17].

Survival rates for TGCT have been improving in the last several decades with 10-year survival rates over 95 % [18]. Quality of life, including fertility concerns, are therefore of utmost importance for these patients. Common treatment strategies in TGCT include orchiectomy, and possible radiation or platinum-based chemotherapy. These treatment modalities may be particularly harmful to overall gonadal function, including impaired spermatogenesis, detrimental effect on sperm quality, and hormonal disturbance. A study of 117 patients [19] with TGCT showed that about 30 %

of men who had attempted to conceive prior to TGCT diagnosis were successful in fathering children. Of the rest, 31 % had oligoasthenospermia, and 13 % had azoospermia. Post-treatment sperm concentration decreased in all treatment groups including surgery and surveillance alone, surgery followed by retroperitoneal lymph node dissection, surgery and chemotherapy, surgery followed by radiotherapy, and surgery followed by chemotherapy and radiation. After treatment for TGCT, 48 % of patients who attempted to conceive were successful, of which 22 % conceived naturally and 26 % with artificial reproductive technology (ART). Of the latter group, 58 % were able to undergo in vitro fertilization using fresh sperm, and 42 % using cryopreserved sperm. Of all the men who did not have children prior to diagnosis, only 22 % banked sperm. This was attributed to lack of adequate information or inadequate sperm parameters for cryopreservation.

Another study of 1433 men [20] with testicular cancer showed that 15-year post-treatment paternity rate was 71 % without the use of cryopreserved semen. The rate was 48 % in men treated with high-dose cisplatin-based chemotherapy (>850 mg cisplatin) versus 92 % in men on surveillance. As discussed above, the effect of chemotherapy on spermatogenesis is dependent on type and cumulative dose of chemotherapy. Another study evaluated 45 patients treated with 1–6 cycles of BEP in TGCT patients. They found that the rate of recovery of spermatogenesis at 2 years after 1–2, 3, 4, 5–6 cycles of BEP chemotherapy was 83 %, 80 %, 67 %, and 0 %, respectively [21].

In the aforementioned study by Brydoy et al. [20], ART was used by 22 % of couples attempting to conceive after treatment. The paternity rates of those treated with retroperitoneal lymph node dissection, radiotherapy, and low-dose chemotherapy (≤850 mg cisplatin) were similar [20, 22]. One hundred seventy-eight men with seminoma were treated with dog-leg or L-field radiation, and 63 % of those men were able to conceive successfully. There was no significant difference in paternity rates amongst<31 Gy, 31–36 Gy, and >36 Gy radiation dose groups. Nine out of 16 patients (56 %) who received a combination of cisplatin-based chemotherapy and infradiaphragmatic radiation with a median dose of 40 Gy had successful conception after treatment.

Ejaculatory function may be affected by certain treatments for TGCT, especially retroperitoneal lymph node dissection. The 10-year paternity rate after treatment amongst men with dry ejaculate was only 10 % compared to 83 % in men with normal ejaculation. The 19-year paternity rate was slightly better at 31 % and 91 %, respectively. In this study, dry ejaculate was the strongest negative predictive factor for achieving paternity [20]. Treatment options for fertility for these men include use of α-sympathomimetic drugs, testis sperm extraction, and transrectal electroejaculation.

Other Malignancies: Prostate, Bladder, and Colon Cancers

Prostate Cancer

Prostate cancer, bladder cancer, and colon cancer can all have varying degrees of impact on fertility depending upon the nature of the treatment involved. Certainly high-grade cancers requiring extirpative surgery carry the likely risk of anejaculation; adjuvant or neoadjuvant chemotherapy or radiation regimens carry additional risk to spermatogenesis itself. Again, while these cancers are typically not thought of as cancers of young men, more men are potentially susceptible to developing one of these cancers prior to fathering children as trends towards delayed family building strengthen. All three cancers are diagnosed in a fashion where pre-treatment sperm banking can be offered to any male patient desiring future fertility without any dangerous delay of treatment.

Prostate cancer is the most common solid malignancy in men; an estimated 220,000 new cases of prostate cancer will be diagnosed in the United States in 2015 [16]. Trends towards increased incidence of the disease are thought not to be due to increasing prevalence of the disease, but rather increased screening as the driving factor. Changes in screening behaviors in the United States are ongoing, and may influence this trend in the immediate future.

Commonly accepted treatment options for men include radical surgery, radiation therapy, hormonal ablative therapy, nonhormonal chemotherapies, and active surveillance. Increasing research into focally ablative therapies including cryotherapy and high intensity focused ultrasound is ongoing. Amongst these treatments, surgery (open, laparoscopic, and robotic assisted laparoscopic radical prostatectomy), radiation (brachytherapy, external beam radiotherapy, and proton beam radiotherapy), and active surveillance represent the more commonly offered treatments for organ confined prostate cancer. Age, comorbidity, and patient preference generally influence the decision to pursue one or another of these treatments if the patient has localized disease. All of these treatments, with the exception of active surveillance, have at least some impact on fertility with surgical treatments causing de facto anejaculation.

Data regarding the age migration of the population being diagnosed with prostate cancer is well established; Adolfsson et al. [23] outlined the shift in prostate cancer demographics observed in Sweden between the years of 1996–2005 within the Swedish National Prostate Cancer Registry. Age-standardized rates of diagnosis increased steadily in the youngest two groups (patients aged 0–49 and 50–59 years), and median age at time of diagnosis dropped from 75 years in 1996 to 70 years in 2005. This trend was reflected in other countries in the western world and was commonly attributed to a number of factors including, but not limited to, increased utilization of prostate-specific antigen screening.

Bladder Cancer

Bladder cancer is another common genitourinary malignancy with a potential fertility impact; approximately 56,000 bladder cancers will be diagnosed in American men in 2015 with 11,500 deaths [16]. Although bladder cancer is generally a cancer of the elderly (mean age at diagnosis is 73 years), 1 out of 10 patients diagnosed with bladder cancer is under the age of 55 years.

Treatment options include endoscopic management (appropriate in cases where disease is confined to the bladder and not locally advanced), surgical extirpation (partial or radical cystectomy with or without the prostate), chemotherapy, and radiation. In locally advanced disease (approximately 35–40 % of cases), combination treatment with neoadjuvant chemotherapy followed by surgical removal of the bladder and prostate is the appropriate treatment. Commonly utilized chemotherapy regimens include platinum-based agents in combination with other drugs (methotrexate, vincristine, doxorubicin, cisplatinum—MVAC; gemcitabine, cisplatinum—GC). Both have a potential risk of damage to spermatogenesis. Radical surgery carries the risk of erectile dysfunction with risk to the cavernous nerves within the neurovascular bundles adjacent to the prostate as well as the risk of anejaculation.

Colon Cancer

Colon cancer (including both adenocarcinoma of the rectum and colon) represents the third most common malignancy in both men and women; there were more than 132,000 cases estimated for the United States in 2015 [16]. Five percent of Americans will develop colon cancer in their lifetime. Increasing success with early diagnosis due to screening colonoscopy as well as increasingly effective treatment modalities have resulted in decreasing cancer-specific mortality; again this makes it more likely that the male patient with infertility related to colon cancer treatment will survive to attempt family building.

Treatment options include partial versus radical surgery, performed either endoscopically (organ sparing), open, laparoscopically, or robotic assisted laparoscopically. Risks of any of these approaches include (1) erectile dysfunction due to injury to the cavernous nerves within the neurovascular bundles adjacent to both the prostate and rectum or (2) ejaculatory dysfunction due to disruption of the pelvic plexus. Although these risks are possible with each of these approaches, they are least with endoscopic management. However, endoscopic management

is not an appropriate treatment choice for locally advanced disease.

Both chemotherapy and radiotherapy are used in conjunction with surgery in many cases [24]. Chemoradiation in combination used in either neoadjuvant or adjuvant fashion has resulted in increased survival for patients with Stages 2–4 colon cancers [25]. There is no consensus yet regarding the standard regimen utilized for these patients. Radiotherapy to the pelvis incurs the risk of vascular injury to the neurovascular bundles potentially resulting in erectile dysfunction.

Chemotherapeutic agents including 5-fluorouracil, leucovorin (or folinic acid), and platinum-based agents such as oxaliplatin are used in many cases in combination [26]. The FOLFOX regimen (combination of the above 3) is thought to have limited lasting impact on fertility. However, there is known risk to testicular function with platinum-based chemotherapeutic agents, and patients with nonobstructive azoospermia after this treatment regimen have been described. There is unfortunately no study documenting the true incidence of hypospermatogenesis after this regimen of chemotherapy.

Fertility Preservation

The etiology of fertility impairment in males with cancers may be due to deleterious effects on hormones, altered metabolism, stress, malnutrition, fevers, release of certain molecules, and direct cytotoxic effects of chemotherapy, radiation, and medications [11]. One study showed that a large proportion of men with cancers had abnormal semen parameters even prior to initiating chemotherapy [27]. In a cohort of 764 males with cancer referred for sperm banking prior to chemotherapy, abnormal semen parameters were found in 64 % of males and no sperm could be banked in 12 % of men [27]. Males with TGCT and extragonadal GCT had the highest likelihood of having poor semen parameters. These tumors may release molecules with endocrine-like function; for example, β-human chorionic gonadotropin (β-HCG) by some TGCT. The authors studied tumor markers and their association with semen quality, and found that inhibin B correlated best with semen parameters, with higher values predictive of better semen quality. There was an association between increased tumor markers (alfa-fetoprotein, β-HCG) and lower inhibin B levels; however, this study did not find a direct correlation between tumor markers and semen quality [27].

For males who are at risk for infertility due to cancer-related etiologies, the only proven successful option to preserve fertility prior to starting treatment is cryopreservation of sperm. This should be offered to all potential candidates per the American Society of Clinical Oncology (ASCO) guidelines [28]. The guidelines state that any health care professional treating cancer including medical oncologists, radiation oncologists, gynecologic oncologists, urologists, hematologists, pediatric oncologists, and surgeons should include a thorough discussion of impact on fertility and fertility preservation options as part of patient education and informed consent for treatment. Threats to present and future fertility should be discussed with patients and/or parents or guardians as early as possible. Those patients who are interested in fertility preservation should be referred to reproductive specialists. The discussion should be documented in the medical chart. Males with limited life expectancy face challenging ethical and psychosocial issues regarding future parenthood. These males should be referred to psychosocial providers to reduce stress and encourage discussion.

The ASCO guidelines [28] state that sperm cryopreservation is the only established preservation method for fertility. Hormonal treatment is not a successful fertility preserving modality and should not be recommended. Other techniques including testicular tissue extraction for cryopreservation should be considered experimental and presented as such to prepubertal males and their parents or guardians. Men should be informed that there may be risk of higher genetic damage in sperm collected after starting chemotherapy. Though not noted in the ASCO guidelines, it may be prudent to discuss postmortem use of cryopreserved sperm and consider obtaining informed consent from patient or guardians

for use of banked sperm, especially in those with guarded prognosis.

One study showed that only 27 % of males of reproductive age with cancer chose semen cryopreservation; however, the main reason for this low percentage was lack of information [29]. Another study showed that 67 % of young men with cancers had successful sperm banking [30]; and the men who did not undergo cryopreservation were of younger age with increased anxiety and reluctance to talk about fertility issues. Therefore, age may have an important impact on sperm banking. In a study of 80 pubertal boys with cancer, 14 of them were not successful in cryopreserving due to azoospermia or asthenospermia [12]. This study also showed that pre-collection endocrine workup failed to predict successful sperm yield, and postulated that it may not have been possible due to stress or lack of sexual experience.

In some of these males, electroejaculation may be used for sperm harvesting. This may be used in adolescents who have reached puberty but are not able to produce semen yet. A small study by Hovav et al. [31] used electroejaculation in 6 adolescents ages 15–18 years, and showed that semen collection was possible in all 6 males. However, the mean sperm count was 16×10^6 (range $0–45 \times 10^6$), and the mean motility was 14 % (range 0–53 %).

Another study [32] of semen cryopreservation in males ages 10–18 years showed that semen collection was possible in 106 out of 114 males with cancer by masturbation alone, of which 78 out of 106 were deemed adequate for cryopreservation. Electroejaculation was done in 11 boys, of which 3 were deemed adequate for cryopreservation. The authors found that testosterone level was higher in males who had adequate semen yield from electroejaculation with a median value of 239 ng/dL (range 150–1154 ng/dL) compared to a median value of 49 ng/dL (range 0–516 ng/dL) in those who did not have an adequate collection for cryopreservation. Additionally, the semen samples collected by electroejaculation had much lower sperm concentration and motility than samples collected by masturbation. Median sperm concentration was 15×10^6 mL^{-1} when collected by conservative method versus 2×10^6 mL^{-1} when collected by electroejaculation. Median motility was 29 % with conservative method of collection versus 3 % with electroejaculation. A review of literature showed that 13 out of 29 reported cases of electroejaculation in adolescents were successful in cryopreserving sperm [32].

It is important to discuss with the patient that semen cryopreservation may result in deterioration of sperm quality. Freezing sperm induces decreased motility in 31 %, altered morphology in 37 %, and abnormal mitochondrial activity in 36 % [33]. Furthermore, the rate of utilization of cryopreserved semen is highly variable, and may be less than 10–15 % [34]. Ultimately, the success rate of ART procedures using banked semen from former cancer patients ranges from 33 to 56 % [34].

The risks to the offspring of cancer survivors may be a concern that should be addressed with patients. The cancer itself, treatment of cancer, cryopreservation of sperm, and use of ART are factors that may pose a risk to the offspring of cancer patients. Theoretically, cancer treatment may result in germ cell mutations posing an increased risk of congenital malformations, growth abnormalities, other diseases, and cancers in the offspring. The literature has not shown this to be true, and in one study, the offspring of male cancer survivors had similar outcomes compared to offspring of non-cancer stricken males in terms of being born prematurely, being small for gestational age, having congenital malformations or altered male to female ratio [35]. In terms of malignancies, unless the malignancy was hereditary, there was no increased risk of cancer to offspring of men treated with chemotherapy or radiation [36]. Most of these children are conceived spontaneously, however, and it remains to be seen whether the outcomes from ART are different between offspring of cancer patients and offspring of non-cancer patients.

Other experimental techniques to restore fertility are under investigation. Successful spermatogonial stem cell transplantation has been done in a rodent model [37]. Another method that has been studied in mice model is testicular allografting, where donor testicular tissue was

extracted from cloned donor mice, and transplanted into testes of recipient nude mice. The donor testicular germ cells were successful in colonizing recipient seminiferous tubules and producing spermatogenesis in some mice [38]. However, for males to undergo this procedure testicular tissue would have to be extracted; the main concern with this technology is that the majority of cancers in childhood may invade testicular tissue due to permeability of the blood–testis barrier. Transplanting this tissue into the adult testicle may introduce malignant cells [39], especially in the case of leukemias, where minimal numbers of cells may be needed for it to recur [40]. Cell sorting techniques are not completely accurate, and therefore, may not be relied upon for complete malignant cell clearance from this tissue. Another experimental method involves harvesting germ cells, maturing them in vitro, and cryopreserving them for future intracytoplasmic sperm injection use [41]. The other main concern with harvesting testicular tissue from prepubertal males is the potential for testicular damage, problems with future puberty, impaired recovery from cancer treatment, and directly causing infertility. Because spermatogenesis recovers at least partially in most males treated for cancer, harvesting testicular tissue may be over-treatment in many males.

In conclusion, some cancers in males of reproductive age may be increasing in incidence, but advances in treatment may be resulting in greater overall survival into adulthood. Impact on fertility and fertility preservation options are an important component of cancer treatment in these males. Therefore, a thorough discussion of fertility preservation prior to initiating cancer treatment with the patient or guardians is paramount. Present studies indicate that this discussion is embarked upon infrequently and with inconsistent results. Efforts to improve this are ongoing. Further studies investigating the impact of several chemotherapeutic options, radiation, and other emerging treatment options for cancers affecting males of reproductive age are needed.

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Demographics of Cancer in the Reproductive Age Female

2

Jason M. Franasiak and Richard T. Scott Jr.

Abbreviations

ART	Assisted reproductive technology
BRCA	BReast CAncer susceptibility gene
CDC	Centers for disease control
ER	Estrogen receptor
FIGO	International Federation of Gynecology and Obstetrics
GnRH	Gonadotropin-releasing hormone
Gy	Gray (SI unit for ionizing radiation)
HER2	Human epidermal growth factor 2
HPV	Human papilloma virus
NSMLC	Non-small cell lung cancer
PR	Progesterone receptor
SCLC	Small cell lung cancer
TNM	Tumor node metastases
WHO	World Health Organization

Introduction

Cancer in reproductive age women represents a significant source of morbidity and mortality. The treatment of cancer in this age group whether it entails chemotherapy, radiotherapy, or surgical resection often results in survivors with impaired or absent reproductive potential without assisted reproductive technologies (ART).

Although primary treatment goals are swift diagnosis, treatment, and follow-up of the primary malignancy, a strong factor in long-term emotional well-being of cancer survivors is the ability to parent a child [1]. Despite this, only about 50 % of cancer survivors report receiving counseling regarding the cancer treatment's impact on their fertility and future options for childbearing [2, 3]. To do this appropriately requires a working knowledge of common malignancies faced in this age group, their treatments at various stages of disease, the treatment's impact on fertility, and the therapeutic options available to patients for fertility preservation and restoration [1]. Ultimately, a collaborative, multidisciplinary team approach will provide optimal management.

Risk of Infertility in Cancer Survivors

Impaired reproductive function can be caused by a number of things related to cancer and its treatment including type of cancer and stage of

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disease; chemotherapeutic agent and cumulative dose of drug; location of radiation and cumulative dose; surgical treatment, which often includes removal of reproductive organs; the disease process itself which can impair both fertility and general health.

Pretreatment counseling is an important part of long-term patient satisfaction. Poor prognostic factors in cancer treatment have been derived from large sibling cohort studies of female cancer survivors. Factors conferring a poor prognosis for future fertility include hypothalamic and pituitary radiation ≥30 Gray (Gy), ovarian and uterine radiation >5 Gy, high cumulative dose of alkylating chemotherapeutic agents, and treatment with lomustine of cyclophosphamide [4].

Several options are available to mitigate risks for infertility in female cancer survivors and are discussed in detail in Chaps. 11, 15 and 16. Briefly, they entail approaches with established efficacy in widespread use, primarily, oocyte, or embryo cryopreservation prior to treatment [5]. Other paradigms, such as ovarian tissue preservation, are offered in primary research settings [6]. Finally, several treatments exist, such as ovarian suppression with gonadotropin releasing hormone analog (GnRH), which have mixed success and are utilized when other more established treatment options are not feasible [7]. The use of gestational carriers can be offered when the reproductive tract was damaged by the cancer or removed as part of its treatment. All available options should be discussed and can be offered alone or in combination.

Female Cancer Demographics

This chapter focuses on the most common malignancies faced by reproductive-aged women and their most common treatments. This overview serves as a foundation for the subsequent chapters and places the subsequent options for fertility preservation in context. First, breast cancer, lung cancer, and cancer of the gastrointestinal tract are reviewed. The incidence and mortality rates among women of all races in all ages are seen in Table 2.1. Data on percent of cases seen in reproductive-aged women from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program from 2007 to 2011 are noted in Table 2.2.

Table 2.1 Most common causes of cancers among women and most common causes of cancer deaths according to the Centers for Disease Control (CDC) [8]

Malignancy	Rate per 100,000 (all races)
Most common cancer among women	
Breast cancer	122.0
Lung cancer	52.0
Colorectal cancer	34.9
Leading causes of cancer deaths among women	
Lung cancer	37.0
Breast cancer	21.5
Colorectal cancer	12.8

Table 2.2 Percentage of new cases in female patients of reproductive age according to the National Cancer Institute's Surveillance, Epidemiology, and End Results [SEER] Program from 2007 to 2011

Cancer among reproductive age women (% of total cases diagnosed)			
Malignancy	<20 (years)	20–34 (years)	35–44 (years)
Breast cancer (%)	0.0	1.8	9.3
Lung cancer (%)	0.0	0.3	1.3
Colorectal cancer (%)	0.1	1.2	4.1
Ovarian cancer (%)	1.2	3.7	7.2
Uterine cancer (%)	0.0	1.6	5.6
Cervical cancer (%)	0.1	13.6	24.9

Breast cancer is commonly seen in patients of reproductive age. Lung cancer and colorectal cancer are seen more commonly in older patients, but occur with high frequency overall and have a number of cases which occur in younger women. Cancers of the female reproductive tract, particularly carcinoma of the cervix, are also seen in women of reproductive age. In the chapter, we review breast carcinoma and hereditary breast carcinoma, cervical carcinoma, uterine carcinoma, and ovarian/primary peritoneal carcinomas.

Breast Cancer

Breast cancer represents the most commonly diagnosed malignancy with over one million cases per year and is the leading cancer-related cause of death worldwide. In the United States, breast cancer is the second leading cause of death in women and the leading cause of death in women ages 20–59 [9]. According to the NCI's SEER database from 2007 to 2011, patients under 20 years of age represent 0.0 % of new cases, those aged 20–34 represented 1.8 % of new cases while patients aged 35–44 represented 9.3 % of new cases, resulting in a total number of 292,297 new cases. A number of risk factors contribute to an increased risk of breast cancer and include: age, ethnicity, history of benign breast disease, personal or family history of breast cancer, use of reproductive hormones, exposure to ionizing radiation, and environmental factors.

Treatment can include surgery, chemotherapy, and radiation therapy and is guided by the histologic subtype and stage according to the tumor node metastases (TNM) staging system. The most common histologic subtypes include:

- Infiltrating ductal carcinoma—accounts for 70–80 % of invasive cancers
- Infiltrating lobular carcinoma—accounts for 8 % of invasive cancers
- Mixed ductal/lobular carcinoma—accounts for 7 % of invasive cancers

Breast cancer is also classified by the presence or absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor 2 receptors (HER2) which guide subsequent adjuvant treatment.

Definitive TNM staging is accomplished during surgery and ultimately given a classification of Stage I–IV which helps to guide subsequent treatment. Stage I–II represents disease confined to the breast while Stage III–IV represents metastatic disease. The stage at diagnosis confers general prognosis, which declines substantially in later stages (Table 2.3). The vast majority of cancers diagnosed in the United States are early stage or locally advanced cancers, corresponding to Stage I–III disease.

Table 2.3 Breast cancer 5-year survival by stage at presentation [10]

Tumor node metastasis (TNM) stage	5-year survival (%)
Stage I	95
Stage II	
IIA	85
IIB	70
Stage III	
IIIA	52
IIIB	48
Stage IV	18

Treatment

As discussed above, the treatment is guided by the staging and molecular characteristics of the breast cancer. In general, early-stage breast cancers undergo primary surgery, either lumpectomy or mastectomy with regional lymph node removal, with radiation therapy after surgery reserved for those at a high risk for local recurrence. Subsequent adjuvant treatment is guided by TNM stage and the presence/absence of ER/PR receptors which may be amenable to endocrine therapy and/or expression of HER2 which may be amenable to HER2-directed treatment such as trastuzumab. For patients with locally advanced breast cancer, neoadjuvant systemic therapy utilizing chemotherapeutic agents, with HER2-directed agents in appropriate patients, is often employed prior to breast surgery and subsequent radiation therapy.

Fertility preservation in women with breast cancer presents the additional challenge of these cancers being hormonally responsive in certain circumstances. Involvement of a reproductive endocrinologist at the outset may allow for safe ovarian stimulation and oocyte retrieval prior to gonadotoxic chemotherapy. Because estrogen levels can rise tenfold more during ovarian stimulation when compared to the natural menstrual cycle, an approach with attempts to minimize systemic exposure should be utilized. Often agents such as letrozole, an aromatase inhibitor, and tamoxifen, a selective estrogen modulator with antiestrogenic actions in breast tissue, are employed during the ovarian stimulation process [11].

Hereditary Cancer Syndromes: BRCA 1/2

Although most breast and ovarian cancers occur sporadically, approximately 10 % of breast cancers and 15 % of ovarian cancers are associated with germ line mutations in tumor suppressor genes [12]. The most common mutations associated with these syndromes in the breast and ovaries are the breast cancer type 1 and 2 susceptibility genes (BRCA1 and BRCA2). Both mutations are inherited in an autosomal dominant fashion with high penetrance.

BRCA1 mutations are associated with cancers of the cervix, uterus, pancreas, esophagus, and stomach as well as breast and ovary. BRCA2 mutations are associated with cancers of the pancreas and possibly the stomach, biliary system, esophagus, and skin as well as breast and ovary. Women who carry the BRCA1 mutation have a 57 % cumulative risk of breast cancer by age 70 and a 40 % risk of ovarian cancer. For those with a BRCA2 mutation, this cumulative risk is 49 % for breast cancer and 18 % for ovarian cancer [13].

Women who are carriers of the BRCA1 or 2 mutations may elect to employ breast and ovarian cancer-reducing screening and/or treatment measures. Risk-reducing surgery for breast cancer involves a double mastectomy which can reduce the risk of breast cancer by as much as 90 %. Risk-reducing bilateral salpingo-oophorectomy decreases ovarian and fallopian tube cancers by approximately 80 % and also serves to decrease the risk of breast cancer by removing the primary endogenous source of estrogen. This is typically done at age 35–40 once childbearing is complete but can be done earlier.

Lung Cancer

Historically, lung cancer had a low prevalence with a death rate similar to that of pancreatic cancer. With the widespread smoking epidemic seen in the twentieth century, it became the leading cause of death first in men in 1963 and then in women in 1985. With anti-smoking campaigns in the United States, lung cancer death rates have begun to decline in both men and women [14]. Although most lung cancers occur after menopause, they still impact women of reproductive age in 3 % of cases [15]. According to the NCI's SEER database from 2007 to 2011, female patients <20 years of age represent 0.0 % of new cases, those aged 20–34 represented 0.3 % of new cases while patients in the 35–44 age group represented 1.4 % of new cases, with total number of new cases at 120,808.

There are four major histologic cell types of lung cancer according to the World Health Organization (WHO):

- Adenocarcinoma—accounts for 38 % of cases
- Squamous cell carcinoma—accounts for 20 % of cases
- Large cell carcinoma—accounts for 5 % of cases
- Small cell carcinoma—account for 13 % of cases

The remainder of the 24 % of cases cannot be fully characterized histologically. The majority of lung cancers present at very advanced stages given the lack of symptoms until that time.

The initial evaluation stage involves determining whether the patient has non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC) as this will guide treatment. The staging of NSCLC follows the TNM staging system while the staging of SCLC utilizes either the Veterans Administration Lung Study Group designation (limited or extensive) or the TNM staging system.

Treatment

For patients who have NSCLC, treatment is guided by the stage of disease and involves surgical resection for early disease and chemoradiation therapy for those with extensive disease. For those with advanced disease, treatment options are limited and primarily involve palliative care.

In cases where SCLC is identified, systemic chemotherapy is the primary modality of treatment as SCLC is disseminated in nearly all cases.

Often thoracic radiation is used in combination with chemotherapy. Of importance when discussing pituitary and hypothalamic radiation, prophylactic cranial irradiation is often employed to decrease the incidence of metastasis to the head.

Cancer of the Gastrointestinal Tract

Cancer of the gastrointestinal tract, in particular colorectal cancer, is the third leading cause of death due to cancer in women. While the majority of cases occur after age 50, 11 % of cases of colon cancer, and 18 % of rectal cancers occur prior to age 50 and the incidence rates among young women have been increasing [16]. According to the NCI's SEER database from 2007 to 2011, female patients under 20 years of age represent 0.1 % of new cases, those aged 20–34 represented 1.2 % of new cases while patients aged 35–44 represented 4.1 % of new cases, where the total number of new cases was 91,411. Approximately 20 % of the cases of young-onset colorectal cancers occur as part of a familial syndrome such as hereditary nonpolyposis colorectal cancer or Lynch syndrome.

Diagnosis is achieved with tissue biopsy, typically obtained during a colonoscopy. The majority of colorectal cancers are characterized histologically as adenocarcinomas. Once the diagnosis is made, staging is accomplished utilizing the TNM staging system and guides surgical resection and chemotherapy.

Treatment

Surgical resection is the mainstay of curative therapy for locally confined colorectal cancer. In patients that have Stage III disease in which lymph nodes are positive, chemotherapy, typically with an alkylating platinum-based analogs, is recommended. In patients with more advanced Stage IV disease, surgery is not helpful and these patients are treated primarily with chemotherapy.

Cancer of the Female Reproductive Tract

Malignancies which affect the female reproductive tract, primarily cervical, uterine, and ovarian/primary peritoneal cancers present unique challenges for fertility preservation. They often occur in women of reproductive age and their therapy may result in the removal of reproductive organs and/or chemoradiation therapy, which is focused on the pelvis. Their demographics are summarized in Figs. 2.1 and 2.2 and the treatments are discussed below.

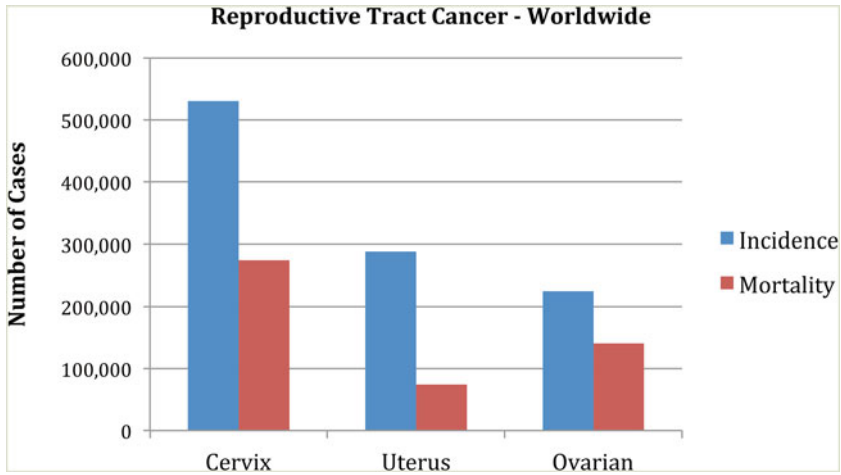

Fig. 2.1 The incidence and mortality of cancer of the reproductive tract worldwide for women of all ages [17]

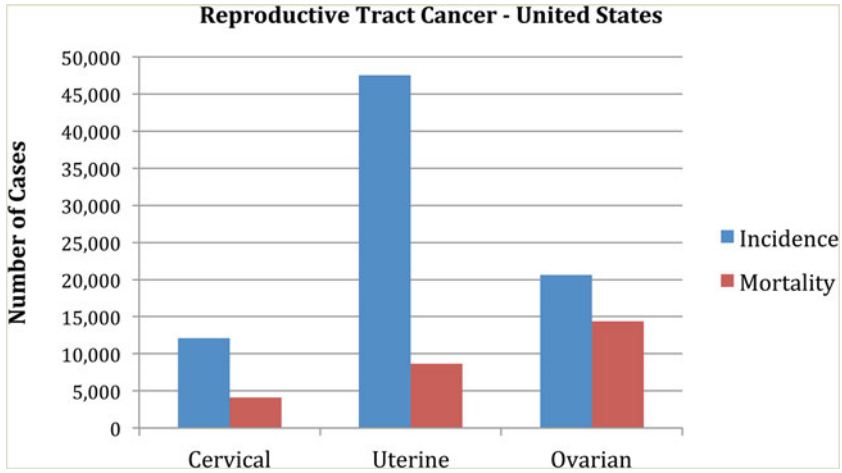

Fig. 2.2 The incidence and mortality of cancer of the reproductive tract in the United States for women of all ages [8]

Cervical Carcinoma

Cancer of the cervix represents the third most common cause of gynecologic cancers both in terms of incidence and mortality in the United States (Fig 2.2). This is not the case in underdeveloped countries of the world that lack robust screening and prevention programs. In these countries, cervical cancer remains the most common type of cancer and the most common cause of cancer deaths among gynecologic cancers (Fig. 2.1).

Although it is the third most common overall, cervical cancer represents the most significant disease burden of female reproductive tract cancers in reproductive age women. According to the NCI's SEER database from 2007 to 2011, patients under 20 years of age represent 0.1 % of new cases, those aged 20–34 represented 13.8 % of new cases while patients aged 35–44 represented 24.9 % of new cases, where total number of new cases was 17,223.

The human papilloma virus (HPV) is detected in nearly all cases of cervical cancer and squamous cell cancer and adenocarcinoma represent the majority of the histologic subtypes [18]. This represents a unique area in cancer prevention given the advent of the HPV vaccination which provides increased immunity to the most common causes of cervical cancer, namely HPV subtypes 16 and 18. It is expected that there will be a further decline in cervical cancer incidence and mortality with some regions with high vaccine utilization showing a decline in the incidence of high-grade dysplasia by 38 % [19].

Treatment

Treatment of cervical cancer depends upon the staging of the disease and extent of invasion. Cervical cancer staging is done according to the International Federation of Gynecology and Obstetrics (FIGO) classification system and takes into consideration clinical findings as well as pathology.

For women with early-stage disease, which represents microinvasive or minimally invasive disease on the FIGO system, women have several options for therapy. Treatment may include definitive therapy with surgery in the form of a modified radical hysterectomy in which the cervix, uterus, upper portion of the vagina, and the tissues closely surrounding these organs. Other options include fertility sparing surgery in which the uterus is preserved and the cancer is resected via cold knife conization or trachelectomy (removal of the cervix). Primary radiation therapy for early-stage disease is reserved for women who are not optimal surgical candidates.

Patients who have locally invasive cervical cancer, FIGO grades which comprise invasive

disease to the cervix, uterus, pelvic sidewalls, bladder, rectum, and outside of the true pelvis, treatment entail primary chemotherapy and radiation therapy. Surgery or radiation therapy alone is not as effective as combined treatment modalities. Chemotherapy is typically undertaken with an alkylating platinum-based analog (commonly cisplatin) which is sometimes combined with an irreversible inhibitor of thymidylate synthase (5-fluorouracil).

Uterine Carcinoma

Uterine cancer represents the most commonly diagnosed gynecologic malignancy in the United States. According to the NCI's SEER database from 2007 to 2011, patients under 20 years of age represent 0.0 % of new cases, those aged 20–34 represented 1.6 % of new cases while patients aged 35–44 represented 5.6 % of new cases, where total number of new cases was 57,667. Because abnormal uterine bleeding is the primary symptom associated with uterine cancer and it is seen in as many as 90 % of women with the disease, the diagnosis is made early by comparison to other gynecologic malignancies and mortality rate when compared to the incidence is quite low [20]. Nearly 70 % of cases are confined to the uterus at the time of diagnosis which confers a 5-year survival rate over 95 %.

While uterine cancers occur most commonly in postmenopausal women, it can occur in women of reproductive age. The majority of uterine cancers are adenocarcinomas which often develop in the setting of unopposed estrogen exposure. Thus, younger women at risk for uterine cancers include those who are obese and those who have chronic anovulation, such as women with polycystic ovarian syndrome. Women who have familial cancer syndromes, such as hereditary nonpolyposis colorectal cancer or Lynch syndrome, are also at an increased risk of uterine cancer.

Treatment

Treatment is guided by endometrial cancer FIGO staging as well as histologic type. Endometrial cancers are classified as Type I or Type II. Type I or endometrioid carcinomas are the most common type, are estrogen responsive, and typically carry a relatively favorable prognosis. Type II carcinomas include carcinosarcomas, serous, and clear cell cancers and have a poorer prognosis.

Surgical treatment alone is typically curative for those with early-stage Type I disease. This entails the removal of the cervix, uterus, fallopian tubes, ovaries, and adjacent lymph nodes. In patients who have higher risk for recurrence, more advanced cancers, or high-grade histopathologic subtypes, adjuvant therapy in the form of chemotherapy and/or radiation therapy is optimal.

Fertility and uterine sparing options exist for a special subset of patients who have low-risk, localized endometrial carcinoma. These patients can be treated with high-dose continuous progestin therapy, either megesterol acetate orally or with the levonorgestrel intrauterine device, with regular close follow-up.

Ovarian/Primary Peritoneal Carcinoma

Ovarian cancer is the second most common cause of gynecologic malignancy in the United States and represents the most common cause of deaths related to gynecologic cancers. According to the NCI's SEER database from 2007 to 2011, patients under 20 years of age represent 1.2 % of new cases, those aged 20–34 represented 3.7 % of new cases while patients aged 35–44 represented 7.2 % of new cases, where total number of new cases was 29,010. The reason for the high mortality relates to the lack of symptoms and late stage of presentation as opposed to that seen with uterine cancer as discussed above.

The majority of ovarian malignancies is derived from the epithelial cells of the ovary and is classified histopathologically as serous, mucinous, endometrioid, clear cell, and transitional cell tumors. These tumors, which comprise 95 % of ovarian cancers, occur most commonly in older patients. The other two layers of the ovary, the stroma and the germ cells, represent the other

cells of origin for ovarian tumors and occur more commonly in younger, reproductive-aged women. Stromal tumors include granulosa cell, thecoma, fibroma, Sertoli cell, and Sertoli–Leydig. The germ cell tumors include dysgerminomas, yolk sac, embryonal, choriocarcinoma, and teratomas. Serous epithelial carcinomas, fallopian tube carcinoma, and primary peritoneal carcinomas are thought to have a similar or common origin and behave and are treated similarly.

Treatment

Treatment of ovarian cancer is guided by histopathology and staging as determined by FIGO. There are many benign subsets of ovarian tumors which require only resection of the ovarian cyst, as is the case with benign teratomas. In other cases, conservative therapy by removal of only one ovary with close follow-up is sufficient, as is the case for many germ cell tumors. For tumors of epithelial origin, treatment involves full surgical staging which includes removal of the cervix, uterus, fallopian tubes, ovaries, surrounding lymph nodes, the omentum, and directed biopsies of lesions on the peritoneum. The goal of these surgeries is optimal cytoreduction and debulking to remove all signs of tumor >1 cm in size.

Treatment after surgery is guided based upon stage and most often includes multi-agent chemotherapy. Chemotherapy is typically undertaken with an alkylating platinum-based analog (commonly carboplatin) which is combined with a taxane that interferes with normal microtubule breakdown during cell division (such as paclitaxel).

Conclusion

The most common cancers in women are breast, lung, and colorectal cancers. Cancers of the gynecologic tract, namely cervical, uterine, and ovarian, are also common in reproductive age women. The treatment of all of these cancers, whether surgical or with chemotherapy and/or radiation therapy, have the ability to impact a women's ultimate reproductive potential.

The epidemiology of these cancers and common treatment paradigms have been discussed here. There are a number of options available to mitigate risks for infertility in female cancer survivors and these are discussed in detail in Chaps. 11, 15 and 16.

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Physiology of Spermatogenesis: Opportunities for Disruption

3

Julie Won-Ching Cheng, Kristene Myklak, and Edmund Y. Ko

Abbreviations

AFP	Alpha-feto protein
ART	Assisted-reproductive technology
ATP	Adenosine triphosphate
DHT	Dihydrotestosterone
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
HD	Hodgkin's disease
HPG	Hypothalamic–pituitary–gonadal
LH	Luteinizing hormone
NHL	Non-Hodgkin lymphoma
ROS	Reactive oxygen species
TNF-α	Tumor necrosis factor-α
WHO	World Health Organization

Introduction

Spermatogenesis is a complex process requiring the orchestration of multiple factors. There are multiple opportunities for disruption to this process and the integrity of spermatozoa within the setting of cancer affecting males of reproductive age. The manifestations of the disease as well as the different modalities used to treat it can influence the hormonal regulation, cellular divisions, and function of spermatozoa.

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Anatomy and Physiology of Spermatogenesis

The Hypothalamic–Pituitary–Gonadal Axis

The hypothalamus is located at the base of the cerebrum and inferior to the thalamus. As a component of the limbic system, it bridges the nervous system to the endocrine system through its actions on the adjacent pituitary gland. The hypothalamus signals the pituitary gland to secrete hormones into systemic circulation in response to diurnal patterns, stress levels, and environmental conditions [1]. The posterior pituitary receives neuronal signaling to secrete oxytocin and vasopressin, whereas the anterior pituitary receives hormonal signaling to secrete its various hormones.

The hypophyseal portal system (Fig. 3.1) enables direct hormonal communication between the hypothalamus and anterior pituitary. The superior hypophyseal artery branches from the internal carotid artery and becomes the primary capillary plexus of the portal system. Hypothalamic

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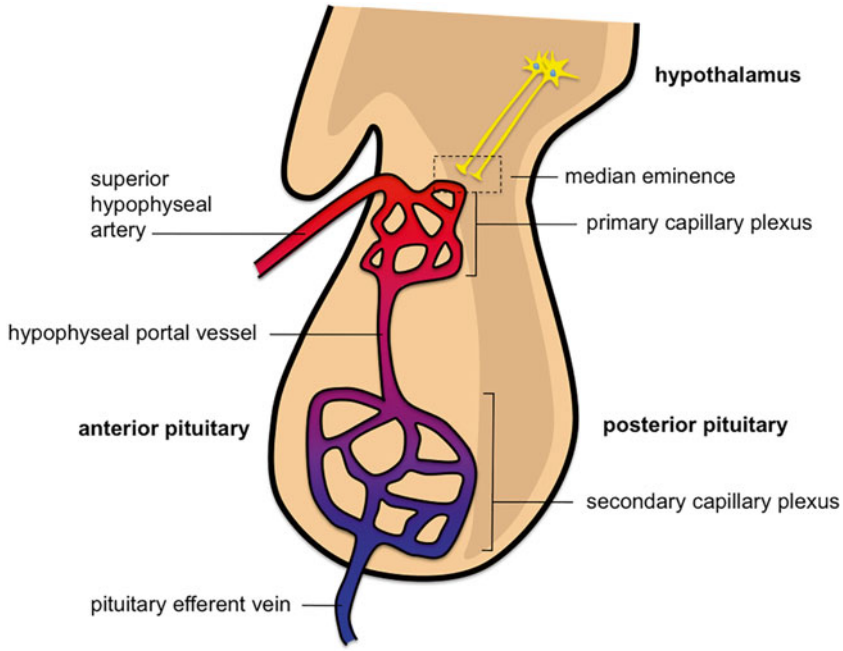

Fig. 3.1 The hypophyseal portal system. Factors released by the hypothalamus at the median eminence diffuse into the primary capillary plexus. These factors are delivered via the hypophyseal vein to the secondary capillary plexus before they diffuse out of circulation to signal cells of the anterior pituitary

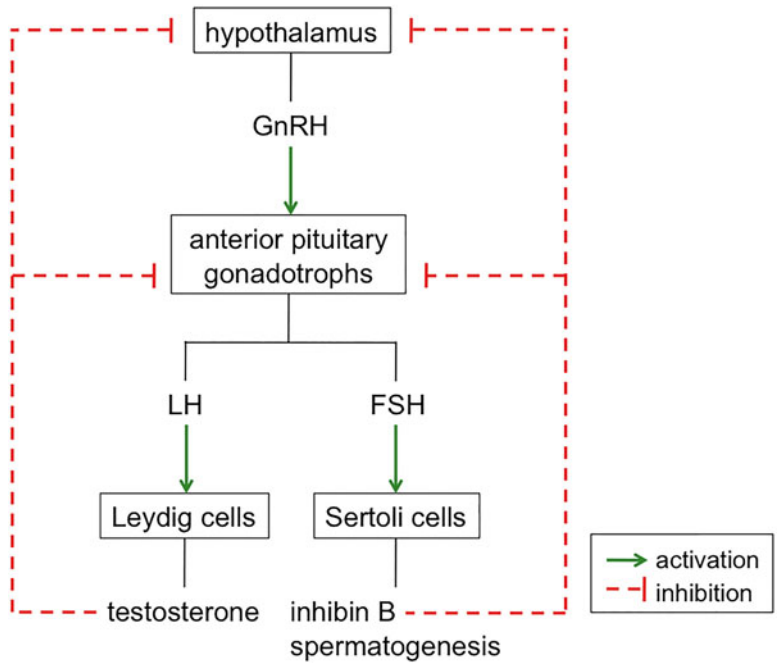

Fig. 3.2 The hypothalamic–pituitary–gonadal axis. The hypothalamus secretes GnRH that triggers the release of LH and FSH from gonadotrophs. Upon reaching testicular circulation, LH acts on Leydig cells to produce testosterone whereas FSH acts on Sertoli cells to promote spermatogenesis and produce inhibin. The testosterone and inhibin produced by the testicles subsequently down-regulates the activity of the hypothalamus and anterior pituitary as a means of feedback inhibition

releasing factors are secreted from the median eminence and diffuse through endothelial fenestrations within capillary walls. Hypophyseal portal vessels then directly deliver these factors to the secondary capillary plexus of the portal system, where they subsequently diffuse through capillary fenestrations to reach the cells of the anterior pituitary.

The hypothalamic–pituitary–gonadal (HPG) axis (Fig. 3.2) is a self-regulating component of the endocrine system that specifically promotes sexual development and reproduction. The hypothalamus releases gonadotropin-releasing hormone (GnRH) in pulsatile rhythmic secretions to act on gonadotropic cells within the anterior pituitary [1–3]. Gonadotrophs produce LH and FSH and release these hormones into systemic circulation via the pituitary efferent vein. These peptide hormones then act on the testis by binding G protein-coupled receptors to activate adenylate cyclase and subsequently increase intracellular concentrations of cyclic AMP. Luteinizing hormone acts on Leydig cells to signal testosterone production whereas FSH acts on Sertoli cells to promote spermatogenesis.

The HPG axis is regulated by feedback inhibition from factors that alter the firing threshold of GnRH neurons as well as gonadotroph sensitivity to activation [4, 5]. Sertoli cells produce and secrete inhibin-B as a peptide protein that suppresses FSH secretion from the anterior pituitary. In contrast, activin produced by various organs stimulates FSH production. Testosterone concurrently acts on both the hypothalamus and anterior pituitary as negative feedback to LH secretion either directly or in the aromatized form of estradiol.

Testicular Anatomy and Physiology

The testes are located in the scrotum and encased by the tunica vaginalis and tunica albuginea (Fig. 3.3). Bilateral testicular arteries directly arise from the abdominal aorta and descend through the inguinal canal to provide the primary

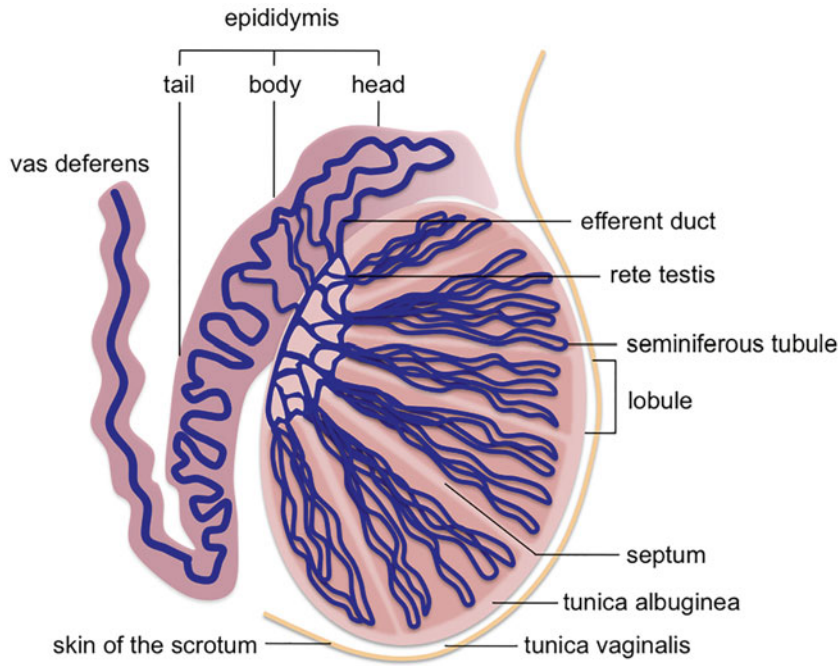

Fig. 3.3 Testicular anatomy. The testicles are encased by the skin, tunica vaginalis, and tunica albuginea. Septa arising from the tunica albuginea divide the testicle into lobules that comprise the seminiferous tubules. The seminiferous tubules arise and end at the rete testis, which is connected to the epididymis through the efferent ducts

arterial supply to the testes with collateral supply from the cremasteric artery and artery of the vas deferens. Venous blood drains into the pampiniform plexus that gives rise to the testicular vein. The para-aortic lymph nodes drain fluid from ducts within the spermatic cord and autonomic innervation arises from para-aortic ganglia.

Each testis weighs approximately 20 grams with an average volume of 18 cm^3, measures 3.6–5.5 cm in length and 2.1–3.5 cm in width, and is compartmentalized by septal divisions into individual lobules [6]. Within these lobules are the two functional components of the testis: the seminiferous tubules and Leydig cells. The seminiferous tubules contain Sertoli cells and germ cells that account for approximately 80 % of testicular volume. Interstitial tissue lies in between these tubules and is composed of Leydig cells, macrophages and mast cells, and neurovascular structures. This tissue accounts for the remaining 20 % of testicular volume.

Leydig cells within interstitial tissue produce testosterone from cholesterol in response to LH (Fig. 3.4). As it is secreted into systemic circulation, testosterone is reversibly bound by albumin and sex hormone-binding globulin for transport. Testosterone is only active and bioavailable in its unbound form when these proteins release it to act on target tissues. As a steroid hormone produced from cholesterol, testosterone is able to diffuse directly into cells, bind androgen receptors within nuclei, and initiate transcription for protein synthesis [7].

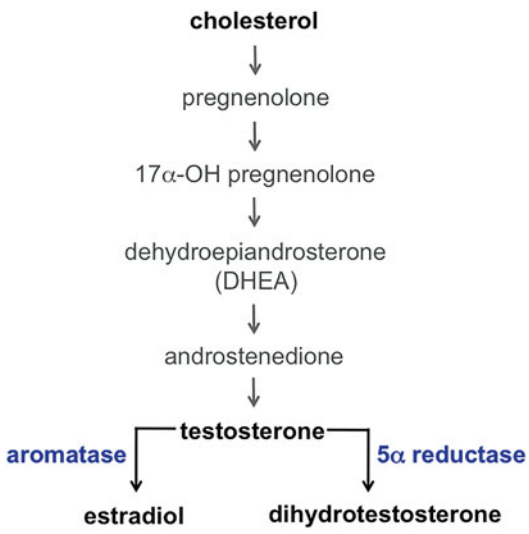

Fig. 3.4 Synthetic pathway of testosterone and its derivatives. Testosterone is produced from cholesterol through a series of conversions. This steroid hormone may act upon its target tissues or be converted into estradiol by aromatase or the more potent androgen DHT by 5α reductase

Testosterone acts at a fundamental male sex hormone that promotes sexual development, growth and maturation, and spermatogenesis [8, 9]. It can also be converted into dihydrotestosterone (DHT) by 5α-reductase. Both androgens provide hormonal influence throughout various aspects of embryonic development, pubertal growth and maturation of primary and secondary sex organs, and maintenance of secondary sex characteristics in adulthood. In addition to amplifying sexual behavior, testosterone has a specific reproductive role of promoting spermatogenesis within the seminiferous tubules.

Testosterone can be converted to estrogen by aromatase. Expression of aromatase and estrogen receptors in parenchyma and germ cells within the testes suggests that estrogen influences spermatogenesis [10, 11]. Targeted disruption of aromatase or the estrogen receptor can impair sperm production, motility, and function despite normal levels of testosterone [12–14]. Furthermore, 17β-estradiol has been specifically shown to inhibit apoptosis of spermatocytes and spermatids, suggesting that estrogen may also act as a survival factor for germ cells [11].

Seminiferous tubules containing Sertoli cells and spermatogonium germ cells are the reproductive tissues responsible for spermatogenesis (Fig. 3.5). These long and convoluted structures originate and end at the rete testis and comprise the majority of testicular volume. A wall of Sertoli cells and developing germ cells surrounds the central lumen within each tubule [15]. Sertoli cells are columnar in shape and attach to the basement membrane of the seminiferous tubules. Developing sperm cells are sandwiched in-between Sertoli cells in the form of spermatogonia, spermatocytes, and spermatids. Tight junctions between neighboring Sertoli cells anchor germ cells to the base-

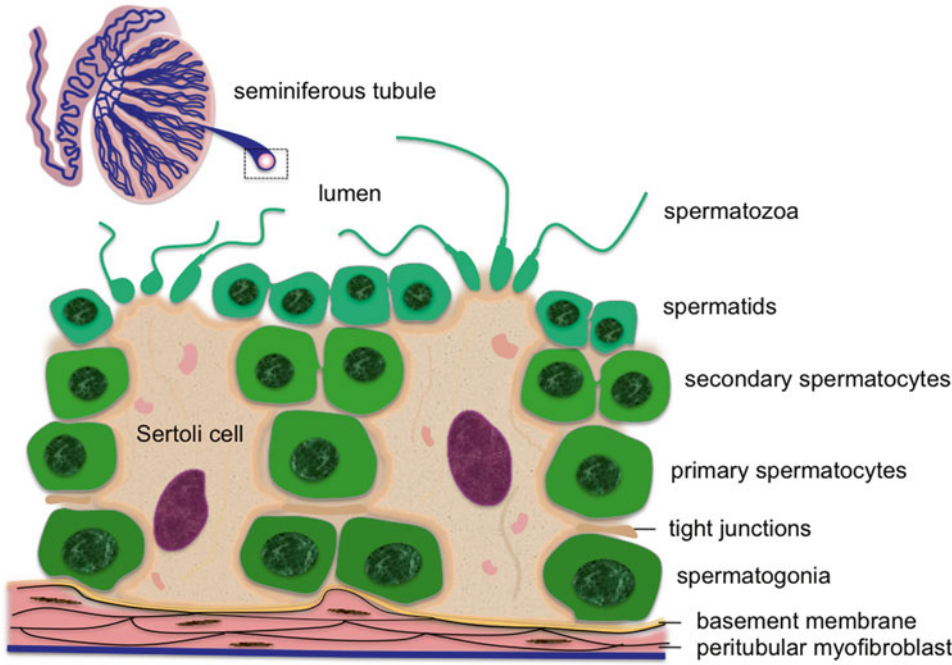

Fig. 3.5 The germinal epithelium. Each seminiferous tubule contains a central lumen surrounded by the germinal epithelium made of Sertoli cells and germ cells at different phases of spermatogenesis

ment membrane and divide the adluminal and basal compartments within the seminiferous tubules. This combination of Sertoli cells and developing germ cells form the germinal epithelium that is encased by peritubular myofibroblasts and extracellular connective tissue.

Seminiferous tubule growth, protein production, and spermatogenesis occur through the action of FSH on Sertoli cells. These cells increase in both total number and size during puberty [16] and directly correlate with testicular size and sperm production [17]. Follicle-stimulating hormone promotes the production of androgen-binding protein that sequesters testosterone in Sertoli cells. This protein maintains elevated intratesticular testosterone levels that can be up to 40- to 100-fold greater than that in systemic circulation [18, 19] and are necessary for spermatogenesis [19, 20]. Despite normal testicular descent and development, knockout mice lacking the androgen receptor at Sertoli cells exhibit spermatogenic arrest, increased germ cell apoptosis, and a reduction in sperm production [21–23]. In fact, suppression of intratesticular levels of testosterone can result in a significant decline in sperm count by up to 98 % [19].

Sertoli cells provide structural and nutritional support for spermatogenesis. Synaptic tight junctions between Sertoli cells form the blood-testis barrier and make the germinal epithelium an immune-privileged site isolated from the immune system [24]. This prevents the formation of antisperm antibodies and leukocytosis directed against antigens specific to meiotic and postmeiotic germ cells. This barrier also regulates the entry of ions, metabolites, and toxic substances from systemic circulation that could negatively impact spermatogenesis. As germ cells develop into spermatozoa, Sertoli cells provide metabolic support in the form of lactate and growth factors through desmosome-gap junctions [25–28]. Sperm development is then assisted through the phagocytosis of residual bodies and cytoplasm shed by spermatids during spermiogenesis. Following completion of spermatogenesis, Sertoli cells secrete fluid that helps release newly formed spermatozoa from the seminiferous tubules into the epididymis.

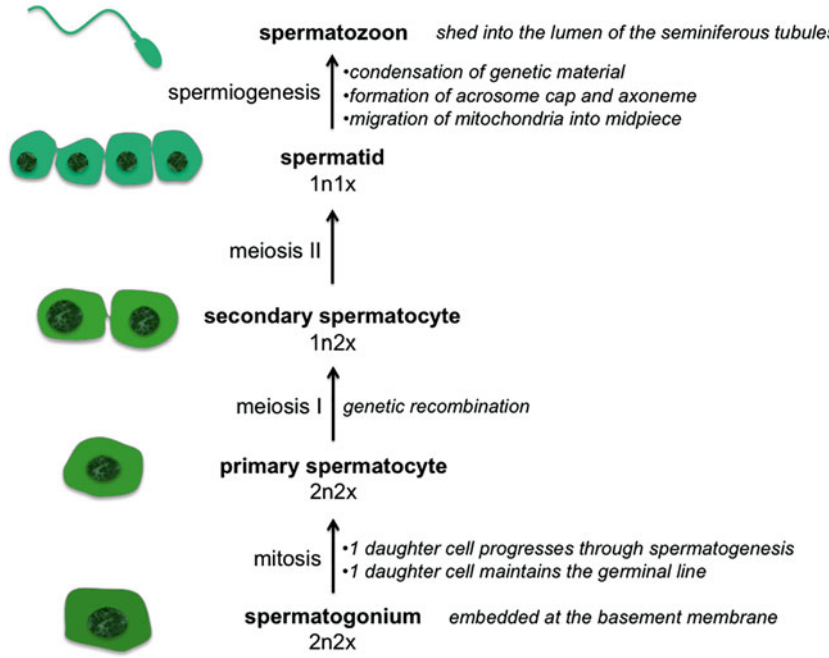

Fig. 3.6 Spermatogenesis. As germ cells progress through each stage of spermatogenesis, there is progressive migration from the basement membrane to the lumen of the seminiferous tubule. Sperm production begins as a spermatogonium germ cell undergoes mitosis to produce a primary spermatocyte. Subsequent meiotic divisions produce secondary spermatocytes and then spermatids. Spermatids undergo spermiogenesis and mature into spermatozoa before being shed into the lumen of the seminiferous tubule

Spermatogenesis

Spermatogenesis is the process in which spermatozoa are produced from primordial germ cells through mitosis, meiosis, and structural differentiation. This continuous process has previously been described to occur over the course of 74 days with 64 days dedicated to spermatogenesis followed by 10 days of maturation in the epididymis [29]. However, more recent investigation has demonstrated that this process may occur over a total course of 64 days and can range from 42 to 76 days depending on the individual [30].

Spermatogonia (2n2x) are diploid germ cells containing 46 autosomal chromosomes and the male XY sex chromosomes. These cells are embedded between Sertoli cells and kept in the basal compartment of the seminiferous tubules by tight junctions. These germ cells remain dormant until puberty when they are signaled by testosterone to proceed through three progressive phases: the proliferative phase, the meiotic phase, and spermiogenesis. As spermatogonia develop into spermatozoa, there is progressive migration from the basement membrane toward the lumen of the seminiferous tubules (Fig. 3.6).

Each spermatogonium undergoes mitosis to produce two identical daughter cells during the *proliferative phase*. One daughter cell is released by the tight junction between Sertoli cells into the adluminal compartment as a primary spermatocyte (2n2x). The tight junction subsequently reassembles and the other daughter cell remains within the basal compartment to maintain the germ cell line for future sperm production.

The primary spermatocyte (2n2x) then enters the *meiotic phase* to produce haploid gametes with a series of two meiotic divisions. Each chromosome is replicated to produce identical chromatids (2n4x) as the primary spermatocyte enters the first phase of meiosis. Homologous chromosomes exchange genetic material through recombination prior to division. This unique aspect of meiosis creates new combinations of alleles for

genetic variation among offspring. The primary spermatocyte with a replicated series of chromosomes (2n4x) then undergoes the first meiotic division to form two haploid secondary spermatocytes (1n2x) with a genetic reduction to 23 somatic chromosomes and a single sex chromosome. Both secondary spermatocytes then undergo the second meiotic division during which sister chromatids separate and four spermatids (1n1x) are formed. As these cells progress through each meiotic division, sister spermatocytes and spermatids remain connected through a cytoplasmic bridge to allow for synchronous development at each stage.

During *spermiogenesis*, haploid spermatids separate and undergo structural changes that transform each round cell with typical cellular anatomy into a differentiated spermatozoon with a head, neck, and tail. As this cell condenses and reshapes into the head of the sperm, residual bodies and cytoplasm are shed and phagocytized by Sertoli cells. Histones within the nucleus are replaced by protamines to tightly compact genetic material. The Golgi apparatus produces an acrosome cap at the head that later facilitates fertilization. As the proximal centriole forms the axoneme that will later act as the backbone of the flagellum, mitochondria move into a helical sheath at the mid-piece of the tail. Upon completion of spermiogenesis, spermatozoa are shed into the lumen of the seminiferous tubules and transported to the epididymis for maturation.

Spermatozoa morphology

Spermatozoa are approximately 60–70 μm in length [31] and are specialized for energy production, movement, and fertilization (Fig. 3.7a). The plasma membrane covers the sperm with

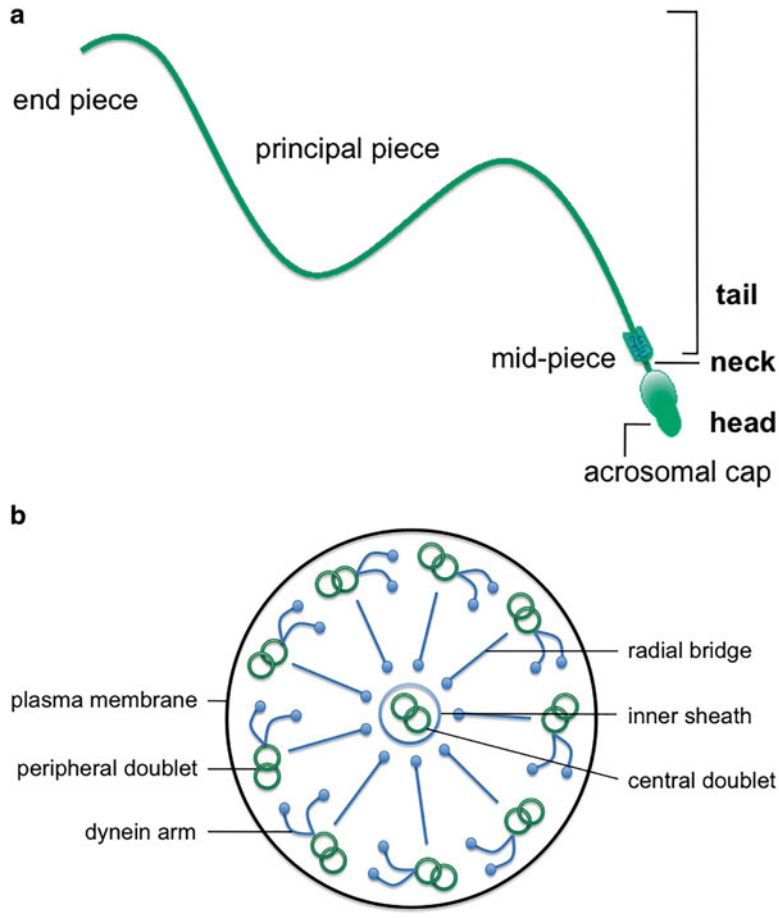

Fig. 3.7 (**a**) **Spermatozoon morphology.** A mature spermatozoon consists of a head, neck, and tail. The head contains tightly compacted genetic material and is covered by an acrosomal cap. The neck contains the proximal centriole that establishes the axoneme. The tail can be further sectioned into the mid-piece, principal piece, and end piece. (**b**) **Axoneme.** In an axial view of the tail, there is a 9 + 2 arrangement in which 9 pairs of microtubules surround a central pair. The peripheral microtubules are connected to the central pair by radial bridges and to each other by dynein arms

exception to end of the tail and regulates transmembrane movement of ions and metabolites.

The head of the sperm is pear shaped with an ellipsoid face and measures 4–5.5 μm in length and 2.5–3.5 μm in width [32]. A condensed nucleus contains a chromatin complex of 23 autosomal chromosomes, one of which is a single sex chromosome, tightly compacted by protamines and interspersed disulfide crosslinks. The head of the sperm is covered by an acrosomal cap, a membrane-bound organelle at the anterior aspect of the head that houses hydrolytic enzymes for fertilization. As a spermatozoon reaches the female oocyte, the acrosomal membrane fuses with the plasma membrane overlying the sperm. This process is referred to as the "acrosome reaction" and permits the release of the enzymes acrosin and hyaluronidase that digest the zona pellucida and oocyte cumulus cells for fertilization.

The neck acts as the connecting piece between the head and the tail. Its proximal centriole establishes the beginning of the axonemal complex that extends through the tail with a 9 + 2 microtubular arrangement (Fig. 3.7b). At the core of the complex is a central pair of microtubules surrounded by nine additional pairs of microtubules. The central pair or doublet is encased by an inner sheath and connected to each outer pair by radial bridges. Along the periphery of the axoneme, dynein arms extend between and connect neighboring microtubule doublets. This dynein protein complex transduces chemical energy from adenosine triphosphate (ATP) into mechanical movement as the microtubules slide and bend the axoneme in different directions.

The tail is a slender flagellum that comprises the axonemal complex surrounded by dense outer fibers. This structure can be divided into three progressive sections: the mid-piece, principal piece, and end piece. The axially arched mid-piece comprises the axoneme surrounded by outer fibers and a helical sheath. Mitochondria within this sheath produce ATP to fuel tail movements by means of oxidative phosphorylation [33, 34]. The principal piece is the main component of the flagellar tail and is encased by peri-axonemal structures. Disulfide bonds within the external outer fibers provide elastic rigidity at the principal piece. These outer fibers then transition to a fibrous sheath comprises longitudinal columns and transverse ribs that encase part of the principal piece and the remaining end piece.

Maturation and Transit Through the Epididymis

After spermatozoa are shed into the lumen of the seminiferous tubules, fluid secreted by Sertoli cells pushes these cells through the rete testis into the efferent ducts. These ducts reabsorb this fluid to assist with forward movement and increase sperm concentration. In addition, epithelial stereocilia and smooth muscle contractions at the efferent ducts help move spermatozoa into the head of the epididymis that lies at the posterolateral aspect of the testis.

Rhythmic contractions of the epididymal duct transports spermatozoa distally through the head, body, and tail. As spermatozoa progress through the epididymis, they undergo structural and functional changes. This period of maturation can vary between individuals, ranging from 2 to 12 days [35, 36]. During this time, the plasma membrane alters its biochemical composition, changes its fluidity, and develops a net negative surface membrane charge [37, 38]. Head size progressively decreases and tail movements advance from random motion to purposeful high-frequency, low-amplitude beats that propel spermatozoa forward [38, 39]. Meanwhile, the ability to bind to and penetrate an oocyte increases [40]. Following the maturation process, spermatozoa are temporarily stored at the tail of the epididymis until ejaculation.

Anatomy of Sperm Expulsion

During ejaculation, stored spermatozoa are expulsed from the tail of the epididymis by smooth muscle contractions into the remainder of the male reproductive tract (Fig. 3.8). The vas deferens is a tubular structure arises at the tail of the epididymis, exits the scrotum, and traverses the inguinal canal within the spermatic cord.

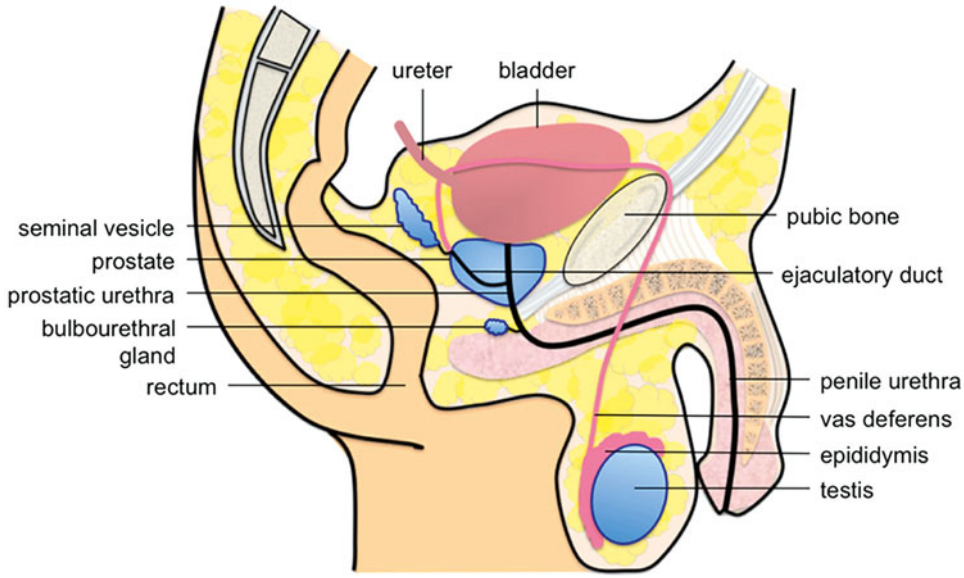

Fig. 3.8 The male reproductive tract. Spermatozoa produced in the testes are stored in the epididymis and propelled into the vas deferens during ejaculation. Spermatozoa then converge with fluid produced by the seminal vesicles, prostate, and bulbourethral glands at the ejaculatory duct prior to expulsion through the urethra

Smooth muscle contractions coupled with epithelial stereocilia propel spermatozoa into the ejaculatory duct to converge with semen.

Simultaneous contraction of the accessory sex organs releases semen during ejaculation. As an alkaline fluid composed of various metabolic and enzymatic components, this opaque viscous fluid promotes the survival of spermatozoa following ejaculation. High levels of inorganic phosphate and proteins alkalinize semen to a pH up to 8.0 to counteract the acidic environment of the female reproductive tract [41]. The seminal vesicles produce the majority of semen volume as it secretes fructose as the primary energy source for ATP production as well as antioxidants and enzymes that protect spermatozoa against oxidative damage [42]. This accessory sex organ also releases prostaglandins, amino acids, and phosphorylcholine [43]. Through hormonal influence from DHT, the prostate releases secretions that comprise phosphatase, citric acid, inositol, calcium, zinc, and magnesium that contribute 15–30 % of semen volume [43]. The bulbourethral glands produce mucus as the remaining 5–10 % of semen that lubricates the penile urethra and assists with motility.

The ejaculatory duct subsequently empties this combination of spermatozoa and semen into the prostatic urethra. The internal urethral sphincter simultaneously contracts to prevent retrograde flow into the bladder and promote expulsion into the penile urethra. Pulsatile ejaculation then occurs under stimulation from the sympathetic nervous system.

Spermatozoa complete maturation in the female reproductive tract through capacitation and hyperactivation. During capacitation, uterine factors interact with the sperm plasma membrane to increase calcium permeability and destabilize the acrosomal membrane in anticipation of fertilization [44]. The increased permeability increases intracellular calcium levels and simultaneously hyperactivates tail movements for motility [45, 46]. Upon reaching the oocyte and digesting the protective layers surrounding it through the acrosome reaction, a spermatozoon is then able to fuse with the female gamete to complete the process of fertilization.

Disruptions in Spermatogenesis and Spermatic Function

The production of functional spermatozoa capable of fertilization is a complex process involving various biochemical, cellular, and anatomic pathways. As there are multiple opportunities for spermatogenesis disruption and impaired male fertility, cancer can impact spermatic function both as a disease and as a target of gonadotoxic treatment. Semen analysis of patients with testicular cancer, lymphoma, leukemia, and gastrointestinal cancer demonstrates decreased sperm count, motility, and velocity prior to treatment [47–54]. For the purpose of focusing discussion on the disruption of spermatogenesis within the setting of cancer, conditions not pertaining to an acquired disorder relevant to cancer will only be briefly mentioned.

Disruptions affecting male fertility can be classified as pre-testicular disturbances in the HPG axis, dysfunction of the testicular parenchyma, or post-testicular obstruction in the male reproductive tract. These can occur individually or in combination with one another. Clinical assessment of testicular volume and changes in hormone levels based on the mechanism of impairment (Table 3.1) can elucidate the etiology underlying male infertility.

Furthermore, there can also be direct dysfunction within spermatozoa as a result of oxidative damage, immunologic factors, and genetic disruption. Sperm count, motility, and morphology can be assessed using semen analysis and measured against guidelines set by the World Health Organization (WHO) based on semen characteristics of men able to induce pregnancy within a 12-month period (Table 3.2). Parameters within

Table 3.1 Hormonal patterns in male infertility

	Definition	FSH	LH	Testosterone	Inhibin
Hypogonadotropic hypogonadism	Endocrinopathy at the hypothalamic–pituitary–gonadal axis	Low	Low	Low	Low
Primary testicular failure	Dysfunction of testicular parenchyma	High	Normal or high	Normal or low	Decreased [55]
Post-testicular obstruction	Disorder in ejaculation and sperm emission	Normal	Normal	Normal	Normal

The mechanism of spermatogenesis disruption can be clinically determined based on hormone levels. Abnormal values of gonadotropic hormones or factors normally produced by the testes would suggest endocrinopathy or testicular dysfunction

Table 3.2 Semen parameters

	Lower fifth percentile (95 % confidence interval)	Terminology
Semen volume	1.5 mL (1.4–1.7)	*Aspermia*—absence of ejaculate
Sperm concentration	15 million/mL (12–16)	*Oligospermia*—concentration less than 15 million/mL
Total sperm count	39 million/ejaculate (33–46)	*Azoospermia*—complete absence of sperm in ejaculate
Vitality %	58 % (55–63)	–
Progressive motility %	32 % (31–34)	*Asthenospermia*—decreased sperm motility
Total motility %	40 % (38–42 %)	
Normal morphology %	4.0 % (3.0–4.0)	*Teratospermia*—decreased percent of morphologically normal sperm

The WHO guidelines of semen characteristics are based on semen analysis of men able to induce spontaneous pregnancy within 12 months [56]

or above the lower fifth percentile are considered normal [56]. Additionally, the structural integrity of spermatozoa can be evaluated by sperm chromatin structure assay to assess for DNA fragmentation and denaturation.

Pre-testicular Disturbance

Pre-testicular causes of spermatogenesis disruption are endocrine in nature and are also referred to as secondary testicular failure or hypogonadotropic hypogonadism. Endocrinopathy at the level of the HPG axis reduces the amount of LH and FSH secreted by the anterior pituitary and ultimately decreases testosterone production and spermatogenesis within testicular parenchyma. This can result from genetic defects or congenital disorders, such as Kallman syndrome, Kleinfelter syndrome, or congenital adrenal hyperplasia as well as acquired causes.

Radiation to the central nervous system in patients with otolaryngological cancer and intracerebral tumors can impair the HPG axis through neurovascular damage and demyelination. Nasopharyngeal cancer, in particular, is targeted by radiation directed at the base of the skull where the hypothalamus and pituitary are anatomically located. Patients receiving cranial irradiation experience hypopituitarism with decreased pituitary hormone production, particularly within the somatotrophs, gonadotrophs, and corticotrophs [57–59]. The degree of endocrine dysfunction following irradiation can be worsened with concurrent chemotherapy [58].

The function of the HPG axis can also be affected by increased prolactin production by mammotrophs in the anterior pituitary. Hyperprolactinemia can inhibit GnRH secretion from the hypothalamus with downstream impairment of gonadal function. Increased serum prolactin levels have also been associated with sexual dysfunction with regard to low libido and premature ejaculation [60]. Prolactin can be produced in excess by a functional prolactinoma or as a side effect of medications, such as dopamine antagonists and antidepressants. Furthermore, hyperprolactinemia has also been identified in patients treated with radiotherapy for intracranial or nasopharyngeal tumors [58, 59].

Endocrine physiology can also be disrupted by excessive production of androgens or tumor markers. Excessive androgen or estrogen production from functional testicular or adrenal tumors increases systemic levels of androgens [61]. The resultant feedback inhibition to the HPG axis limits LH secretion and subsequently decreases testosterone production by Leydig cells. While androgen levels may remain elevated within systemic circulation, production of the intratesticular testosterone necessary for spermatogenesis would be diminished.

Testicular germ cell tumors may produce specific proteins oftentimes measured at elevated levels at the time of diagnosis. Seminomatous or non-seminomatous testicular tumors can each produce human chorionic gonadotropin (hCG) whereas non-seminomatous tumors may also produce alpha-fetoprotein (AFP). Although no association was identified between tumor markers and direct testicular dysfunction on histological evaluation [62], these tumor markers can disrupt spermatogenesis by means of feedback inhibition. Human chorionic gonadotropin produced by testicular germ cell tumors can suppress LH and FSH secretion with a respective decrease in Leydig function and spermatogenesis [62–64]. Testicular cancer patients with elevated hCG were found to have lower LH and FSH levels as well as decreased sperm motility [62]. These patients were also found to have increased estrogen and prolactin levels compared to testicular cancer patients without elevated hCG [63]. Orchiectomy in men with elevated hCG resulted in a decrease in this tumor marker, increase in FSH, and decrease in systemic testosterone and estradiol [65]. Elevated AFP has also been correlated with decreased sperm count [66] but has not been examined as extensively with regard to its effect on testicular function.

Testicular Dysfunction

Primary testicular failure occurs when defective parenchymal tissue within the testes is no longer able to adequately produce spermatozoa. This is

also known as hypergonadotropic hypogonadism where an intact HPG axis continues to produce elevated levels of FSH and LH in response to decreased feedback inhibition from low testosterone and inhibin. As Sertoli cells directly determine testicular volume [17], loss of these cells can result in decreased testicular size on clinical examination. Decreased testicular volume is associated with decreased sperm concentration, proportion of spermatozoa with forward motility, and proportion of spermatozoa with normal morphology [67].

The loss of volume and function at the testes and seminiferous tubules can vary in severity depending on the extent of damage to the germinal epithelium. Maturation arrest can arise as a functional disorder in which spermatogonia are present but unable to mature past a certain point of spermatogenesis. Testicular biopsies of males with nonobstructive infertility identified maturation arrest in primary spermatocytes with faulty recombination during meiosis [68].

Spermatogenesis can also be disrupted through a spectrum of germ cell loss, which includes hypospermatogenesis, germinal aplasia, and end-stage testis. Sperm count can be decreased with hypospermatogenesis in which normal spermatogenesis occurs despite a reduced number of germ cells. There can be a decrease in seminiferous tubule size with a complete absence of germ cells in germinal aplasia, also known as Sertoli cell-only syndrome. End-stage testis is at the end of the spectrum as the testes are diffusely scarred with thickened basement membranes, tubular sclerosis, and an absence of both Sertoli and germ cells.

Disrupted spermatogenesis in cancer can be partially attributed to a baseline defect in testicular function in some patients. Men with a previous history of cryptoorchidism or testicular dysgenesis have poorly developed gonads and demonstrate a greater prevalence of testicular nodules and cancer [69]. Furthermore, some cancer patients not previously diagnosed with testicular dysgenesis have been found to exhibit carcinoma-in-situ, immature tubules, microcalcifications, and Sertoli-cell-only patterns on biopsy of the contralateral testis during orchiectomy [70]. These findings suggest that a proportion of patients with testicular cancer already have preexisting fertility issues with baseline testicular abnormalities affecting spermatogenesis. Patients without these preexisting deficits, however, can nevertheless experience disrupted spermatogenesis and impaired fertility within the setting of cancer.

Elevated Scrotal Temperature

Elevations in body and scrotal temperature can impact testicular function. The testes within the scrotal sac are physiologically 2 °C cooler than core body temperature [71, 72]. This slight decrease is maintained by countercurrent heat exchange in testicular vasculature, evaporative heat loss at the thin-skinned scrotum, and adaptive contraction and relaxation of the cremasteric muscles surrounding the testes. These mechanisms can protect testicular function from long-term occupational heat exposure or mildly increased scrotal temperature when wearing athletic supports with no measured effect on semen parameters [73, 74].

Despite the ability of the testes to acclimate, however, semen analysis following acute febrile illness up to 40 °C led to a temporary decrease in sperm concentration, total count, and motility with no change in semen volume [75, 76]. The effect of increased temperature corresponds to the phase of spermatogenesis during which fever occurs and is indicative of the significance of each specific phase. Sperm concentration is decreased when fever occurs during meiosis whereas motility and morphology are affected when fever occurs during spermiogenesis and epididymal maturation [75].

In contrast with a transient febrile episode, cancer patients can experience intermittent fevers from a systemic inflammatory response. Fevers, night sweats, and weight loss are referred to as the systemic “B symptoms” associated with lymphoma. Fevers and night sweats have each been shown to decrease motility and sperm concentration in lymphoma patients prior to chemotherapy with a greater effect at higher temperatures [77, 78].

Scrotal temperature can also be increased without a corresponding rise in body temperature through increased blood flow at cancerous lesions or venous stasis in varicoceles. Retrospective review of testicular sonograms of men with testicular cancer or lymphoma demonstrated increased Doppler flow indicative of hypervascularity at cancerous lesions [79, 80]. This increase in flow can ultimately impair the countercurrent heat exchange that maintains testicular temperatures at physiologic levels.

As a common cause of infertility, varicoceles are defined as dilated and tortuous veins in the pampiniform plexus that result from defective valves or compression from abdominal or pelvis masses. These masses can involve primary tumors, enlarged lymph nodes, and metastatic disease in the setting of cancer. Venous stasis limits countercurrent heat exchange in varicoceles and can increase scrotal temperature [81]. Impaired sperm concentration, motility, and morphology as well as higher FSH levels have been demonstrated in men without cancer that had varicoceles and elevated scrotal temperature [81–83]. As a means of treating this condition, varicocelectomy has been shown to improve testicular function with a corresponding increase in sperm motility and concentration [83]. Although the exact mechanism of testicular dysfunction from varicoceles and increased temperature remains to be elucidated, semen samples demonstrated decreased antioxidant activity and expression of proteins involved in mitochondrial ATP production [83].

Localized Damage and Tissue Loss

In addition to tumor marker production, the inflammatory response, and local mass effect, cancer can directly disrupt spermatogenesis through damage to testicular parenchyma. Elevations in inflammatory markers may directly injure the germinal epithelium [78, 84]. Furthermore, local testicular infiltration has been demonstrated on histological evaluation [62, 64, 85–88].

Lymphomas are hematologic malignancies with various subtypes that are classified as either Hodgkin's disease (HD) or Non-Hodgkin lymphoma (NHL) based on the presence or lack thereof of Reed-Sternberg cells on histological assessment. Postmortem evaluation of children with hematologic malignancies demonstrated diffuse testicular infiltration with leukemic or lymphocytic cells, destruction of seminiferous tubules, and thickened basement membranes [85]. Pretreatment semen in HD and NHL patients demonstrated significantly poorer concentration, motility, and morphology compared to healthy controls [48–53, 84, 89]. Although there was no difference in testicular size and hormone concentrations between the two classifications of lymphoma, patients with NHL may exhibit better sperm quality than those with HD [89].

Testicular tumors can be identified as malignant germ cell tumors or generally benign stromal tumors derived from Leydig or Sertoli cells. Germ cell tumors comprise the majority of testicular tumors and are further classified as seminomatous or non-seminomatous cancers based on their histological composition. While both benign and malignant tumors can impact testicular function, there is a greater decrease in spermatogenesis in specimens with malignant germ cell tumors than those with benign lesions [86]. Conflicting studies have suggested that the degree of disruption on spermatogenesis may vary based on histology, with either non-seminomatous tumors [90, 91] or seminomatous and mixed tumors [64, 66] causing greater impairment.

Regardless of subtype, spermatogenesis in either the affected or contralateral testis can be reduced [62, 88]. Histological evaluation of orchiectomy specimens with seminomatous or non-seminomatous testicular cancer identified local structural damage and disrupted spermatogenesis [62, 64, 86–88]. There was a greater deficit in spermatogenesis observed in testicular tissue in a closer vicinity to the tumor [86, 87]. This impairment is also correlated with larger tumor size [62] and later stage of diagnosis [64]. Decreased rates of spermatogenesis in the contralateral testis have also been identified on biopsy

[88] and may be attributable to bilateral involvement, preexisting testicular dysgenesis [70], or production of cytokines or tumor markers.

Radical orchiectomy of the cancerous testicle ultimately removes half of the germinal epithelium. Semen analysis studies have demonstrated a decrease in sperm concentration, total sperm count, and inhibin levels with a compensatory increase in FSH and LH immediately following radical orchiectomy for testicular cancer [64, 65, 91–93]. The decrease in sperm concentration following orchiectomy was less than half but not proportional to the removal of half of the germinal tissue, which suggests a greater degree of dysfunction at the affected testis [64]. Despite an initially decreased production of spermatozoa, sperm morphology and motility as well as testosterone production did not appear to be affected [65, 92, 94, 95]. In contrast, other studies have shown that orchiectomy has a minimal negative impact on concentration, motility, and morphology [94, 95]. Although Sertoli cells have been thought to stop proliferating after puberty [96], compensatory hypertrophy from increased endogenous FSH and normalized sperm count and concentration have been observed in patients with early-stage seminomatous testicular cancer by the second and third year following orchiectomy [92].

Gonadotoxic Treatments

Testicular dysfunction can also be induced by gonadotoxins that directly injure the germinal epithelium. These can include antibiotics, various medications, and pain medications that may be involved in treating complications of cancer, such as infection or pain. A significant concern of gonadotoxicity within the setting of cancer arises during treatment with systemic chemotherapy and irradiation to the testes.

Chemotherapy targets rapidly dividing cells regardless of their malignant characteristics (Tables 3.3 and 3.4). Actively proliferating spermatogonia are therefore particularly sensitive to chemotherapy. This is supported by the observation that sexually mature males are more sensitive to gonadotoxic treatment than prepubertal males with inactive spermatogenesis [97].

Spermatogenesis disruption, however, is not immediately evident after the initiation of chemotherapy. Impaired sperm concentration, motility, and morphology have been observed

Table 3.3 Chemotherapy agents

Class	Individual agents	Mechanism of action
Alkylating agents	Cyclophosphamide	Attach to alkyl groups on DNA to damage DNA through crosslink formation
	Dacarbazine	
	Mechlorethamine	
	Procarbazine	
Glycopeptide antitumor antibiotics	Bleomycin	Fragment strands of DNA
Intercalating agents	Doxorubicin	Damage DNA by inserting between DNA bases
Platinum analogues	Cisplatin	Crosslink DNA and trigger apoptosis
Topoisomerase inhibitors	Etoposide	Prevent DNA from unwinding for replication and DNA synthesis
Tubulin binders	Vinblastine	Inhibit the formation of microtubules during mitosis
	Vincristine	

Chemotherapy agents vary in their mechanisms of action and are used in combination to treat cancer. The listed agents are those that are used to treat cancers commonly diagnosed in young males

Table 3.4 Gonadotoxicity of chemotherapy

Regimen	Treatment	Agent	Mechanism of action	Effect
ABVD	Hodgkin's disease	Adriamycin (doxorubicin)	Intercalating agent	• Temporary impairment with more than 90 % of patients recovered at 24 months after treatment [99] • Less toxic than MVPP [53]
		Bleomycin	Glycopeptide antitumor antibiotic	
		Vinblastine	Tubulin binder	
		Dacarbazine	Alkylating agent	
BEP	Testicular cancer	Bleomycin	Glycopeptide antitumor antibiotic	• Temporary impairment [93, 95] • Most significant decrease in sperm parameters at 3 months [98] • Cisplatin toxicity [107–109]
		Etoposide	Topoisomerase inhibitor	
		Platamin (cisplatin)	Platinum analogue	
CHOP	Non-Hodgkin lymphoma	Cyclophosphamide	Alkylating agent	• Temporary with 61 % recovered at 24 months after treatment [99] • Dose-dependent damage by cyclophosphamide [97, 103–105, 107] • Greater decrease in motility and vitality [99]
		Hydroxydaunorubicin (doxorubicin)	Intercalating agent	
		Oncovin (vincristine)	Tubulin binder	
		Prednisone	Steroid	
MOPP or MVPP	Hodgkin's disease	Mechlorethamine	Alkylating agent	• Permanent gonadal damage associated with procarbazine [106] • Greater decrease in motility and vitality [99] • MOPP with permanent gonadal damage [55]
		Oncovin (vincristine)	Tubulin binder	
		Procarbazine	Alkylating agent	
		Prednisone	Steroid	

The short-term and potential long-term effects of commonly used chemotherapy regimens on spermatogenesis can vary depending on the agents involved

after the first 3 months to 1 year after initiation of treatment for testicular cancer and lymphoma [93, 95, 98, 99]. This delayed effect corresponds with the 2–3 month cycle of spermatogenesis following an initial insult to the germinal epithelium. Testicular biopsy following chemotherapy for acute lymphoblastic leukemia demonstrated reduced spermatogonia, infiltration of leukemia cells, interstitial fibrosis with abnormal Leydig cell maturation, and basement membrane thickening [100, 101]. Testicular dysfunction can be further demonstrated by a corresponding increase in LH and FSH during treatment [97, 102]. As Leydig and Sertoli cells are exposed to gonadotoxic agents, decreased production of testosterone and inhibin diminishes the negative feedback acting on an intact HPG axis.

Among the various chemotherapy agents used to treat lymphoma, alkylating agents have been specifically implicated in causing testicular dysfunction. Agents such as cyclophosphamide and procarbazine damage DNA in rapidly dividing cells by binding to an alkyl group on DNA and forming crosslinks. The immediate and long-term effects of these agents on sperm concentration and gonadal damage are dose-dependent in males previously treated for childhood cancer as well as those treated as adults [97, 103, 104]. Cancer survivors treated with high-dose cyclophosphamide for childhood sarcoma exhibited long-term gonadal dysfunction with significantly decreased sperm count regardless of pubertal status at the time of treatment [105]. These agents are also oftentimes used in the treatment of both HD and NHL. Although lymphoma patients experienced decreased spermatogenesis with chemotherapy, those treated with cyclophosphamide and procarbazine demonstrated a greater decrease in sperm motility and vitality [99]. Cumulative doses of procarbazine used to treat HD were associated with a greater frequency of long-term gonadal dysfunction in both men and women [55, 106]. These patients exhibited a permanent increase in FSH and LH with decreased inhibin and sperm concentration [55]. Individual alkylating agents can differ with regard to the extent of their gonadotoxicity. For example, the chemotherapy regimen ABVD includes the alkylating agent dacarbazine but has been found to be less toxic than regimens with cyclophosphamide or procarbazine [53].

Cumulative doses of cisplatin used to treat testicular cancer have also been associated with long-term gonadotoxicity [107–109]. This platinum-based analogue works in a similar fashion as alkylating agents by crosslinking DNA and causing subsequent damage to rapidly dividing cells. Patients treated with this agent demonstrated decreased testicular function in addition to cardiovascular, nephrologic, and neurologic complications [109]. Despite the impact on other organ systems, gonadotoxicity is the most frequent long-term toxicity identified following cisplatin treatment with dose-dependent gonadotropin elevations, Leydig cell deficiencies, and decreased paternity rates [107–109].

Table 3.5 Gonadotoxicity of radiation

Dose (cGy)	Effect during treatment	Long-term effect
<20	No effect on FSH or sperm count [111]	–
20–100	Transient dose-dependent increase in FSH and decrease in sperm count [111, 112]	Recovery within 12–30 months [111, 112]
>100	Azoospermia during treatment [113]	• Azoospermia 8–34 weeks after treatment with recovery at 44–77 weeks [110]
		• No evidence of recovery at 15 months in some patients [113]

Scattered radiation likely exhibits a dose-dependent effect with regard to disrupting spermatogenesis

In addition to chemotherapy, irradiation for cancer treatment has been demonstrated to be gonadotoxic [110–113]. Low-dose scattered radiation can be applied to the inguinal and testicular region for lymphoma and testicular cancer at varying doses (Table 3.5). Although the toxicity of chemotherapy manifests 3 months following initiation of treatment, the effects of radiation on semen analysis becomes evident 6 months following initiation of therapy for testicular cancer [98] and lymphoproliferative disorders [93]. The degree of damage to the germinal epithelium occurs in a dose-dependent manner and can be measured by time to recovery [111, 114]. While spermatogenesis was preserved at radiation doses less than 20 cGy, Leydig cell function can be preserved with doses up to 70 cGy [111]. Impaired spermatogenesis may be transient in moderate doses of testicular irradiation [111, 112] but can become permanent at higher doses [113]. However, radiation dose may not affect time to recovery and testicular function may be preserved if shielding techniques are properly employed during treatment [115].

Post-testicular Infertility

Causes of male infertility that are post-testicular are primarily ejaculatory disorders and are not necessarily attributable to disruptions in spermatogenesis. Ejaculatory dysfunction can prevent proper sperm emission through mechanical reproductive tract obstruction, neurologic dysfunction, or psychogenic sexual dysfunction.

Obstruction along the reproductive tract from the testis to the urethral meatus can occur by various mechanisms. The tract may not be patent due to a congenital absence of the vas deferens associated with cystic fibrosis or by iatrogenic ligation from an elective vasectomy [116–118]. Infectious processes or malignant masses can also obstruct sperm emission [119]. Similar to venous compression in varicoceles, local mass effect from primary tumors, enlarged lymph nodes, or metastatic disease can obstruct sperm emission at any point in the reproductive tract.

Post-testicular male infertility can also arise as a result of neurologic dysfunction. Organic erectile dysfunction can occur with neuropathy, such as in patients with poorly controlled diabetes [120–123], neurologic disorders [124–126], and head or spinal cord injury [127]. Within the setting of cancer, neurologic dysfunction can occur as a consequence of treatment. Unintentional nerve injury to the autonomic nervous system during retroperitoneal lymph node dissection or mass excision can impair the ability to achieve an erection or ejaculation [128]. Furthermore, nerve injury may also impair urethral sphincter contraction. In this situation, semen would be ejaculated in a retrograde manner into the bladder instead of through the penile urethra and result in a dry orgasm [128].

The psychological stress of cancer also cannot be overlooked when evaluating infertility. In addition to organic causes, erectile dysfunction can be psychogenic in nature. Despite the physical capability of achieving an erection, stress or anxiety can limit sexual function in a patient diagnosed with and being treated for a life-threatening disease. Psychological stress and emotional distress have been associated with reduction in sperm concentration, motility, and velocity [129–131]. Although these changes in semen parameters may be attributable to multiple physiologic sequelae related to stress, the association between stress and fertility should nevertheless be acknowledged when considering potential disruptions in spermatogenesis.

Defects in Spermatozoa

The normal range for semen analysis parameters is within the lower fifth percentile for fertile men to be considered normozoospermic [56]. This allows for a considerable overlap between fertile and infertile men and semen analysis may be equivocal when assessing for male factor infertility. Immunologic, biochemical, and genetic disruptions can affect spermatic function even without any clinically detectable disturbances in spermatogenesis and should therefore be considered when evaluating fertility.

Immunologic Inhibition

The blood-testis barrier formed by tight junctions between Sertoli cells isolates the germinal epithelium from the immune system. Violation of this barrier can expose the various antigens on developing germ cells and spermatozoa to the immune system. This could elicit a response through antibody formation or leukocytosis that subsequently impairs spermatic function.

Tumor necrosis factor-α (TNF-α) potentially contributes to the physiologic release of tight junctions to allow spermatocyte migration during spermatogenesis [132]. Administration of this cytokine has been shown to transiently disrupt the blood-testis barrier and allow passage of dye from systemic circulation into the adluminal compartment [132]. Elevations in inflammatory cytokines including TNF-α in cancer may prolong tight junction release and expose developing germ cells to the immune system. Furthermore, gonadotoxic injury may directly damage the germinal epithelium and further expose this immune-privileged site to systemic circulation.

Upon immune exposure, different surface antigens on spermatozoa activate serum antibod-

ies to inhibit sperm function through immobilization or agglutination reactions [133]. Immunoglobulins in the form of IgG, IgA, and IgM can affect fertility by binding the plasma membrane and inhibiting motility, the ability to penetrate cervical mucus, or fertilizing capacity [134]. These antibodies have been specifically detected in patients with seminomatous and nonseminomatous testicular cancer with a consequential decrease in serum levels following orchiectomy [135, 136]. However, serum antisperm antibodies are likely to persist after orchiectomy in patients with advanced disease [136]. Although antisperm antibodies are associated with decreased sperm concentration and motility as well as reduced fertilization rates [137], the percentage of antibody-bound spermatozoa has not been correlated with fertilization, pregnancy, or miscarriage rates in assisted-reproductive technology (ART) thus far [138].

In addition to antibody formation, white blood cells may be directly involved in spermatic dysfunction. The WHO defines leukocytospermia as 1×10^6 WBC/mL on semen analysis. While there is no correlation identified between antisperm antibodies and this elevation in white blood cell count [139], leukocytospermia has been associated with genetic defects within spermatozoa, a reduction in sperm count and motility, and abnormal morphology [139–141]. Furthermore, individual types of leukocytes have been found to affect semen parameters in different ways. Monocytes and macrophages have been associated with reduced ejaculate volume, whereas T lymphocytes have been associated with reduced sperm velocity [139].

Oxidative Damage

Reactive oxygen species (ROS) are a product of metabolism and the physiologic chemical reactions that occur during capacitation and the acrosome reaction [142]. Excessive ROS can cause oxidative damage to spermatozoa; however, as high levels of oxidative stress markers in spermatozoon DNA have been associated with male factor infertility [143, 144]. Semen analysis in men with elevated markers specifically demonstrated impaired sperm motility, morphology, and concentration [143–146] as well as a dose-dependent increase in DNA fragmentation [146]. Furthermore, experimental administration of excessive ROS resulted in membrane peroxidation, DNA strand breaks, sperm head abnormalities, and impaired fertility [42, 147].

The mechanism by which spermatozoa are impaired by the immune response may also include oxidative damage, as greater rates of ROS production have been correlated with leukocytospermia [140]. Reactive oxygen species produced by seminal leukocytes may lead to a significant reduction in intracellular ATP [148], which would ultimately impair sperm motility by limiting the chemical energy available for mechanical movement.

The harmful effects of oxidative damage may be potentially counteracted by the consumption of antioxidants, including vitamin C, vitamin E, glutathione, beta-carotene, and zinc. Supplementation with antioxidants in non-cancer patients with oxidative DNA damage may significantly reduce oxidative damage markers, increase sperm concentration, and decrease the percentage of spermatozoa with fragmented DNA [144, 149–151]. Antioxidant supplementation can also improve gestational outcomes [152]. However, other studies did not demonstrate improved sperm motility, reduction in DNA fragmentation, or improvement in chromatin structural integrity with dietary consumption of antioxidants [153, 154] and additional studies are needed to determine the role of antioxidants on spermatic structure and function.

Genetic Defects

Genetic defects within spermatozoa can occur in the form of chromosomal mutations, structural fragmentation, and impaired chromatin condensation during or after the process of spermatogenesis. Alterations to the content or structure of DNA may be reflected through impaired morphology or motility but can also occur independent of normal sperm development.

Alterations to the genetic content of DNA have been implicated in male infertility. Spermatogenesis can be impaired by maturation arrest with poor chromosomal recombination during meiosis as pre-

viously discussed [68]. Furthermore, nondisjunction during chromosomal segregation results in aneuploidy when chromosomes do not appropriately separate during mitotic or meiotic daughter cell formation. This inappropriate number of chromosomes has been negatively correlated with sperm concentration and percentage of morphologically normal spermatozoa [155, 156]. Furthermore, chromosomal aneuploidy has also been associated with recurrent pregnancy loss [157] and lower rates of fertilization with ART [158]. Microsatellites occur throughout the genome and can serve as sites of genetic mutation. Fortunately, microsatellite mutations have not been demonstrated following testicular irradiation compared to pretreatment semen samples [159], but additional studies are needed to assess the impact of cancer and gonadotoxic treatment on mutation risk.

In addition to mutation risk, the structural integrity of DNA may be compromised by breakage within individual strands. DNA fragmentation has been associated with increasing paternal age [160–163], oxidative stress [42, 146, 147, 164], leukocytospermia [140, 141], and acute febrile illness [76] even in men with normal semen parameters during infertility assessment [161, 165]. Sperm chromatin alterations and DNA fragmentation have been specifically identified in cancer patients prior to treatment [99] as well as after gonadotoxic damage from chemotherapy and radiation [95]. These alterations to DNA structure have been correlated with decreased sperm concentration, motility, and percentage of spermatozoa with normal morphology [161, 166, 167] in addition to decreased testicular volume [67]. Various studies have associated DNA fragmentation and chromatin structural defects with prolonged time to pregnancy by natural conception [162, 163, 166, 168–170], difficulty achieving and maintaining pregnancy with ART [171–174], and recurrent pregnancy loss [157, 175, 176].

Genetic material is normally compacted within the head of the spermatozoa until fertilization. The formation of a tightly packed chromatin complex within the nucleus allows for size restriction and appropriate morphology. Appropriate chromatin condensation may be representative of the structural integrity of genetic material as poor chromatin condensation is associated with DNA fragmentation [167]. Impaired chromatin condensation has been identified in cancer patients prior to and after treatment [95, 99] and associated with leukocytospermia [140, 141]. Not only is this associated with poor sperm quality [141, 167], but it has also been associated with lower rates of fertilization and pregnancy with ART [158].

Experimental and population studies have begun to evaluate the ultimate question of whether genetic aberrations are passed down to offspring and affect embryonic development. There was a reduction in early embryonic growth in offspring fertilized by mouse testes exposed to prolonged heat [177]. However, there was no overall growth or developmental deficiency as compensatory growth was observed later in gestation [177]. No association was otherwise identified between paternal gonadotoxicity or genetic aberrations with impaired zygote or embryonic morphology [174]. Offspring of childhood cancer survivors previously treated with chemotherapy did not exhibit a greater rate of abnormal karyotypes or incidence of Down syndrome or Turner syndrome [178, 179]. In the age of ART, however, selection for genetic integrity reflected in motility or morphology may be bypassed and additional studies are needed to further evaluate the impact of genetic sperm defects on offspring.

Recovery Versus Permanent Gonadal Damage

Recovery of spermatogenesis with improved sperm concentration has been found to occur up to 2 years after treatment for testicular neoplasia or lymphoma [99]. Sperm count prior to chemotherapy [95, 99] and radiation therapy [114] has been recognized as a predictive factor of spermatogenesis recovery. However, other studies have shown that recovery may not be related to pretreatment semen analysis in testicular cancer [54, 98]. Although testicular cancer patients may have a greater risk of reduced pretreatment sperm concentration, this cancer was associated with the lowest risk of azoospermia after treatment compared to other malignancies [54]. The time to recovery may also depend on the number of chemotherapy treatments administered [95], cancer

stage at diagnosis [53], and post-treatment reduction in sperm concentration [54]. Furthermore, combined treatment with chemotherapy and radiation therapy appears to have a compounding effect in prolonging the recovery period following treatment [114].

Unfortunately, a small percentage of patients can remain azoospermic at long-term follow-up after lymphoma treatment [55, 99]. Furthermore, a considerable proportion of childhood cancer survivors treated with chemotherapy and radiation have remained azoospermic with decreased testicular volume in adulthood [104, 180]. This risk of prolonged recovery or permanent gonadal damage warrants discussion with patients regarding the risk for infertility and fertility preservation should be considered in patients with cancer.

Conclusion

Spermatogenesis is an orchestrated process with multiple opportunities for disruption. The regulation of the HPG axis depends on extrinsic factors in addition to feedback inhibition. Cellular divisions must involve genetic recombination and the proper reduction of diploid genetic material in anticipation of integrating with a complementary female gamete. Spermatozoa should be properly structured and differentiated for energy production, forward motility, and the acrosomal reaction. Following functional maturation, spermatozoa converge with semen containing a balanced composition of metabolites, proteins, and antioxidants. This combination is then ejaculated through a patent male reproductive tract prior to entering the female reproductive tract to attempt fertilization.

The disease process of cancer as well as its treatments can easily disrupt spermatogenesis as well as the structural and genetic integrity of spermatozoa through various mechanisms (Table 3.6). In addition to increased inflammatory markers and local structural disturbance, surgical excision of reproductive tissue and damage to the sensitive germinal epithelium from chemotherapy and radiation can reduce fertility for a prolonged period of time. Despite reports that azoospermia may be temporary, preservation of male fertility should nevertheless be considered within the setting of cancer.

Table 3.6 Cancer and spermatogenesis

	Testicular cancer	Lymphoma
Risk factors	History of cryptoorchidism and testicular dysgenesis increase risk of developing testicular cancer [69, 70]	–
Endocrine and systemic disturbance	• Androgen production by benign Leydig tumors or elevated hCG may cause feedback inhibition [63, 65] • Decreased sperm count associated with AFP [66]	Elevated ESR associated with decreased semen parameters [78, 84]
Testicular dysfunction	• Preexisting dysfunction associated with cancer [69, 70, 88] • Hypervascularity [80] potentially increasing temperature • Local damage adjacent to tumor [62, 86, 87] • Greater impairment with larger tumors [62] at advanced stages [66]	• Systemic B symptoms [77, 78] and hypervascularity [79] may affect spermatogenesis through increased scrotal temperature • Infiltration with leukemic or lymphocytic cells, destruction of seminiferous tubules, thickened basement membranes [85] • Advanced stage with poorer semen parameters [84]

(continued)

Table 3.6 (continued)

	Testicular cancer	Lymphoma
Spermatic dysfunction	• Serum anti-sperm antibodies in patients with seminomatous and non-seminomatous testicular cancer [135, 136] • Altered chromatin structure and DNA fragmentation with chemotherapy treatment [95]	Altered chromatin and DNA fragmentation prior to treatment [99]
Orchiectomy	Removal of germinal epithelial tissues [64, 65, 91–93] with compensatory hypertrophy [92]	–
Chemotherapy	• Toxicity from BEP [93, 95] with cisplatin-specific toxicity [107–109] • Most significant impairment at 3 months [98]	• *Hodgkin's disease*: toxicity from MOPP/MVPP [55, 93, 99] with procarbazine-specific toxicity [106] whereas ABVD is less gonadotoxic [53] • *Non-Hodgkin's*: Toxicity from CHOP with cyclophosphamide-specific toxicity [93, 99, 106]
Radiation	• Most significant impairment at 6 months [98] • Dose-dependent damage [95, 112, 114] • Altered chromatin structure and DNA fragmentation [95] • No microsatellite mutations detected after radiation [159] • Radiation dose may not affect recovery if shielding techniques are employed during treatment [115]	• No effect on Leydig cell function with doses up to 70 cGy [111] • Significant decrease in sperm parameters evident at 6 months [93, 98] • Dose-dependent damage [111, 112] with permanent damage at higher doses [113]
Recovery	Recovery depends on pretreatment sperm quality [95, 114], the number of chemotherapy treatments received [95], radiation dose [114]	Mean recovery at 24 months with persistent azoospermia in some patients [99] and depends on pretreatment sperm quality [99]

Spermatogenesis can be disrupted within the setting of cancer through various mechanisms affecting the endocrine system, testicular parenchyma, and spermatozoa

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Fertility Assessment in the Male

4

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Introduction

The fertility evaluation of the male has three important goals: to identify conditions or illnesses that may impact the health of the patient, identify conditions that may impact the health of offspring and finally, to help the patient obtain a pregnancy. In the case of patients with cancer, there is the added goal of preserving the ability to have children throughout and after treatment. As patients are surviving longer with current cancer treatments, fertility preservation is becoming more important to patients and practitioners. This preservation often requires obtaining sperm samples prior to the initiation of therapy, no matter if that treatment is chemotherapy, radiation, or surgery. Each of these treatments can affect fertility in their own way and will be discussed later in this book. It is important to be able to perform a full assessment of a patient's fertility prior to therapy as a proper evaluation allows the practitioner to identify patients who may be more susceptible to injury during treatment, or who may already have fertility issues that may make preserving fertility prior to treatment more difficult.

Medical History: Reproductive History

Similar to evaluations for any medical issue, a thorough history can glean a significant amount of the necessary information about the reproductive system. The most important question in the fertility evaluation is whether or not the patient has previously fathered a child or caused a pregnancy. No other question gives you more insight into that patient's fertility status, especially since there is no definitive lab test for fertility. After this, all other questions stem from identifying changes in health or exposures since that time.

If a couple has not previously conceived, or has in the past, but it was some time ago; identifying those whose lack of pregnancy represents pathology is important. The current accepted definition of infertility is the inability to conceive a child by 1 year of intercourse without contraception [1], though there is some variation among professional organizations with some authors extending this time to 2 years because of the

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relatively low monthly fecundity even in couples who will eventually conceive [2]. Because fertility, especially female oocyte quality, declines with aging, in some couples, waiting for 2 years may lead to a significant decline in success rates by the time treatment is attempted [3]. Thus, the trigger for initiating infertility evaluation and treatment must be tailored to the couple. When attempting to determine how long a couple has been trying to conceive, it is important to clarify if and for how long the couple has been using birth control. Often couples will state that they have only been actively "trying" to have a child for the last 6 months, but have never used birth control during the entire 5 years of their marriage. Patients often confuse "trying" with conscious intent to have a child, forgetting that there is no biologic difference between "trying" and having frequent intercourse without birth control.

If the patient or couple have achieved a pregnancy before, but not had a child, the number of pregnancy losses and when they were lost can offer insight into possible dysfunction. While recurrent pregnancy loss is commonly an issue with the female partner, male factor infertility has been found to play a role as well. In some patients, this is due to translocations where the offspring might not inherit a complete genome [4, 5]. Poor sperm DNA integrity with increased DNA fragmentation has also been shown to be positively correlated with repeat pregnancy losses [6–8].

Frequency of intercourse is also an important part of the reproductive history. Some patients will claim a long period of attempting pregnancy, but have infrequent attempts. The optimal time for intercourse is at least every other day during the 7 days in the periovulatory period [9–11]. Ovulation timing can be estimated by urinary ovulation prediction kits, basal body temperatures measurements, calendar prediction, analysis of the cervical mucus, or ovarian ultrasound [11]. While ensuring that patients are having intercourse within this window, it is important that psychological stress, which is already increased in couples undergoing fertility evaluation, is minimized [12, 13]. This stress can not only strain the couple's relationship, but there is also evidence that emotional stress can impair spermatogenesis [14]; in fact, significant decreases in sperm counts were seen in men after hurricane Katrina [15]. Additionally, the stress of infertility can lead to sexual dysfunction in men, potentially limiting frequency of intercourse and emotional intimacy within the relationship [16].

There are no recommended sexual positions to optimize fertility, and patients should only be encouraged to do what they feel comfortable with. The use of lubricants though has been found to impair sperm function [17]. While it does not appear to influence fertility rates in fertile couples, it is not recommended for those with issues obtaining a pregnancy [18]. While there are no entirely sperm-friendly lubricants, not even saliva, some are worse than others [19]. Those that are most problematic are among the most commonly used lubricants such as KY™, Surgilube™, and Astroglide™, which are all water based [20]. More sperm-friendly lubricants include those that are vegetable oil, glycerin, or petroleum oil based [21]. If at all possible, patients should be counseled to avoid lubricants all together (Table 4.1).

General Medical History

Like all patients presenting to a practitioners office, a detailed history of other medical conditions should be obtained. This is done for two reasons. Other medical conditions can cause sexual dysfunction (such as erectile dysfunction, anejaculation, or retrograde ejaculation) or impair sperm production and function itself. Patients who have cancer may have other disorders either exacerbated or induced by their treatment that may affect their fertility.

Taking a history in chronological order is a convenient way of introducing order into a history that may end up being quite detailed. The first part of such a history is the developmental history. This can commonly uncover syndromic disorders, especially those of testosterone production and metabolism. In a study of 34,910 children, disorders of the sex chromosomes were found in 1 per 426 births. The most common of these being

Table 4.1 History for fertility evaluation

Question	Implication	Question	Implication
Reproductive History		*Social History*	
Have you previously caused a pregnancy? If not, how long have you been attempting a pregnancy?	Defines infertility	Have you ever used any tobacco product?	Impairs of spermatogenesis
Has your partner ever lost a pregnancy?	Defines recurrent pregnancy loss	Have you utilized any illicit drugs?	Interferes with hormone production/metabolism
– How early?	Uncovers possible chromosomal abnormalities	How much alcohol do you typically intake in a week?	Interferes with hormones and impairs sperm productions
– Were products of conception analyzed?		What is your occupation?	Exposure to gonadotoxic medications
Are you using methods to predict ovulation?	Discovers ovulatory issues and ensures proper timing of intercourse	– Have you ever been exposed to hazardous chemicals?	
How often and when are you having intercourse?	Ensures proper timing of intercourse	*Surgical History*	
Are you using lubricants?	Exposure to spermicidal agents	Have you ever had a genital surgery?	
General Medical History		– Inguinal hernia repair, open or laparoscopic? Unilateral/bilateral?	Injury to testicle or vas deferens
Did you go through puberty at the same time as peers?	Discovers disorders of testosterone or genital development	– Orchiopexy/hernia repair as child?	
Do you have recurrent pneumonias or sinusitis	Discovers microstructural disorders of sperm motility	Have you ever had pelvic or abdominal surgery?	Scaring and secondary obstruction or ejaculatory dysfunction
Do you have diabetes?	Potential for ejaculatory or erectile disorders	Have you had surgery within the last year?	Acute illness impairs spermatogenesis
Do you or anyone in your family have Cystic Fibrosis	Should screen patient for CBAVD	– Bariatric surgery?	Possible significant affect on sperm production
Do see a physician on a regular basis for treatment of any medical issue?	Possible other chronic disorders that can affect spermatogenesis	*Medication History*	
Have you seen sick within the last 3–6 months?	Identifies acute diseases affecting spermatogenesis	Have you started any new medications in the last 3–6 months?	Possible gonadotoxins
Genitourinary History		Have you ever taken testosterone or anabolic steroids?	Suppresses HPG axis and spermatogenesis
Have you ever had a urinary tract infection?	Identifies chronic inflammatory conditions or anatomic abnormalities (secondary obstruction due to scarring)	Do you take medications for urination (alpha blockers, 5ARIs)?	Impairment of spermatogenesis and ejaculatory dysfunction
Have you ever had an infection or trauma to the scrotum or testicle (especially with swelling and/or fever)?		Have you recently taken any antibiotics?	Possible gonadotoxins and acute illnesses
Do you experience pain in your pelvis/genitals?			
Do you experience any urinary issues; dysuria, hematuria, urgency/ frequency?			
Have you ever had a STI?			

STI sexually transmitted illness, *CBAVD* congenital bilateral absence of the vas, *5ARI* 5 alpha reductase inhibitors, *HPG* hypothalamic, pituitary, and gonadal

Klinefelter's was found in 1 per 576 boys [22]. These patients can present as having incomplete masculinization or delayed puberty. The symptoms of significantly delayed or absent puberty suggest complete failure of testosterone production, which is the classic presentation for Kallmann's syndrome, Fertile Eunuch syndrome, and hypogonadotropic hypogonadism, all of which are less common than Klinefelter's but may present as infertility in their 20s and 30s, especially if the patients did not have ready access to medical care earlier in their lives [23–25].

Other congenital disorders that influence fertility may not have any androgenic symptoms. An entire class of syndromes affecting sperm motility may initially present earlier in a patients life with seemingly unrelated pulmonary issues. Syndromes such as Young's syndrome and Kartagener's syndrome are both disorders of fertility that are associated with increases in pulmonary and sinus infections, the first due to tubular obstruction from thickened secretions and the second from nonfunctional cilia [26, 27]. More commonly, a practitioner may see a patient with a personal or family history of Cystic Fibrosis (CF), in which the disease and carrier state is associated with Congenital Absence of the Vas Deferens (CBAVD) [28, 29]. Conversely, patients who are found to have CBAVD should be screened for genetic mutations associated with CF to assess the risk to the offspring.

Not only do genetic or congenital disorders affect the male reproductive system, medical conditions acquired throughout life can affect fertility and lead to sexual dysfunction which then interferes with successful intercourse. Diabetes is one such disease; it is increasing in prevalence throughout the world, especially in children and adolescents occurring in as many as 5300 per 100,000 in some studies of adolescents [30]. This means that there is a significant population of individuals who may experience diabetes associated ejaculatory and erectile dysfunction during their reproductive years [31]. Additionally, diabetes itself may affect the production and function of sperm [32].

Transient illnesses can have a significant effect on sperm production, especially those with a fever. Patients may not take such short-lived illnesses seriously and may not initially inform the physician about them. Studies of semen analyses after acute febrile illnesses have found that these illnesses can adversely affect all aspects of sperm production and function. It typically takes at least 2–3 months for sperm parameters such as concentration, motility, and DNA fragmentation to return to normal ranges [33–35].

Genitourinary-specific illnesses can also affect fertility. Under normal conditions, the male reproductive system is typically sequestered from the immune system. Breakdown in this barrier can lead to decreased sperm functioning or even necrospermia. In a study of 60 men, the presence of leukocytes was positively correlated with markers of oxidative stress and apoptotic markers [36]. Any infections of the genitourinary system can put one at risk for this.

The most obvious infection of the genitourinary system that may affect sperm function and production is epididymoorchitis. This can affect fertility in two ways: it can both introduce leukocytes into the reproductive system, but subsequent epididymal scarring can lead to obstruction and need for reconstruction or IVF [37]. Many patients believe that any pain in the groin is epididymoorchitis, and many have actually been diagnosed with such during evaluations by different practitioners. A more convincing history includes the combined symptoms of erythema, induration, swelling, and even fever. Pain in the genital region can originate from a variety of systems: genitourinary, musculoskeletal, gastrointestinal, or neurological, and it is important to try to distinguish between these before attributing a patient's infertility to epididymoorchitis [38].

Chronic prostatitis is a more insidious inflammatory condition that also negatively affects male fertility. A recent meta-analysis of 999 patients with chronic prostatitis and 455 controls found significantly decreases in sperm concentration, progressively motile sperm and sperm morphology in those with prostatitis. While total motile sperm counts were not different, it does suggest that these chronic inflammatory conditions of the genitourinary system are important to discover and treat [39]. Chronic prostatitis can also be a cause of sexual dysfunction impairing successful

intercourse [40]. Signs of chronic prostatitis can be difficult to elucidate and include pelvic pain, dysuria, pain with defecation, pain with sitting, urinary frequency and/or urgency. For identification and evaluation of these patients, the utilization of the validated classification system, UPOINT, has been shown to be effective [41]. Rarely, a patient may have minimal to no symptoms and an analysis of expressed prostatic secretions may help find the cause of significant leukocytospermia [42].

Social History

Like many aspects of health, a patient's behavior can have a major impact on fertility. Smoking, alcohol, and illicit drugs can all impair sperm production. By one estimate, over 20 % of Americans smoke tobacco on a regular basis, a rate that has not changed significantly from 2005 to 2009. By-products of tobacco smoke are detectable within the semen and have been related to changes in sperm morphology [43]. DNA fragmentation is higher in smokers with a mean sperm DNA fragmentation of 32 % in smokers compared to 25.9 % in non-smokers in a study of 108 patients [44]. There is some evidence that even prenatal exposure may decrease sperm counts in offspring [45]. Smoking is even important among couples who choose to undergo IVF with ICSI, as there are well-demonstrated decreases in clinical pregnancy rates in those men who smoke [46].

Alcohol

Alcohol use is another commonly used drug that can impair sperm production [47]. Chronic use has been well linked to liver disease, which can alter the hormonal milieu [48, 49]. This alteration can significantly affect fertility [50]. Additionally, alcohol intake appears to affect the metabolism of essential micronutrients to sperm production such as vitamin A [51]. Even without chronic use and the subsequent sequelae, short-term increased use of alcohol has been found to reduce sperm production. In a study of 1221 Danish men, a reduction in sperm concentration, total motile count (TMC), and morphology were seen in as little as 5 drinks per week. This effect increased with higher usage as greater than 40 units of alcohol consumption weekly was found to reduce TMC by 33 %, with an associated increase in free testosterone and a reduction in sex hormone-binding globulin (SHBG) [47].

Surgical History

As discussed previously, recent illnesses and surgeries can affect sperm production thus this aspect of the history can be very important. Some surgeries interfere with sperm delivery while others affect sperm production itself.

Surgeries affecting sperm delivery include those that affect the anatomy of ejaculation, such as transurethral resection of the prostate [52]. Additionally, any pelvic surgeries that may disrupt the sympathetic innervation to the bladder neck or genitourinary system can cause retrograde ejaculation or even anejaculation [53]. Inguinal hernia repairs either as a child or adult risk vasal obstruction by direct injury to the vas at the time of surgery or dense fibrotic reaction to the surgical mesh. Over time, especially in pediatric cases, this obstruction may affect spermatogenesis itself [54, 55]. Hernia repairs can also cause direct injury to the vasculature of the involved testicles, though commonly associated with atrophy [56].

Other surgeries and conditions can directly affect sperm production; pediatric inguinal hernias are commonly associated with undescended testicles; and patients may only know about the hernia repair, but could also have had a simultaneous orchiopexy. While injury to the vas is a possible risk in this surgery, cryptorchid testes suffer decreased function, even after orchiopexy. A study of 11 adult men who had orchidopexy as children had significant decrease in metabolic activity in those testicles even 20 years later as measured by testicular (18) F-fluoro-2-deoxyglucose ((18)F-FDG)-uptake on PET scan [57]. This represents significant disruption in the number of spermatogonia which is correlated with subsequent oligospermia or even azoospermia [58]. Additionally, cryptorchidism

increases the incidence of testicular cancer, which not only can be a cause of infertility, but also needs immediate treatment in its own right. In a meta-analysis of 2281 cases and 4811 controls, cryptorchidism had a relative risk of 2.9 for the development of testis cancer [59]. While this is predominantly in the affected testicle, even contralateral testes are at risk; 21 % of testis cancers in patients with cryptorchidism occur in the contralateral testis as evidenced in a meta-analysis of 11 papers [60].

There is also some evidence that bariatric surgery can affect fertility. While hormonal parameters may greatly improve after surgery, there are case reports of development of severe oligospermia or even azoospermia [61–63]. More rarely, central nervous system surgeries, especially those of the pituitary, may lead to hypogonadotropic hypogonadism, which in turn impairs spermatogenesis due to low levels of testosterone and gonadotropins. It is important to realize that in addition to fertility implications of their surgery, these patients should also be screened for osteoporosis, as the associated hypogonadism can be severe [64].

Medications

Medications can significantly affect fertility. The effect of antineoplastic agents on sperm production has been well documented and will be discussed in more detail later. There are other medications that are well known to affect fertility besides chemotherapy drugs. These medications are either gonadotoxic, or affect the hormonal axis, indirectly interfering with sperm production. Studies on the effects of medication on sperm production are limited due to small patient numbers or questionable clinical applicability.

Many commonly prescribed medications can disrupt the hypothalamic–pituitary axis. The most obvious and most controversial currently is testosterone. Through negative feedback, exogenous testosterone decreases endogenous production of LH, follicle-stimulating hormone (FSH), and testosterone. In the testicle, high concentrations are required for spermatogenesis, much higher than serum, thus testosterone supplementation significantly lowers intratesticular testosterone and subsequently impairs sperm production [65, 66]. Testosterone prescriptions have risen over the last decade, and while they are well known to suppress endogenous testosterone production, studies have shown they are prescribed in as many as 30 % patients who are trying to obtain a pregnancy [67]. It is not uncommon to have patients referred for microsurgical sperm retrieval due to azoospermia who are found to be prescribed testosterone.

Other medications that affect testosterone metabolism are 5-alpha reductase inhibitors. In many patients, this can cause a reduction in sperm counts, but this may not be long lasting [68]. Low-dose finasteride, typically used for hair restoration, rarely causes issues, though there have been case reports of it negatively affecting spermatogenesis and so cessation should be discussed with patients [69]. Chronic opioid use has also be shown to affect male hormones with decreasing free testosterone and increasing prolactin, which may lead to a decrease in sperm production in chronic users [70, 71]. Similarly, ketoconazole, an antifungal, can significantly affect fertility with declines in sperm concentration and motility through alterations in the hormonal system [72].

Other medications have been found to have direct impairment of sperm function, though much of the evidence for these are limited to in vitro studies. The applicability to patients is therefore not entirely known. These medications include antibiotics such as co-trimoxazole, erythromycin, amoxicillin (only at high doses), tetracycline, and chloroquine [73]. Psychotropic drugs such as lithium have been shown in animal and in vitro studies to inhibit sperm motility [74, 75]. In one of the few studies on humans, paroxetine has been shown to negatively affect sperm DNA fragmentation [76].

Lastly, other commonly prescribed medications that do not affect sperm production, but instead sperm delivery, are the alpha-blockers, specifically, tamsulosin [31, 77]. In a double blind 3-way crossover study of 48 men found significant decreases in sperm concentration with

tamsulosin compared to alfuzosin and placebo [78]. In patients who are on tamsulosin and have good urinary relief, often the switch from tamsulosin to alfuzosin is enough to resolve the retrograde ejaculation but preserve the urinary efficacy.

Physical Exam

Physical exam may be one of the most underutilized aspects of the male exam, providing important insight into the functioning of the biology within. To start, male exams are best initiated in the standing position. Not only does this allow for easily accessing all parts of the exam, but also allows for evaluation of varicoceles which are much more difficult to detect in the supine position.

The first aspect of a male genital exam is overall appearance. One is evaluating for normal gross genital anatomy as well as proper development and pubertal stage. Obvious abnormalities such as sparse genital hair or abnormally small genitals may be the first indication of abnormality. While these findings are not pathognomonic for any one condition, they can give the practitioner insight into the development and maintenance of the male reproductive system. Because this system is testosterone dependent, alterations in testosterone metabolism can cause developmental issues with poor virilization as well as interfering with spermatogenesis.

In addition to the overall evaluation of the genital region, one should pay special attention to the presence of scars. Scars in the inguinal region or scrotum may indicate a previous hernia operation, hydrocelectomy, or orchiopexy as a newborn, sometimes unbeknownst to the patient. Scars indicative of laparoscopic surgery may indicate a history of orchiopexy of an abdominal testicle. History of these surgeries is important as they can affect fertility, as previously discussed. While most patients are aware of having had these surgeries, many are unaware of the exact operation performed or laterality.

Penis

The exam of the penis can uncover issues that a patient may not have ever been aware of or been comfortable sharing with a physician. Disorders of the penis can be divided into two categories, issues that prevent intercourse and issues that impair intravaginal delivery of sperm.

When examining the penis, the first aspect to examine is the meatus. In order to accomplish this, any foreskin must first be retracted. Inability to retract the foreskin, or pain while doing so can both interfere with successful intercourse. Once the foreskin has been retracted, the meatus is examined. Orthotopic placement is important. While glandular hypospadias is not felt to interfere with semen deposition, more proximal hypospadias may, especially if the meatus if located on the shaft or even more proximal [79]. Some patients with uncorrected hypospadias may have associated sexual dysfunction and should be screened for this [80]. In addition to placement, caliber of the meatus may interfere with ejaculation. Typically, patients will complain of dysuria if they have severe meatal stenosis, though patients who have had this for some time may never notice that they have a pathologic voiding pattern.

The primary objective in the exam of the penis is to uncover anatomic abnormalities that would interfere with intercourse such as Peyronie's disease. Peyronie's disease involves the development of plaques along the tunica albuginea of the corporal bodies of the penis which can cause significant angulation of the erect penis. To detect plaques of Peyronie's disease, the penis is palpated in a systematic way along the longitudinal axis. Plaques are typically on the dorsal side of the penis. If such as plaque is found, it is not necessarily indicative of a problem, but should warrant a further detailed history about curvature.

The physical exam for patients with erectile dysfunction is commonly normal. One exception is patients who have a history of trauma in which an arteriovenous shunt may lead to inability to obtain an erection. For this, vascular thrills may be detected along the penis, and even along the corporal bodies into the perineum, where such

malformations may exist as a consequence of pelvic fracture or other such trauma [81].

Testes

Approximately 80 % of testicular volume comprises cells associated with the process of spermatogenesis [82]. Of the many aspects of the physical exam, this offers us a clue as to whether patients may have significant alterations in spermatogenesis and may require a surgical sperm retrieval to obtain sperm.

There are multiple methods of measuring the testicle on physical exam. One can either measure the long axis of the testicle with a caliper, or estimate volume itself with the help of a Prader orchidometer (the orchidometer most commonly used). Each of these methods has been shown to have some intraobserver variability [83]. This has led some practitioners to rely upon testicular ultrasound to more objectively measure volume. This is considered the gold standard for measurements, but some studies have shown even this can have some level intraobserver variability [84].

Median testicular volumes are 13.9–18.9 as measured by ultrasound depending on the volume equation used [85]. Because seminiferous tubules comprise approximately 80 % of the testicular volume, they can give insight into issues with production. Testicular volumes of less than 12 cm [3] have been correlated with abnormalities in semen parameters [82]. Especially small testicles may be suggestive of congenital issues of spermatogenesis or testosterone metabolism, such as Kallmann's syndrome or Klinefelter's syndrome [24, 86]. Additionally, testicular size can be evidence of a previous vascular injury such as after inguinal hernia repair or testicular torsion [56].

Smooth and regular contour is very important to evaluate for and document in patients who are presenting for infertility. Testis tumors are known to affect fertility, and there is an increased incidence of testis cancers in the infertile male [87]. If there is any doubt in the feel of a testicle, or if there are difficulties in the exam, especially in those at high risk of malignancies (such as a history of undescended testicles), one should have a low threshold to augment their exam with an ultrasound.

In addition to the testicular exam, one needs to ensure the presence of the genitourinary ductal system. In the testicle, this means confirming the presence of an epididymis and palpating for its level of fullness. Due to differences in embryonic development, the head of the epididymis is rarely missing, but a missing midbody or tail of the epididymis can be indicative of either CBAVD (important for patients due to its relation to cystic fibrosis) or a Wolffian duct abnormality. Unilateral absence of the epididymis and vas may indicate such Wolffian duct abnormalities which are associated with lack of development of the kidney on that side (due to lack of the ureteric bud, a component of the Wolffian system) [88]. If a patient has unilateral absence of the vas deferens, renal ultrasound should be performed so that those with a solitary kidney can be appropriately counseled about avoiding injury to it.

Once the testicle has been examined, special attention should be given to the spermatic cord. This is examined by typically gently compressing it between one's fingers and allowing the contents to slowly slide through the fingers. One should pay attention to the vas deferens and the veins of the cord. In examining the vas, not only does its presence need to be confirmed, but its quality as well. In patients with previous surgery or developmental abnormalities, the vas may be present but functionally impaired due to either injury or poor development. In these cases, it may be difficult to feel and quite diminutive. Additionally, the presence of any gap in the vas or sperm granuloma is suggestive of a vasectomy. While one would hope this would be discovered during the history portion of the visit, patients may not be aware of its permanence or have forgotten this prior procedure.

Examination of the spermatic veins is performed to determine whether a varicocele is present and its severity. Varicoceles are graded on a scale from I to III based on the exam {Society For Male Reproduction 2014}. Grade III varicoceles are visible through the scrotal skin, grade II are palpable without Valsalva and grade I are only felt with Valsalva. One must be careful that when the patient performs the valsalva maneuver, the pressure they feel is not the contraction of the cremaster muscle as

this can be difficult to differentiate from a varicocele, especially in smaller varicoceles. After a practitioner has found a varicocele, one must ensure that it reduces when the patient lies in the supine position, especially if it is large or right sided. While it is felt that most varicoceles are due to the insertion of the gonadal vein into the renal vein or IVC, rarely they can be due to retroperitoneal disease processes [89]. Those due to retroperitoneal processes are less likely to reduce in the supine position due to the nature of the obstruction. Due to the higher pressure in the renal vein, where the left gonadal vein insert, and the right angle at which the gonadal vein inserts, left-sided varicoceles are much more common than right-sided ones.

The final aspect of the exam for a fertility evaluation is the prostate exam. This is primarily reserved for patients with either severe oligospermia, pyospermia, low volume ejaculate, or anyone with voiding complaints. Aside from the typical aspects of a prostate exam, one should pay special attention to signs of obstruction or infection. Midline cysts and dilated seminal vesicles can indicate obstructive processes with the prostate, either a Mullerian duct remnant or an ejaculatory duct cyst. Tenderness of the prostate may indicate an acute or chronic inflammatory process. This may be difficult to assess for though, especially in the young male who may be uncomfortable with such as exam.

Semen Analysis

Semen analysis is currently the most commonly used tool to gain insight into a man's ability to father a child. It has to be remembered that it does not directly measure many aspects of sperm function related to fertility, and this has to be taken into account when interpreting a semen analysis. One should also examine more than a single semen analysis because the variation over time and season can be substantial. A study of 12 patients who had a semen analysis monthly over the course of a year found average variations in semen concentration of 4.8 ± 4.3 fold and variations in motility of 2.8 ± 1.4 fold. Morphology had the least variation of 1.9 ± 0.4 fold [90].

The World Health Organization has set the standard for modern semen analysis interpretation. There have been previously updated parameters in 1980, 1987, 1992, and 1999 [91]. In 2010, the WHO released new guidelines for the normal values. These ranges were established based on a multi-institutional study evaluating the semen analyses for 4500 men from 14 different countries. These men had proven fertility and had previous children, with a time-to-pregnancy of less than or equal to 12 months. As there is no known upper limit of semen parameters, one-sided lower limits were established as defined by the 5 % percentile of these fertile men [91].

Despite the fact that these new criteria were based upon rigorous evaluation of a widely applicable population, it has been somewhat controversial. One of the issues many practitioners have with the new criteria is that while the time-to-pregnancy was well documented, there were no comparisons to men who were actually infertile [91]. Because this is being used to define a pathologic state, many believe that this is a critical omission [92]. This change in reference ranges is important because as many as 15.1 % of men who previously were defined as infertile now are defined as normal, which can significantly affect a patient's access to care [93, 94].

One must also caution the use of the semen analysis alone in evaluating infertile men. In a study of 473 men, when analyzing the standard criteria for semen analysis, shorter time to pregnancy was associated only with sperm concentration and TMC. The fecundity odds ratios were quite close to one for each of these parameters; TMC had a fecundity odds ratio of 1.2 (1.06–1.36); and sperm concentration was similar at 1.19 (1.2–1.34) [95]. While semen parameters do seem to be able to predict fertility and time to pregnancy, it does not appear definitive. Thus, one needs to take the entire presentation into account.

Collection

In order to ensure the most accurate semen analysis, one must have a proper collection. Specimens

must be transported to the lab at body temperature as quickly as possible, at least under 1 h and processed promptly. As is recommended with normal intercourse, lubricants should be avoided.

Prior to being asked to produce a specimen, patients should abstain from intercourse for at least 2 days, and no longer than 7 [96, 97]. When semen analyses are done less that 2 days after an ejaculation, count may be adversely affected especially with sperm concentration and overall volume where an additional day of abstinence can increase volume by 0.62 mL and concentration by 17.6 M/mL [98]. Total antioxidant capacity (TAC) and DNA fragmentation are worse in specimens made with only 1 day of abstinence [99, 100]. Conversely, when collections are made after a longer period of abstinence, the quality of the sperm may also be affected, motility has been found to be inferior in specimens made after long periods of abstinence [101]. A study of 422 patients found significantly better sperm motility on abstinence days 4–5 compared with 2–3 and 6–7. Additionally, sperm with defects of the tail were more common with 6–7 days of abstinence [102].

Volume

Volume of the ejaculate is important as a factor in the absolute number of sperm being delivered (concentration × volume), but also as a measure of other processes that may be taking place. Normal volume is currently defined as 1.5 mL [91]. As with all seminal parameters, there is no upper limit. Poor collection is a common cause of low volume, many patients will report not being able to collect the entire specimen, and an astute lab should record this in their report, but still this should be confirmed with the patient, especially in cases where the rest of the analysis is normal. As previously stated, not abstaining from ejaculation for at least 2 days can also adversely affect volume [98].

A low seminal volume is characteristic of three classifications of conditions: poor production of seminal fluid, obstruction. and retrograde ejaculation. Reduction in production can be a marker of severe hypogonadism either primary or secondary to a syndrome such as Klinefelter's syndrome [103]. It may also represent a decrease in volume that is typically seen as people age; though this is rarely severe, this reduction can be as high as 22 % [104]. If the decrease in volume is secondary to a production issue, treating the underlying hormonal condition will commonly solve the fertility issue. In cases of severe hypogonadism, this treatment focuses on increasing gonadotropins, typically done with clomiphene citrate or HCG and HMG, or more rarely GnRH infusion, all depending on the function of the pituitary [105, 106].

Other causes of lower volume include anejaculation or retrograde ejaculation. To differentiate cases of obstruction or retrograde ejaculation, one only need obtain a postejaculate urine. Many patients have small amounts of sperm in their urine after an ejaculation, but infertile patients are more likely to have greater portion of their total sperm in the urine [107]. Retrograde ejaculation can be a consequence of a general medical condition such as diabetes or other similar condition affecting the innervation of the male genitourinary system, or as a consequence of medication such as alpha-blockers [31, 77]. Treatment includes cessation of inciting medications or on-demand pseudoephedrine or imipramine, which can restore antegrade ejaculation in a significant number of patients [108].

Concentration

Sperm concentration is an important part of the semen analysis. Currently, according to the 2010 WHO guidelines for sperm concentration, the threshold of normal is 15 million sperm/mL. As previously stated, this is defined as the lower limit of the bottom fifth percentile of men with proven fertility [91]. While this is a good description of the characteristics of natural conception in the fertile male, it is less helpful in describing the infertile male as well as the role of assisted reproduction. There have been some studies attempting to determine the threshold for concentration that provides a reasonable

sensitivity and specificity, which is not easily done because of the significant overlap in these two populations.

One study by Guzick et al. evaluated the semen concentration of 765 infertile and 696 fertile men. Comparing these two groups, they found that subfertile ranges were less than 13.5 M/mL. It should be noted though that the majority of men presenting with infertility had a concentration of greater than 25 M/mL, and isolated oligospermia was associated with an odds ratio of 2.9 (2.2–3.7) [109]. In another study of 1055 men presenting to an infertility center, the median concentration for those who achieved a pregnancy was 65 M/mL compared to 25 M/mL for those who did not. A sperm concentration of less than 20 M/mL was associated with a relative risk ratio of 1.51 for not having a pregnancy [110].

One of the most helpful aspects of the concentration is determining whether a patient is azoospermic or not. In the age of IVF/ICSI, only a single sperm is required for fertilization of an egg. Cryopreservation techniques have also improved in the past decade to allow for preservation of very small numbers of sperm, and even single sperm freezing [111]. Thus, for the cancer patient looking to cryopreserve and examining fertility options in the future, the exact models to predict time to pregnancy may not be as helpful as; "does the patient produce sperm, and what will the patient require in the future to have a child".

Motility

After concentration, motility is the characteristic most valued in the prediction of fertility. The 2010 WHO guidelines set the normal level for motility at 40 %. The previously mentioned study by Guzick et al. found that subfertility was associated with a motility of less than 32 %. Like all parameters, the overlap between fertile and infertile men is significant with the majority of infertile men having sperm motility of greater than 42 % [109]. A study of 83 patients found that after varicocele ligation improvement in motility was the only predictor of pregnancy [112].

Motility is often considered in conjunction with sperm concentration and volume as the TMC. In a study of 1177 couples presenting with infertility without known female factor, of which 514 achieved a pregnancy, only TMC was associated with a spontaneous pregnancy. Men with a TMC of <1 million and 1–5 million had a significantly lower spontaneous pregnancy rate than those with a TMC of 5–10 million (OR 0.371) [113]. TMCs of less than 10 million have been found to lead to significantly lower pregnancy rates with intrauterine insemination (IUI) [114].

Morphology

While other semen parameters have only changed over time in the value of "normal," morphology has changed in its actual definition and the tools used to analyze it. The 2010 WHO semen parameters utilized the Kruger criteria for morphology [91]. This is also referred to as "strict" morphology as the parameters are more stringent and the level for normal is much lower than prior criteria. Normal is considered greater than 4 % normal forms. These criteria were developed as a tool to predict IVF success in semen analyses that were otherwise normal [115]. Initially, the normal value was 14 % as using that level demonstrated a significant correlation to IVF outcomes in a study of 96 patients [115].

Since its initial development and validation, the normal value has been moved from 14 to 4 %. This threshold is felt to be more predictive of fertility via natural conception. While it is difficult to determine the pregnancy rate with spontaneous intercourse due to variations in frequency and follow-up, it is not difficult with assisted reproductive techniques, the least invasive of which is IUI. A systematic review of IUI success predictors found that 11 of 16 papers utilized greater than 4 % as the cutoff for normal, which had a high specificity of predicting failure [116].

Seminal pH

Seminal pH is rarely clinically used except in cases of low volume azoospermia. Contributions to the semen from the various glands of the male reproductive tract have different pH values. The prostatic secretions are

acidic, and seminal vesicle secretions are typically alkaline [117–119]. This can help determine whether there is obstruction or a failure of development of one of these organs. In cases of obstruction or failure of development of the seminal vesicles, the pH is acidic, typically less than 6.8. This is commonly the case with CBAVD or ejaculatory duct obstruction.

Advanced Semen Parameters

In patients with marginally abnormal histories or physical exam findings, especially those with partners who have no demonstrable issues, advanced semen testing can help determine whether male factor may be playing a significant role in a couple's subfertility. Such advanced semen testing typically entails seminal Reactive Oxygen Species (ROS), TAC, and sperm DNA fragmentation. These have not yet been incorporated into the standard male evaluation, but are starting to gain traction as useful tools in clinical decision making. These may be especially useful in those patients with malignancies and may contribute to the understanding of the mechanism of infertility in this population. The most well known of these tests is sperm DNA fragmentation.

Sperm DNA fragmentation is commonly performed with either the COMET or TUNEL assays, depending on the lab. There is some controversy in the utilization of these additional parameters for care of the infertile male, but sperm DNA fragmentation has been shown to be highly elevated in those presenting for IVF/ICSI [120], and may be related to recurrent pregnancy loss [121]. Data on outcomes with IVF are mixed, though studies suggest patients may have worse outcomes with IVF if they have elevated sperm DNA fragmentation [122], while others have not [123]. Interestingly, this may be one of the mechanisms by which cancer suppresses fertility. Patients with testicular or systemic malignancies have significantly increased sperm DNA fragmentation as demonstrated in a study of three groups: patients with a testicular malignancy ($n=39$), non-testicular systemic malignancy ($n=50$), and controls ($n=20$) [124]. For these groups, sperm DNA fragmentation was 17.8 ± 2.2, 21 ± 3, and, 9 ± 0.9 respectively. This effect has been demonstrated in other studies as well [125].

ROS and TAC are intimately related, and are commonly associated with white blood cells penetrating into the reproductive system. Patients with leukocytospermia have elevated ROS and DNA fragmentation, even in the absence of abnormalities in standard semen parameters [126]. Additionally, antioxidants within the sperm and semen may be important for preventing lipid peroxidation by ROS, which can adversely affect membrane fluidity and sperm motility [127–130]. It is through this mechanism that antioxidant therapy is thought to improve sperm function in the face of oxidative stress as shown in a systematic review of 17 trials including 1665 patients [131].

Hormone Analysis

In conjunction with the semen analysis, laboratory blood tests give significant insight to the functioning of the male reproductive system (Table 4.2). Because of the well-defined feedback

Table 4.2 Common diagnoses and their associated hormone findings

Condition	LH	FSH	Testosterone	Estradiol
Klinefelter's syndrome	High	Very high	Low	High
Kallman's syndrome (hypogonadotropic hypogonadism)	Very low	Very low	Very low	Very low
Fertile Eunuch syndrome	Low	Normal	Low	Low
Testicular failure	High	High	Low	Low
Testosterone use	Low	Low	High or normal	High
Late onset hypogonadism	Low or normal	Low or normal	Low	Normal or high relative to T
Obesity	Normal	Normal	Low	High

system that controls both testosterone production and spermatogenesis, a few laboratory tests, especially when combined with the semen analysis and physical exam, can often let practitioners know not only the likely cause of issues, but also any potential treatments. The initial serum laboratory investigation typically includes: total testosterone, luteinizing hormone (LH), FSH, and estradiol. Depending on the results of those, some practitioners may obtain other hormones such as prolactin and thyroid-stimulating hormone. In patients with severe oligospermia, genetic analysis for karyotype and Y chromosome microdeletion is also warranted.

Testosterone is one of the core labs commonly ordered for the infertile male. When ordering testosterone, it is important that the blood sample is obtained early in the day. Testosterone exhibits a diurnal variation with the highest values in the morning, an effect that is most pronounced in men under the age of 40 [132]. Among older men, this circadian rhythm decreases, with less pronounced variation as seen in a study of 3006 men who had blood drawn throughout the day [133]. Because of this variation, normal values are set against the morning value. Thus, a serum testosterone may appear low if obtained at three or four in the afternoon, but could be normal when checked at the appropriate time. Many labs may not record the exact time when a lab is drawn and so confirmation with the patient is important. Testosterone is so important as sperm express testosterone receptors throughout their maturation which are critical for sperm development [134]. The concentration of testosterone required for spermatogenesis is several times higher than serum Thus, endogenous production is required and exogenous replacement is not sufficient for maintenance of spermatogenesis [65].

Many people question whether free testosterone is a useful test to obtain. The term free testosterone refers to the amount of testosterone that is free to react with the steroid receptors within target tissues. The vast majority of circulating testosterone is bound to albumin and SHBG and therefore unable to react. Free testosterone can be measured directly, but is commonly calculated from measurements of total testosterone. There are a variety of equations that have been developed and found to be quite accurate obviating the need for a directly measured free testosterone [135]. At this time, its use is best limited to patients with normal total testosterone and signs of hypogonadism, or in patients with borderline low total testosterone [136, 137].

LH is important is determining the cause of disorders of testosterone, and therefore should be ordered in conjunction with it. Whenever testosterone is low, the critical question is then: is this a disorder of the testicle or the hypothalamus/pituitary? Patients whose LH is low or low/normal in the face of a symptomatically low testosterone may require further investigation into pituitary dysfunction. Depending on other symptoms the patient may have, this could involve obtaining other pituitary labs (discussed later in this section), or possibly pituitary imaging.

As testicular volume was important in the physical exam for offering insight into spermatogenesis, FSH gives one understanding as to the state of sperm production. Late in spermatogenesis inhibin B is released which provides negative feedback for FSH; receptors of which are expressed on sperm throughout their production [138]. Thus, if sperm do not advance to this late stage of development, FSH levels, without the suppression of inhibin B, rise [139]. This is especially important in azoospermic patients, for differentiating between obstructive azoospermia in which FSH is normal and nonobstructive azoospermia in which FSH would be elevated [139, 140]. Ninety-six percent of men with obstructive azoospermia have an FSH of less than 7.6 or a testicular length of greater than 4.6 cm [141]. Combining FSH and the physical exam in this way, one can virtually eliminate the need for a diagnostic testicular biopsy.

Estradiol is another important hormone in the infertile male evaluation. It is produced through aromatization of testosterone, which can occur in adipose tissue [142, 143]. Because of this, it can be significantly elevated in obese patients, an issue that is becoming more common in today's society [144]. It is not merely the elevation in estradiol that has deleterious effects on sperm production, but instead the ratio of testosterone to

estradiol [144]. It has been found to be lowered in patients with abnormal semen analyses [145]. This is important diagnostically and therapeutically as it is also a potential target for therapy. The use of aromatase inhibitors to correct this ratio can greatly improve semen parameters, especially concentration [146, 147].

Due to the importance of the pituitary hormones LH and FSH in spermatogenesis, disorders of the pituitary can impair sperm production. Prolactin is the most common pituitary hormone that has been implicated in male infertility. While the exact mechanism is unknown, elevated prolactin levels have been shown to suppress GnRH, LH, and FSH release and subsequently decrease the secretion of testosterone. A mass lesion can directly interfere with the release of gonadotropins. Elevated serum prolactin may be caused by prolactin secreting tumors of the pituitary as well as a variety of medications [148]. The practitioner should be aware that there is marked variability in serum prolactin levels so an abnormal value should always be confirmed with a repeat test.

Other Laboratory Evaluations

Besides the hormonal analysis, more intensive testing is required for those with more significant seminal abnormalities. This testing includes genetic testing for abnormal karyotype, AZF microdeletion and in those with physical findings of an absent vas deferens, cystic fibrosis testing. The purpose of this genetic is to identify illnesses within the individual that might affect their medical care (e.g., Klinefelter's syndrome or cystic fibrosis), provide a prognosis for fertility outcomes (e.g., AZFa and AZFb microdeletions), and provide information to the patient about risk of inheritance to the offspring.

Genetic abnormalities, specifically karyotype abnormalities and AZF microdeletions, are rare, occurring in about 7 % of all patients presenting with infertility. They are much more common in those with azoospermia and seminal concentrations < 1 million sperm/mL. Only 4 % of these genetic abnormalities occur in patients with >1 million sperm/mL, and 1 % occur in those with >5 million/mL. Because of this, testing is rarely done for those with >5 million sperm/mL and many practitioners lower their threshold closer to 1 million/mL. As this testing can be expensive, lowering the threshold for testing has been shown to save money while missing very few patients with genetic abnormalities [149, 150].

Imaging

Imaging has become crucial to the practice of modern medicine. In the evaluation of the infertile male, few imaging tests are routinely used, usually being limited to specific clinical scenarios. The typical imaging modalities used in male fertility are ultrasound (US), magnetic resonance imaging (MRI), and more rarely computer-aided tomography (CT).

US is the main imaging tool for use in the male. US of the testicles is used to obtain accurate sizing of the testicles, evaluation for masses, and to evaluate the vasculature of the testicle. It is considered the gold standard for evaluating the testicle and should be ordered if there is any physical exam concerning for a mass. The resolution of the testicular parenchyma with US makes it the optimal modality for evaluating for testis masses.

In measuring the size of the testicle, practitioners utilize a variety of orchidometers to assist with physical exam, or an US measurement. Comparing US measurements to the Prader orchidometer, there is a higher correlation to the actual water displacement volume with US measurements, though the Prader orchidometer also gave a fairly accurate volume [151]. In fact, there is a strong correlation between Prader orchidometer measurements and US measurements, though orchidometry can overestimate the size of the testicle by as much as 5.5 cm [3]; hence, normal values used should be specific to the method of measurement [152].

Transrectal ultrasound is rarely used in the infertile male. Its role is limited to evaluating the seminal vesicles and prostate in an attempt to diagnose ejaculatory duct obstruction. Enlarged seminal vesicles associated with a large prostatic cyst suggest obstruction at the level of the prostate

while diminutive or absent seminal vesicles are pathognomonic for CBAVD [153, 154].

MRI and CT are rarely used. MRI is most useful in evaluating patients in which an anatomic or developmental abnormality is suggested by history and US is either unable to be performed, or is not diagnostic, in such conditions as Mullerian duct remnants, prostatic obstructions, non-palpable testis, or CBAVD [155, 156]. Additionally, MRI is the imaging modality of choice for evaluating for masses or developmental abnormalities in the pituitary when they are suggested by the hormonal profile [157, 158].

Conclusion

The infertility evaluation is an important part of treating those who have recently been diagnosed with cancer and those who have already undergone treatment. As treatments have extended the life span of patients with cancer, and people have been delaying when they start their families, preserving fertility through treatment and restoring it after has become a larger concern [159, 160]. Because of this, proper early evaluation for disorders of fertility is important to identify those who may require assistance for reproduction or may require more than an ejaculated seminal sample to preserve fertility.

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Fertility Assessment in the Female

5

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Definitions and Prevalence of Infertility

According to the American Society for Reproductive Medicine, "Infertility is a disease, defined by the failure to achieve a successful pregnancy after 12 months or more of appropriate, timed unprotected intercourse or therapeutic donor insemination" [1]. Therefore, in the absence of ovulatory dysfunction or other known infertility factors, couples should be counseled to delay intervention for infertility evaluation and treatment until they have been trying for at least a year as 85 % will succeed spontaneously within that time frame. Evaluation and treatment are recommended after just 6 months for women over 35 years old due to the natural age-related decline in fertility.

Data derived from the National Survey of Family Growth (NSFG) conducted between 2006 and 2010 showed that 1.5 million married women aged 15–44 were infertile (6.0 %) [2]. This data set also indicated that from 2006 to 2009, 7.4 million women between the ages of 15 and 44 (12 %), or their partners, received some level of infertility services [3]. Increasing numbers of patients sought infertility services over the past three decades. This may be explained by the effects of age on fertility as more women are delaying childbearing, as well as the greater availability of infertility services and the fact that treatment success rates have increased dramatically. Overall, approximately 75 % of couples will achieve a pregnancy with treatment [3]. In addition, there is much greater public awareness of infertility which no longer carries the social stigma it once had.

Primary Evaluation of the Infertile Female

The evaluation of the infertile women begins with a comprehensive medical history and a physical examination looking for specific signs and symptoms listed below. Elicit the duration the couple has been trying to conceive, whether each partner has established a pregnancy, together or with other partners, the pregnancy outcomes, as well as the results of prior infertility testing and treatments. Basic fertility testing is then performed to document ovulatory function

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and to assess the uterine cavity and patency of the fallopian tubes. Additional testing may be recommended based on the history and physical as well as the results of the initial tests. In addition, the male partner's medical history is also obtained at the initial consultation, and a semen analysis is performed concurrently with the woman's evaluation.

History

(a) *Obstetrical history*: To review each pregnancy outcome as well as the time required to conceive, whether pregnancy was established with the current partner as well as if they were achieved spontaneously or with treatment.
(b) *Menstrual history*: Age at menarche, cycle intervals and duration, blood loss and menstrual symptoms. Abnormal menstrual cyclicity implies ovulatory dysfunction. Intermenstrual bleeding could be a result of a submucosal myomas or endometrial polyps. Heavy menstrual flow (menorrhagia) may be due to myomas or bleeding diathesis whereas light blood flow may indicate intrauterine adhesions. Dysmenorrhea and dyspareunia are common symptoms of pelvic endometriosis [4].
(c) *Sexual history*: Frequency of sexual intercourse, sexual dysfunction, or dyspareunia and the use of lubricants as well as risky sexual behaviors or prior sexually transmitted infections.
(d) *General medical history and medication use*: It is of primary importance to ascertain whether the patient has any medical conditions that would jeopardize her health during pregnancy or with infertility treatment. Several medical conditions, and their treatment, may affect fertility. The pregnancy category of each medication should be checked and patients advised to switch to agents with a better safety profile in pregnancy if doing so will not compromise their health care. In addition, all patients should be advised to take at least a 400 μg folic acid supplement daily to reduce the risk of neural tube defects. Document the patient's blood type and immune status to rubella and varicella and test if unknown. Also, assure that the PAP smear is up to date and inquire about the management of any abnormal results.
(e) *Surgical history*: Indication and outcome, especially abdominal or pelvic surgeries. Postoperative pelvic adhesions may contribute to infertility by compromising oocyte pickup by the fallopian tubes [5]. Prior tubal surgery increases the risk of ectopic pregnancy.
(f) *Family history*: Especially inheritable medical conditions that may affect the patient such as endometriosis or breast cancer. Also, inquire about any potential genetic mutations that may affect the child. Genetic counseling and/or preimplantation genetic diagnosis should be offered if family history is positive.
(g) *Lifestyle factors* such as diet, exercise, smoking, alcohol and recreational drug use, exposure to environmental toxins and radiation should be discussed and modifications recommended.

Physical Examination

The patient's height, weight, body mass index (BMI), and blood pressure are recorded. The skin is checked for signs of androgen excess, i.e., acne and hirsutism. Be aware that hirsutism may not be apparent as most patients will shave, wax, use depilatories, electrolysis, or laser therapy to remove hair. Acanthosis nigricans, a hyperpigmented thickening of the skin, typically in the posterior nuchal region as well as in the axillary and inguinal folds, is indicative of insulin resistance. The thyroid is assessed for enlargement, nodules, or tenderness, and a breast examination is performed to elicit masses, nipple discharge, or tenderness.

On pelvic examination, the external genitalia are checked for lesions and clitoromegaly. A speculum is then inserted to rule out vaginal or cervical abnormalities or pathologic discharge. A pap smear and cultures for gonorrhea and

chlamydia should be performed as indicated. A bimanual exam is performed to assess the size, position, and mobility of the uterus; elicit cervical motion, uterine or adnexal tenderness, and the presence of an adnexal mass or pelvic nodularity.

Post-coital Test

Post-coital Test (PCT) has been used to evaluate a cervical factor in infertile couples by assessing the ability of sperm to penetrate and survive in the periovulatory cervical mucus.

However, the test lacks clinical validity as it fails to distinguish between fertile and infertile couples or influence management decisions. Therefore, it is of historic interest only [6].

Evaluation of Ovulation

Ovulatory dysfunction accounts for up to 40 % of cases of infertility [7]. Ovulatory dysfunction may manifest as irregular, infrequent, or absent menses as well as frequent or prolonged cycles. In addition, very light or heavy menstrual flow could be indicative of underlying pathology. The following methods may be used to assess ovulatory function.

(a) *Menstrual diary*

Recording the days of menses will help to establish the cycle intervals and duration. Normal menstrual cycles are from 24 to 35 days, lasting 3–7 days. Cycle intervals should not vary by more than a week. The volume of blood loss per cycle is difficult to assess by history. The presence of pre-menstrual and menstrual symptoms, also known as molimina l symptoms (including dysmenorrhea, breast tenderness, mood liability, bloating, etc.) are further evidence of ovulatory cycles. Since ovulation occurs approximately 2 weeks prior to the onset of menses (cycle day 1) and conception can occur up to 5 days prior to ovulation [8], patients are instructed to time coitus every 1–2 days for a week starting about 17 days before the expected menses based on their shortest cycle. For example, if a woman has 28–30 day cycles, ovulation would occur between cycle days 14 and 16, and they should initiate attempting to conceive by cycle day 11.

(b) *Basal body temperature (BBT)*

An increase in the BBT is due to the thermogenic effect of progesterone during the luteal phase. Patients are instructed to measure their body temperature first thing in the morning before getting out of bed. Ovulatory cycles are associated with a biphasic curve, i.e., temperatures are higher in the luteal phase, while anovulatory cycles are monophasic. However, BBT charting is inaccurate and can be difficult to interpret. Thus, ovulatory women may not demonstrate a biphasic curve [9]. Furthermore, BBT charting is unable to predict when ovulation will occur and can be a daily source of stress for patients dealing with infertility.

(c) *Urinary-luteinizing hormone (LH) evaluation*

There are many brands of over-the-counter urinary ovulation prediction kits that detect the preovulatory LH surge. A positive test result is associated with greater than 90 % probability that ovulation will occur within 24–48 h [10]. Pregnancy rates are the highest with intercourse or intrauterine insemination on the day of ovulation with high rates on the day of the LH surge as well. However, ovulation predictor kits have never been shown to improve pregnancy rates in women attempting to conceive spontaneously and many ovulatory women have difficulty detecting the surge leading to lost opportunities.

(d) *Endometrial biopsy (EMB)*

Historically, late luteal phase endometrial biopsies were performed to diagnose luteal phase deficiency, i.e., a lag in the endometrial development. The endometrium was assigned a cycle day based on the histologic features of the tissue which was then correlated with the day of the patient's menstrual cycle. The test has been abandoned as it was painful, expensive, suffered from high inter and intra-assay variability and fertile women

also had a high rate of luteal phase deficiency. EMB should only be performed when endometrial pathology such as hyperplasia or cancer is suspected.

(e) *Serum progesterone level*

Serum progesterone levels are low (<1 ng/mL) during the follicular phase. A luteal phase progesterone level greater than 3 ng/mL confirms that ovulation has occurred. While some have suggested that levels above 10 ng/mL are indicative of better luteal phase quality, the absolute level above 3 provides no additional information beyond documenting ovulation, as progesterone secretion is pulsatile and the levels fluctuate widely. There is no clinical test to diagnose luteal phase deficiency.

Anovulation

The evaluation of irregular, infrequent, or absent menses begins with a history and physical examination as outlined above and ruling out pregnancy with a urine or serum hCG. All patients should have a serum TSH and prolactin level obtained with appropriate follow-up testing and treatment if persistently abnormal. A total testosterone level may be assessed in women with virilization or severe or rapidly progressive hirsutism to exclude an ovarian or adrenal tumor. A testosterone level should also be obtained in women without signs of hyperandrogenism, such as acne and hirsutism, as elevated values would make a diagnosis of polycystic ovary syndrome (PCOS). A flow chart outlining a rational sequence of testing for abnormal ovulation is included (Fig. 5.1). Once a diagnosis is established, ovulation may be induced using the most appropriate agent. Since many patients with ovulatory dysfunction conceive within the first few cycles, further infertility testing can usually be postponed for several months.

Polycystic Ovary Syndrome

PCOS is the most common endocrine abnormality in reproductive age women, affecting up to 5–10 % [11]. Approximately 75 % of anovulation is due to PCOS. The criteria for making a diagnosis of PCOS had been modified several times over the years. The most current was established by the Androgen Excess and PCOS Society in 2006 [12]. It includes hyperandrogenism and/or hyperandrogenemia AND chronic anovulation OR polycystic ovaries on ultrasonography.

Other causes of anovulation with hyperandrogenism or hyperandrogenemia should be excluded. A 17-hydroxyprogesterone level can be considered to diagnose late onset congenital adrenal hyperplasia in women with a family history or early onset hirsutism, or those in high-risk ethnic groups such as Ashkenazi Jews, Hispanics, Mediterranean, Slavic, and Yupic Eskimos. Women with clinical signs of Cushing's syndrome should be screened with an overnight dexamethasone suppression test or 24 h urinary cortisol level.

While not required for diagnosing PCOS, 50–75 % of PCOS patients will have insulin resistance, 35 % will have impaired glucose tolerance and 10 % will be diabetic [13–16]. Therefore, women with PCOS should be evaluated with a 2 h oral glucose tolerance test, though many order a hemoglobin A1C level instead. While many tests for insulin resistance have been proposed, they are impractical or lack clinical validity and therefore, testing is not advised.

Amenorrhea

Patients with primary amenorrhea typically present in adolescence with the absence of menarche. The most common causes are androgen insensitivity syndrome, mullerian anomalies such as uterine and/or cervical agenesis, a transverse vaginal septum or imperforate hymen; and gonadal dysgenesis such as Turner's syndrome. Secondary amenorrhea is defined as cessation of menses for the period equivalent to three previous menstrual cycles or 6 months.

The prevalence of amenorrhea not related to pregnancy, lactation, or menopause is around 3–4 % [17]. In the absence of endometrial trauma from a D&C, or removal of endometrial polyps or

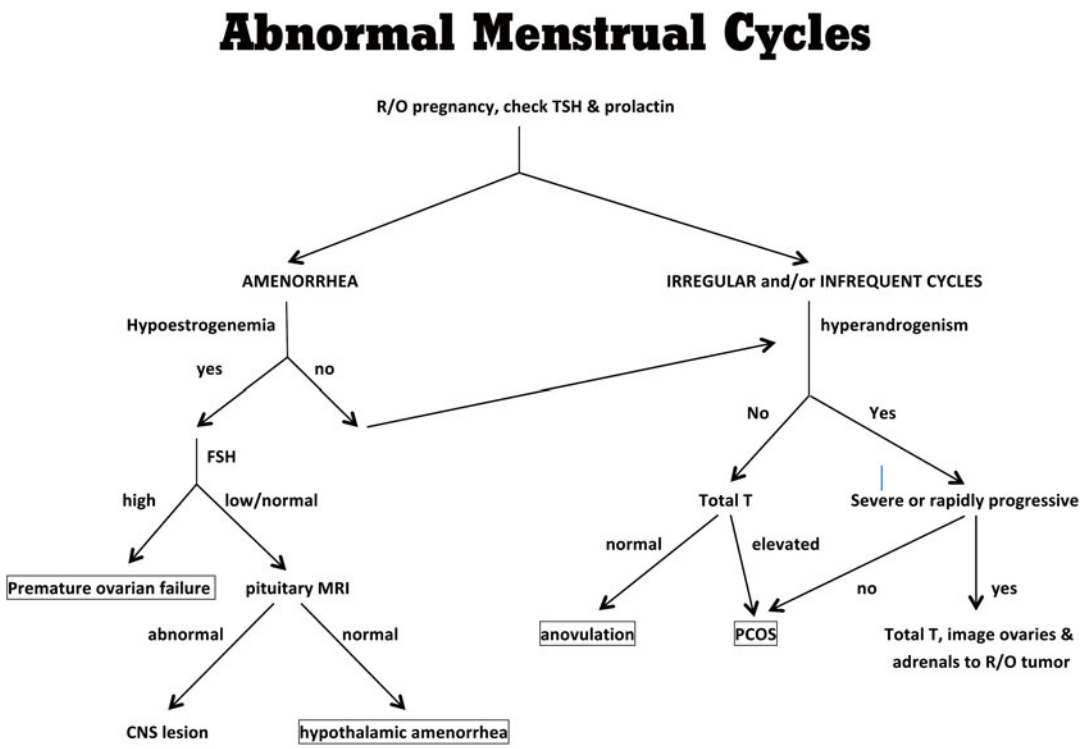

Fig. 5.1 Evaluation of abnormal menstrual cycles

submucus myomas which may lead to intrauterine adhesions (Asherman's syndrome), a uterine cause of secondary amenorrhea can be excluded based on history. Serum FSH and estradiol levels should be obtained in addition to hCG, TSH, and prolactin. A high FSH and low estradiol (hypergonadotropic hypogonadism) indicates premature ovarian failure. Since ovarian failure may be the result of an autoimmune polyendocrinopathy, antithyroid, and anti-adrenal antibodies should be obtained. Karyotypic analysis should also be performed in women under 30 year of age to rule-out a Y chromosome which would place the patient at significant risk for a gonadal malignancy requiring bilateral gonadectomy.

In women with hypoestrogenemia with a low or normal FSH, a magnetic resonance imaging (MRI) of the pituitary should be ordered to exclude a CNS lesion. Those with normal imaging have hypothalamic amenorrhea which is a diagnosis of exclusion. Often the history and physical will reveal the potential cause of the condition such as extremes of body weight, significant weight change, eating disorders, strenuous exercise, and significant stress. Amenorrhea with normal estrogen levels is due to chronic anovulation or PCOS.

Ovarian Reserve Testing

The goals of ovarian reserve testing are to determine the number and quality of the oocyte pool as well as potential for success with fertility treatments. However, testing for diminished ovarian reserve does not predict when a woman will enter the perimenopausal transition. Moreover, a diminished ovarian reserve cannot predict the inability to conceive and therefore, should not be used to deny patients access to fertility treatment with their own oocytes. Currently, the only clinical value of ovarian reserve testing is to help select the most appropriate gonadotropin stimulation protocol for In Vitro Fertilization (IVF).

The fact that there are multiple tests for assessing ovarian reserve is a clear indicator than none has emerged as the gold standard. Studies of the various tests suffer from small sample sizes and heterogeneity [18]. A systematic review of ovarian reserve testing concluded that the accuracy of predicting the ovarian response to gonadotropin stimulation was only modest while the predictability for achieving pregnancy was poor [19].

The original test for ovarian reserve was a basal FSH level in the early follicular phase. Elevated values were associated with a poorer response to gonadotropin stimulation. However, FSH has considerable intra- and inter-cycle variability, limiting its reliability. Dynamic stimulation with clomiphene citrate (The clomiphene citrate challenge test) failed to significantly improve predictability beyond the basal value. Basal estradiol and inhibin B levels are similarly compromised by high cycle variability, making them poor screening tests as well.

More recently, serum anti-mullerian hormone (AMH) has emerged as a more clinically useful test. AMH is secreted by the granulosa cells of early gonadotropin-independent follicles and thus can be measured at any time of the cycle, even on hormonal contraceptives. Low levels are predictive of poor response to gonadotropin simulation while high levels are associated with an increased risk for developing the ovarian hyperstimulation syndrome (OHSS). The antral follicle count (AFC) is another test with good consistency between cycles. It is calculated by adding the number of 2–10 mm follicles in both ovaries as measured by transvaginal ultrasonography in the early follicular phase. An AFC below 4 predicts poor response to ovarian stimulation for IVF. Conversely, a high AFC is frequently seen in high responders at risk for OHSS, including patients with the PCOS.

Anatomic Assessment

Hysterosalpingography

The gold standard for evaluating the female reproductive organs is diagnostic hysteroscopy and laparoscopy with chromotubation to document tubal patency. However, it is expensive, requires general anesthesia, and carries all of the intrinsic risks for surgical complications as well as a postoperative recovery. For these reasons, hysterosalpingography (HSG) is the first-line test in the initial work up of infertility along with documenting ovulatory status and obtaining a semen analysis. It is performed in the radiology suite by injecting an iodine-based contrast medium while observing by fluoroscopy. The procedure provides an accurate image of the uterine cavity size and shape in addition to tubal patency (Fig. 5.2). It also has a therapeutic effect with higher fecundity rates for several months afterwards.

Uterine cavity abnormalities detected by HSG include congenital anatomical abnormalities (unicoruate, bicornate, septate, or didelphys uteri) as well as adhesions, submucosal leiomyomas, and endometrial polyps (Fig. 5.3) An abnormal cavity on HSG should be further assessed with 3D ultrasonography, with or without saline enhancement, MRI or hysteroscopy depending on the suspected pathology.

The observation of contrast filling the fallopian tubes then spilling freely in the pelvis establishes that the tubes are patent. Loculation of contrast after exiting the tubes is suggestive of peritubal adhesions. Proximal tubal occlusion at the uterine cornua is usually due to spasm of the utereo-tubal ostia, especially when unilateral. True proximal anatomic blockage may be due to plugging by mucus and debris, salpingitis isthmica nodosa or fibrosis. Dilation of distally occluded tubes makes a diagnosis of hydrosalpinges (Fig. 5.4). The extent of dilation and the presence of mucosal folds help to predict whether the tube may be opened by laparoscopic neosalpingostomy or if the patient would be better served by laparoscopic salpingectomy followed by IVF.

Transvaginal Ultrasonography and Saline Hysterography

The standard two-dimensional (2D) transvaginal ultrasonogram is complementary to HSG for assessing the ovaries, the endometrium, and

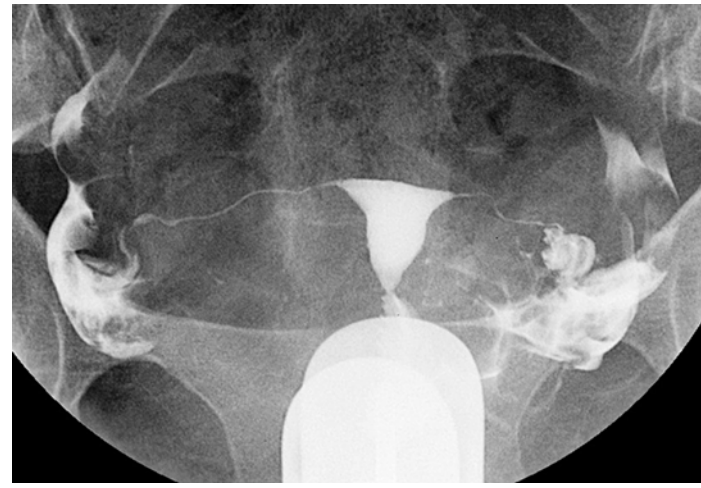

Fig. 5.2 Normal HSG

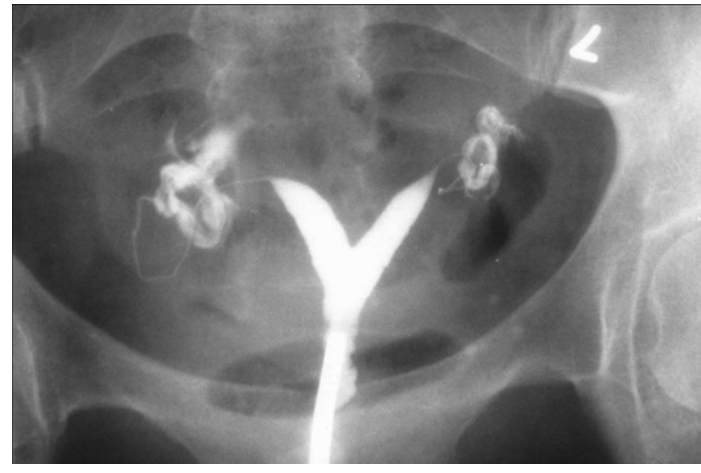

Fig. 5.3 Septate/bicornuate uterus

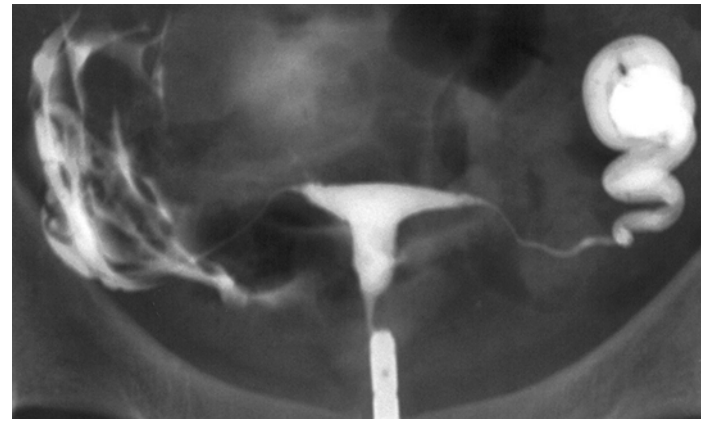

Fig. 5.4 Unilateral hydrosalpinx

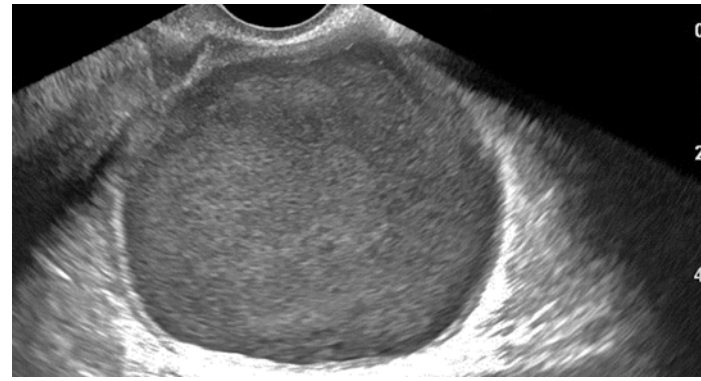

Fig. 5.5 Endometrioma on ultrasonogram

extracavitary myomas. Most ovarian cysts in reproductive age women are functional cysts of ovulation (follicular or hemorrhagic corpus luteum cysts) that resolve spontaneously over several weeks. Persistent complex cysts are usually endometriomas or benign dermoid cysts (mature cystic teratomas) (Fig. 5.5). Paratubal cysts and large hydrosalpinges may also be diagnosed by ultrasonography. An ultrasonogram performed around ovulation can assess the endometrial thickness and appearance as a crude marker for receptivity for embryo implantation. 2D ultrasonography provides images in the sagittal and coronal planes. 3D adds the frontal projection which is needed to distinguish between a septate and bicornuate uterus by showing the external myometrial contour since the cavities look similar on HSG.

Sonohysterography (SHG) is a technique in which sterile saline is injected into the uterus using a thin flexible catheter placed through the cervix. The sonolucent saline provides an acoustic window to delineate lesions within the cavity. It can differentiate between polyps and myomas (Fig. 5.6). Since it also reveals the myometrium, it can demonstrate how much of a submucosal myoma is intracavitary and how much is intramyometrial. This helps with preoperative planning as myomas that are over 50 % intracavitary may be resected hysteroscopically while those with a greater intramural component would need to be removed abdominally by laparoscopy or laparotomy. Intrauterine adhesions can also be diagnosed with SHG.

By using saline with microbubbles and a balloon-tip catheter to occlude fluid from leaking out of the cervix, tubal patency can be observed. Unlike HSG, sonohysterosalpingography, can be performed in the office and avoids the risk of allergic reactions to the contrast media as well as ionizing radiation exposure. However, it does not show the tubal architecture and will miss salpingitis isthmica nodosa. Also, it may not be associated with the higher pregnancy rates that occur for several months following an HSG.

Magnetic Resonance Imaging

Pelvic MRI is performed with and without the administration of intravenous gadolinium. It may be obtained to distinguish between a septate or bicornuate uterus noted on HSG as it provides a frontal view of the external uterine contour. However, 3D ultrasonography should be the preferred modality as the images are at least as good as MRI; it may be performed in the office, does not require IV contrast, and is less expensive. MRI is most useful for clarifying the size and location of myomas for preoperative myomectomy planning (Fig. 5.7).

Hysteroscopy

Hysteroscopy is well tolerated in the office without anesthesia by placing a small diameter rigid or flexible hysteroscope transcervically

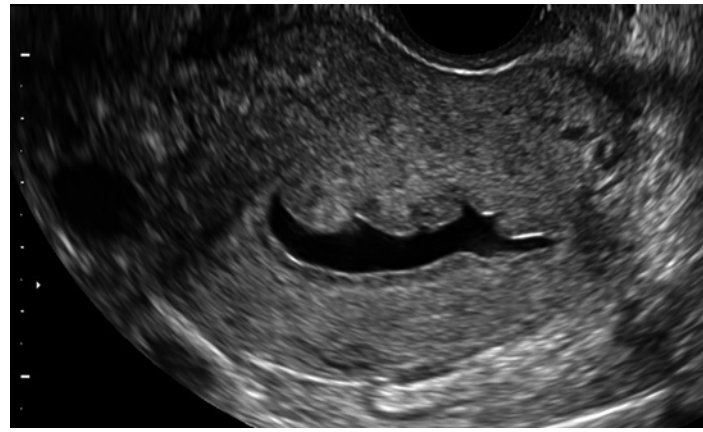

Fig. 5.6 Polyps on sonohysterogram

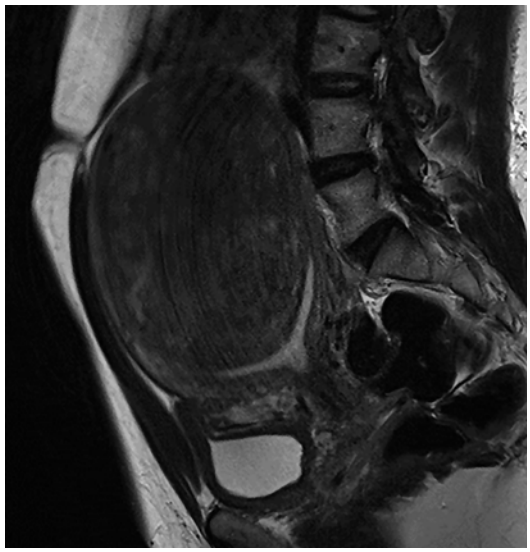

Fig. 5.7 Myoma on MRI

using saline or CO_2 to distend the cavity. It is usually performed to better diagnose and treat uterine filling defects noted on HSG. It may also be used to assess the cavity in cases where tubal status is irrelevant, such as in preparation for IVF. It can reveal a fundal indentation from a septate or bicornuate uterus but cannot distinguish between them. It can demonstrate the extent of intrauterine adhesions as well as the size, number, and location of polyps and myomas but cannot assess the intramural component of the myomas. Many of these abnormalities may also be treated in the office with the rigid scope which has an operating channel for scissors and graspers. More extensive lesions may require treatment in an operating room under general anesthesia.

Laparoscopy

While the above imaging modalities can diagnose uterine, tubal, and ovarian abnormalities that may impair fertility, only laparoscopy can diagnose endometriosis and pelvic adhesions. It should be reserved for those patients with a high probability of finding pelvic pathology. These include women with chronic pelvic pain, severe dysmenorrhea and/or dyspareunia, prior pelvic surgery, history of pelvic infection, tender nodularity on bimanual examination, or a persistent complex ovarian cyst on ultrasonography. Any pathology found at laparoscopy can be treated at the same time. In asymptomatic women with a negative history and a normal pelvic examination and imaging, the potential benefit from laparoscopy is low. If we consider that treating stage 1–2 endometriosis only marginally improves fertility and that about 30 % of women with infertility will have endometriosis, the number needed to treat to achieve 1 additional pregnancy is 44 [20].

For women with ovulatory cycles, either spontaneous or induced, evaluation for anatomic factors can be pursued using the following flow chart (Fig. 5.8).

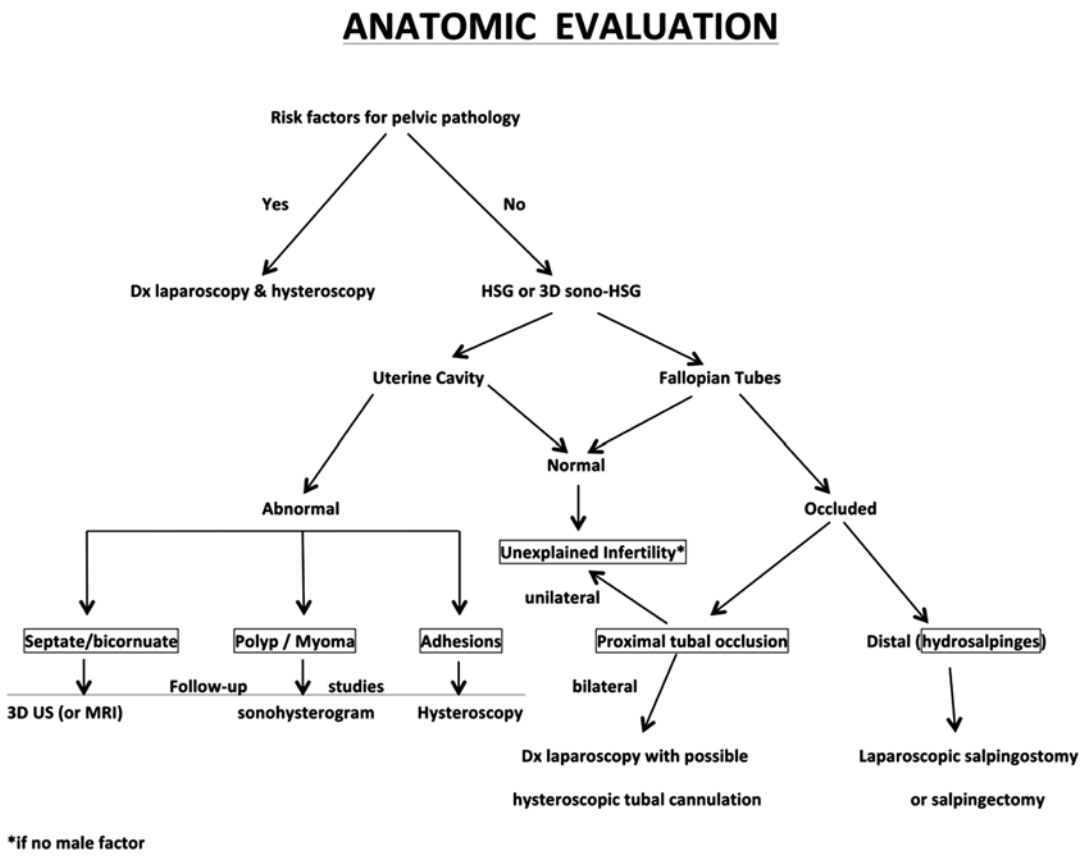

Fig. 5.8 Anatomic assessment flow chart

Conclusion

The discussion above applies to the evaluation of the female partner of an infertile couple. The approach for the woman about to undergo cancer treatment is different. The assessment of the uterine cavity and fallopian tubes is unnecessary prior to embryo, oocyte, or ovarian tissue cryopreservation. A semen analysis is only required if IVF with embryo freezing is the selected method for fertility preservation. For the man who has banked sperm prior to cancer treatment, his partner must have documentation of normal pelvic anatomy and regular ovulatory cycles, either spontaneous or induced, before thawing the samples for intrauterine insemination or IVF.

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Fertility Conditions Associated with Cancer Development

6

Kevin A. Ostrowski and Thomas J. Walsh

Introduction

After puberty, spermatogenesis requires rapid and organized cell division, the likes of which are not seen in other cell lines of the human body [1]. As a result of this rapid cell division, spermatogenesis is exquisitely sensitive to a variety of genetic, hormonal, and environmental insults. The physiology of spermatogenesis is covered in more detail in Chap. 3 of this book.

Although research is ongoing, these insults may place individuals at an increased risk for the development of systemic illnesses (cancer, cardiovascular, or endocrine disease). Due to the tremendous cell turnover, declines in spermatogenesis may be the first marker of a significant insult, long before systemic disease occurs. Recent data has found that the phenotype for certain genetic abnormalities includes both infertility (or spermatogenic failure) and cancer [2]. Unfortunately, our understanding of male infertility, from an epidemiologic standpoint, is limited by our inability to assemble cohorts of infertile men, follow their reproductive and health outcomes, and compare them to men with apparently normal fertility. As a result, little population-based data is available to establish the association between male infertility and systemic diseases. We will explore historical and contemporary data linking male infertility and cancer, with an emphasis on urologic cancers (TGCT and CaP). Further, we will suggest the work that lies ahead to further our understanding of this association and the mechanisms that underlie it.

Infertility

Infertility is defined as the failure to conceive despite 1 year of unprotected intercourse. Overall, approximately 15 % of couples will experience infertility and this prevalence rises with advancing age. Of these couples, 20 % will only have a male factor cause of infertility and male factors will contribute to an additional 30–40 % of cases [3].

With the advent of intracytoplasmic sperm injection (ICSI), pregnancy may be achieved without urologic expertise since it only takes one motile sperm per oocyte. However, clinically significant medical diseases that underlie male infertility may be missed [4–7]. In a landmark study, Honig and colleagues studied more than a thousand infertile men and identified diseases, including cancer, that required intervention in ten cases (0.8 %) [4]. Similarly, Kolettis evaluated 536 infertile men and identified 33 (6 %) with medical diseases, including both prostate and testicular cancer [8]. These findings initiated a

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new era of research focused on understanding the association between male infertility and cancer.

Testicular Germ Cell Tumors

TGCT are the most common cancer among young men in industrialized countries. The incidence of these tumors has risen over the past five decades, without evidence of plateau. The absolute risk of TGCT remains low relative to other malignancies, with the highest incidence in the world occurring in Denmark with 10 per 100,000 person-years. However, the incidence has increased in the USA from 2.5 per 100,000 person-years in 1990 to 5 per 100,000 person-years in 2007 [9, 10].

Epidemiologic Data Linking Infertility and Testicular Germ Cell Cancer

The works by Honig and Kolettis were suggestive of an association between male factor infertility and testicular cancer. However, the cross-sectional design of these uncontrolled studies failed to answer two important questions:

1. Are men with male factor infertility truly at higher risk for TGCT than men without male factor infertility?
2. Does occult TGCT necessarily precede infertility, or is it possible that impaired spermatogenesis may occur before any evidence of malignancy?

Early work suggested that infertility was a risk factor for TGCTs via the intermediary, Carcinoma-in-Situ (CIS). In 1983, Pryor and colleagues first examined the role of male infertility and CIS [11]. The investigators examined 2,043 males of infertile couples who had undergone a testicular biopsy between 1955 and 1982. Of these men, 8 were found to have CIS and 6 subsequently developed invasive TGCT suggesting that CIS may be associated with both failure of spermatogenesis and subsequent cancer. This has been expanded showing that CIS is a key transformation change leading to both impaired spermatogenesis and cancer [12, 13].

Petersen et al. confirmed that men with TGCT and CIS often have abnormal semen analysis [14]. They examined the contralateral gonad in men undergoing orchiectomy for TGCT to determine if CIS conferred global testicular dysfunction in men with TGCTs. Sperm concentration in men with CIS was 300-fold lower than those without CIS. This prompted the authors to conclude that CIS may cause poor semen quality, but they failed to determine if abnormal spermatogenesis precedes the development of CIS in select cases.

Testicular cancer has many risk factors. Dieckmann and colleagues performed a review of the evidence for risk factors in TGCTs. There was level 1 evidence for cryptorchidism increasing relative risk (RR) for TGCT. There was level 2 evidence for contralateral TGCT and infertility and level 3 evidence for familial TGCT and marijuana smoking increasing RR for TGCT [15–20]. Work by Dieckmann and others has attempted to identify men at risk who can be screened to detect and cure early TGCT.

Most of the data for infertility conferring risk for cancer was from Scandinavian countries, where national healthcare data registries track patient diseases over time [12, 14, 21, 22]. Moller and Skakkebaek presented data in 1999 from a case–control study showing that men with TGCTs fathered fewer children prior to their diagnosis of testis cancer compared to age-matched controls [22]. Men with prior paternity had 0.63 times the risk of TGCT compared to controls. Strengths of the study include that it controlled for marital status and educational level. Also, it accounted for reverse-causality (the presence of an occult testicular tumor-causing infertility) by assessing paternity 2 years prior to diagnosis of TGCT. However, studies that rely upon paternity as a measure of fertility are difficult to interpret. The underlying reason that a man is childless can be due to lack of opportunity, choice, female factor infertility, or male factor infertility.

More definitive work by Jacobsen and colleagues in 2000 confirmed the association between infertility and TGCT among 32,442 Danish men using a large, population-based cohort who presented for semen analysis [23]. In this study, evaluated men were 1.6 times (Standardized incidence ratio (SIR) 1.6, 95 % CI 1.3–1.9) more likely to develop TGCT. Furthermore, men with abnormal semen parameters were at higher risk still. This increased risk for cancer persisted up to 11 years beyond their semen testing, suggesting that abnormal semen quality may have been an early marker for cancer.

Given the high incidence of TGCT in Denmark relative to other countries (10 per 100,000), some have speculated that this data may not be generalized to US men [24]. Examination of the association between infertility and TGCT has proven difficult in the USA due to the lack of longitudinal, centralized data registries that track male reproductive health.

Several attempts to characterize this association among US men have been made. Doria-Rose and colleagues presented data in 2005 from a case–control study that found men with a history of infertility have increased the risk of TGCT. They showed that men with a prior diagnosis of infertility had an Odds Ratio (OR) of 2.4 (95 % CI 1.00–5.77) for developing TGCT. This elevated risk was not accounted for by cryptorchidism alone [25]. This data is highly suggestive but difficult to interpret given the recall bias that is likely introduced by the case–control design.

Raman and colleagues found an 18-fold (SIR 18, 95 % CI 18.0–18.8) increased risk for TGCT among men presenting for specialized infertility care in New York [26]. The rate of TGCT among men presenting for fertility evaluation was compared to the rate among an age- and geography-matched group of men in New York. While this markedly elevated risk is intriguing, it may be overestimated given the inclusion of both prevalent and incident tumors, thus not adequately accounting for reverse-causality.

The largest US-based cohort study examined more than 22,000 men who sought infertility evaluation from 1968 to 1998 at multiple infertility clinics in California [27]. Twenty-four percent of men had male factor infertility (abnormal semen analysis). Men with male factor infertility were linked to statewide cancer registries and found to be nearly three times more likely to develop TGCT compared to age- and geography-matched men (SIR 2.8; 95 % CI 1.5–4.8). Further, among cohort members, those with male factor infertility were 2.8 times more likely to develop TGCT compared to those without after controlling for age, duration of infertility and fertility clinic location (HR 2.8; 95 % CI 1.3–6.0). Similar to previous studies, seminoma was the most common histopathology and most tumors were confined to the testis at time of diagnosis. Men diagnosed with cancer within 1 year of their infertility evaluation were excluded from analysis to avoid issues of reverse-causality and the average follow-up time was more than 11 years. This data provides compelling evidence for a significant increase in the risk of TGCT among infertile US men.

Although the mechanism linking TGCT and infertility remains uncertain, current data provide strong evidence for the independent association between these two diseases in multiple countries (Table 6.1).

In the USA, large nationally based infertility registries with tissue samples able to be linked to cancer registries (e.g., SEER) will allow investigation and possible early intervention among infertile men at highest risk for TGCT. Given the evidence that male infertility confers increased risk of TGCT prior to the diagnosis of a testicular tumor, these men merit early evaluation and counseling by a urologist.

Prostate Cancer

CaP is the most common malignancy diagnosed in men and the second most common cause of cancer-related mortality in the USA. However, its etiology remains poorly understood [28, 29] and the most agreed upon risk factors remain age, family history, and race [30].

Due to persistent limitations in our understanding of the risks for CaP, efforts have been made to explore novel factors that increase risk

Table 6.1 Evidence for the association between infertility and testicular germ cell cancer [22, 23, 25–28]

Author	# Pts	Year	Country	Design	Findings
Moller et al. [22]	1,234	1999	Denmark	Case Control	– RR 1.98 (CI 1.43–2.75) – Increased RR for TGCT for men with lower than expected number of children for age
Jacobsen et al. [23]	32,422	2000	Denmark	Cohort	– SIR 1.6 (CI 1.3–1.9) – Men in couples with fertility problems increased risk for TGCT
Richiardi et al. [28]	16,846	2004	Sweden	Case Control	– OR 0.71 for men with TGCT to have fewer children – OR 0.49 for lower frequency of dizygotic twinning
Doria-Rose et al. [25]	1,001	2005	WA, USA	Case Control	– OR 2.4 (CI 1.00–5.77) – Prior diagnosis of infertility confers increased risk of TGCTs
Raman et al. [26]	3,800	2005	NY, USA	Case Control	– SIR 18.2 (CI 18.0–18.8) – Increased risk for infertile men to develop TGCT.
Walsh et al. [27]	42,274	2009	CA, USA	Cohort	– SIR 2.8 (CI 1.5–4.8) – Male factor infertility conferred a significantly higher risk of TGCT

for this disease. These risk factors include genetic variation, prostate infection and inflammation, androgen and androgen receptor variants, nutrition, and reproductive health [29–37].

Epidemiologic Data Linking Male Reproductive Health and Prostate Cancer

Fatherhood and Prostate Cancer Risk

Table 6.2 provides a summary of the studies evaluating the association between paternity and CaP. These studies were designed to explore the theory that androgen sensitivity is common to both CaP and fertility [38].

In 2002, Dennis and Dawson consolidated 18 studies of paternity and CaP [39]. The primary risk factor of interest was prior sexually transmitted infections; however, this meta-analysis did not identify a statistically significant association between the number of offspring and CaP. Giwercman and colleagues provided evidence from a cohort study of 48,850 Swedish men that childless men were 20 % less likely to develop CaP compared to men with children (ORs 0.83, 95 % CI 0.81–0.86) [40]. A smaller study by Negri of 1249 Italian men found no association between men with few or no children and CaP, but highlighted the importance of controlling for key social factors such as marital status [41]. In a variation of this, Harlap found that men with a history of fathering stillborn offspring were at higher risk of developing CaP [42].

Table 6.2 Epidemiologic studies of the association between paternity and prostate cancer

Author	# Pts	Year	Country	Design	Findings
Dennis et al. [39]	18 studies	2002	Multiple	Meta-analysis	– OR 1.01 (CI: 0.92–1.12) – No association found
Giwercman et al. [40]	48,850	2005	Sweden	Case Control	– OR 0.83 (CI: 0.81–0.86) – Reduced CaP risk in men without children
Negri et al. [41]	1294	2006	Italy	Case Control	– OR 1.10 (CI: 0.74–1.62) – No association found
Harlap et al. [42]	15,268	2007	Israel	Cohort	– RR 1.71 (CI: 1.07–2.73) – Stillbirth history increased risk of CaP
Jorgensen et al. [38]	51.6 M[a]	2008	Denmark	Cohort	– RR 0.84 (CI: 0.90–1.08) – Reduced CaP risk in men without children
Wirén et al. [43]	679,972	2013	Sweden	Case Control	– OR 0.83 (CI: 0.82–0.84) – Reduced CaP risk in men without children

[a]Million person-years of follow-up

Given these conflicting results, Jorgensen and colleagues performed the largest population-based cohort study on Danish men and reported on 51.6 million person-years of follow-up. In this study, 3,400 cases of CaP were identified and childless men had a 16 % relative reduction in CaP diagnoses (RR 0.84, 95 % CI 0.90–1.08). Interestingly, the authors found that among fathers, CaP risk was highest among those with the fewest children in a dose–response relationship, whereby with each additional child, CaP risk further decreased [38]. This was repeated in Sweden by Wirén and colleagues who after adjusting for marital status and education found a decreased risk of CaP in childless men compared to fathers with an OR of 0.83 (95 % CI: 0.82–0.84). Low-risk CaP had an adjusted OR = 0.87 (95 % CI = 0.84–0.91), whereas OR for metastatic cancer was an adjusted OR = 0.92 (95 % CI = 0.88–0.96) [43].

Eisenberg evaluated the relationship between offspring number and CaP risk among 161,823 men enrolled in the NIH—American Association of Retired Persons Diet and Health Study [44]. The study identified 8,134 cases of CaP and found that overall there was no relationship between fatherhood and incident CaP [hazard ratio (HR) 0.94, 95 % confidence interval (CI) 0.86–1.02]. After stratifying for CaP screening, unscreened childless men had a lower risk of CaP (HR 0.73, 95 % CI 0.58–0.91) compared with unscreened fathers. These data suggest a similar relationship between paternity and CaP among US men (seen in Danish and Swedish men) and emphasized the importance of assessing PSA screening when investigating CaP risk.

Offspring Gender and Prostate Cancer Risk

Table 6.3 summarizes the studies of the association between offspring gender and CaP.

In the 1980s, investigators reported on the association or lack of association between offspring gender and CaP with mixed results [45–47]. Since that time, the deletion of Y-chromosome-specific genes has been implicated in the development of CaP. In addition to their requirement for male sex determination, Y-chromosome-specific genes are a known cause of impaired spermatogenesis [48]. Harlap and colleagues hypothesized that a Y-chromosome locus might be common etiology for both the inability to sire men and CaP. The authors utilized the Jewish Perinatal Cohort Study of 38,934 men and found that an absence of male offspring conferred a 40 % increased risk (RR 1.40, 95 % CI 1.04–1.91) for CaP [49]. Among all men who developed CaP, mortality was highest among men who did not father sons. This suggests a possible association with more aggressive cancers. Bermejo and colleagues reported on more than 3.1 million men of which 120,812 developed CaP. In this larger study, the authors failed to find an association between offspring gender and CaP risk [50].

Eisenberg and colleagues reported the association between offspring gender and CaP in their study of US men [44]. Among men unscreened for CaP with PSA, the inability to father daughters conferred a weak but statistically significant increased risk for CaP.

Table 6.3 Epidemiologic studies of the association between offspring gender and prostate cancer

Author	Year	Country	Association between offspring gender and CaP
Hill et al. [46]	1985	Canada	Yes
Le Marchand et al. [45]	1986	USA	No
Spitz et al. [47]	1986	Canada	No
Harlap et al. [49]	2007	Israel	Yes
Bermejo et al. [50]	2007	Sweden	No
Eisenberg et al. [44]	2010	USA	Yes

Male Infertility and Prostate Cancer Risk

The results of studies of paternity and gender of offspring have been inconsistent. Each of these studies relied upon the number or gender of offspring in the absence of a specific fertility evaluation. A man's ability to father children is intimately related to the fertility potential of his partner, his socioeconomic status, and his personal choices. Thus, the number of children fathered may not accurately reflect their biologic fertility. A US-based cohort study of men evaluated specifically for infertility found an association between male factor infertility and CaP [51]. 22,562 men evaluated for infertility in California were linked to statewide cancer registries to determine their subsequent risk of CaP after a median follow-up of 11 years. Overall, men evaluated for infertility (not necessarily male factor) did not have increased risk of CaP relative to the general population (SIR 0.9; 95 % CI 0.8, 1.1). When CaP was stratified by grade, risk was significantly higher for men with male factor infertility who developed high-grade CaP (SIR 2.0; 95 % CI 1.2, 3.0). In multivariate analysis, men with male factor infertility had no more risk of low-grade CaP than those without male factor infertility (HR 1.5, 95 % CI 1.0, 2.3). However, men with male factor infertility were nearly three times more likely to be diagnosed with high-grade CaP (HR 2.8, 95 % CI 1.5, 5.0). This data suggests that male factor infertility may be an early and identifiable risk factor for clinically significant CaP. Further, the difference in risk between low- and high-grade cancers suggests that CaP screening alone does not account for the increased cancer risk.

Reconciling Differences in Study Findings

The compiled epidemiologic data are heterogeneous and as a result, we do not have a clear picture of how a man's reproductive health may predict his risk for CaP. Differences in the study findings arise from multiple sources. The most important difference is in the definition of both the exposure, a male reproductive event, and in the outcome, cancer.

With regard to defining the exposures of interest, the number of children a man has fathered may not be an accurate reflection of his reproductive biology. While each of these "reproductive events": paternity, offspring gender, and biologic infertility may be predictors of CaP (or protection against a future cancer) the mechanisms through which each predictor is etiologically related to cancer may not be the same. Thus, each of these factors may provide a unique window into the future prostate health of each man. The disparities in study findings may also result from differences in the assessment of outcome, CaP. Data from large observational cohorts of men diagnosed with CaP has identified a subset of men at very low risk for prostate cancer-specific mortality during their lifetime. Autopsy studies have described the occurrence of clinically indolent CaP in men dying of other causes [52–54]. Because of these aspects of CaP, it is possible that we are dealing with two distinct diseases: low-grade CaP which is indolent and high-grade CaP which is potentially life threatening. CaP risk factor studies should be aimed at identifying predictors of high-risk disease, in healthy men who are most likely to benefit from aggressive therapy. Furthermore, predictors of CaP, of which two thirds are low grade, may not be the same as the predictors of isolated high-grade CaP.

In spite of these varied findings, the association between male reproductive health in a man's fourth decade and his development of aggressive CaP in his sixth decade should not be ignored. Rather these findings, combined with the robustness of the potential common underlying mechanisms, should serve as the foundation of future longitudinal studies of male reproductive health that are more specific and directed in their approach to answering questions about the association between male reproductive failure and future systemic disease.

Mechanism Underlying Infertility and Cancer

Spermatogenesis involves rapid and organized cell division that is very sensitive to a variety of genetic, hormonal, and environmental insults [55]. These same insults may place individuals at higher risk for cancer. Because germ cell renewal and meiosis accelerates at a young age, declines in spermatogenesis seen with abnormal sperm quality may be the first marker of an insult, long before cancer is detected [56]. This section provides a brief survey of potential etiologies linking poor sperm quality and subsequent cancer. To date, some of these etiologies remain hypothetical but provide a foundation for our understanding and future research.

Mechanisms Linking Male Infertility to Testicular Cancer

Advances in molecular and computational biology as well as microarrays may yield insight into the link between male infertility and TGCT. Postulated connections linking male infertility with TGCT include Testicular Dysgenesis Syndrome (TDS), the Hiwi protein and chromosome 12, DNA mismatch repair (MMR), and Y-chromosome instability [57–59]. A full examination of the genetics and epigenetics of infertility and cancer is beyond the range of this chapter; however, there are several excellent reviews on the topic [55, 56, 60, 61]. The data clearly shows that perturbations in testis stem cell regulation and DNA fidelity can lead to both impaired spermatogenesis and TGCT. Furthermore, these perturbations may come from intrinsic genetic abnormalities (Hiwi or MMR) or external environmental factors (TDS).

Testicular Dysgenesis Syndrome

The aforementioned Danish studies have culminated in TDS initially proposed by Skakkebaek and colleagues [62–64]. TDS does not describe a biologic basis for disease. However, TDS is a theoretical construct that attempts to relate environmental modulators, genetics, and infertility in the development of testis cancer [64].

TDS theory poses that certain male reproductive disorders such as cryptorchidism, hypospadias, infertility, and TGCT may be manifestations of a fundamental alteration of gonadal development

related to environmental toxin exposure combined with an underlying genetic predisposition [65]. A common underlying exposure is suggested from the rising occurrence of these problems in industrialized countries [63–67]. This is further supported by the fact that germinal epithelium is exquisitely sensitive to cytotoxic drugs, environmental toxins, and radiation [68–70].

Central to TDS is the concept that CIS is a precursor to TGCTs and CIS is associated with aneuploidy of 12p [12]. CIS is felt to underlie a spectrum of disorders of gonadogenesis embryologically and these disorders give rise to hypospadias, cryptorchidism, and TGCTs [64]. Evidence for an environmental role in TDS and TGCTs comes from geographic distributions of rates of TGCTs, semen quality, hypospadias, and cryptorchidism. Depending on geographic region examined, these rates are uniformly either all high or all low [10, 71, 72]. Phthalates, which can induce testicular dysgenesis in utero and a TDS in rats, have been implicated as one possible environmental endocrine disruptor [73, 74].

Epigenetics and Hiwi

In 1998, Cox and colleagues cloned the Drosophila gene piwi. This is a highly conserved amino acid protein whose human homolog hiwi is found on chromosome 12 and expressed in germ line cells. This protein acts as a governor for stem cell self-renewal, gametogenesis, and RNA interference in multiple diverse organisms [75, 76].

Piwi and hiwi have been found to be essential for asymmetric division of germ line stem cells to produce and maintain daughter stem cells. However, they are not essential for further division of committed daughter cells [75, 76]. The location of hiwi (band 12q24.33) displays genetic linkage to development of TGCTs. This is particularly true for transformation of CIS to TGCT and future research could provide a biologic explanation for the link to chromosome 12 aneuploidy enabling the transition of CIS to TGCT.

Qiao and colleagues [75] stated that hiwi expression is only present in germ cells and enhanced hiwi expression was found in 12 of 19 sampled seminomatous TGCT and 0 of 19 non-seminomatous TGCT. Thus, mutations of hiwi could render men both infertile and susceptible to TGCT and explain the linkage between chromosome 12 aneuploidy and TGCT seen in TDS. Perhaps in susceptible men, environmental endocrine disruptors such as phthalates disrupt the Hiwi pathway and fuel infertility and TGCT in germ line stem cells.

DNA Mismatch Repair

The MMR system is a DNA repair mechanism which corrects mispaired bases during DNA replication errors and is crucial for maintaining DNA fidelity. The mutation rate for cancer cells deficient in these MMR proteins is increased 10^2–10^3-fold. Defective DNA repair has been associated with colon cancer, retinoblastoma, and melanoma and is suspected to play a role in certain gastric, breast, and ovarian cancers [2, 55, 56, 61, 77].

Data from mice studies suggests that mutations in genes needed for DNA repair (*PMS2, Mlh1*) lead to infertility characterized with a pattern of maturation arrest seen in testis pathology [78]. Male infertility characterized by azoospermia, germ cell maturation arrest, and Sertoli cell only syndrome may also be associated with abnormalities in DNA mismatch repair. Men with these conditions have lower numbers of viable MMR proteins and higher rates of detectable defects in recombination [59, 79–84].

Increasingly, errors in DNA mismatch repair are being linked to human cancers. Inactivation of Exonuclease 1 protein in mice, which is critical for the excision step of DNA mismatch repair, has been shown to cause both infertility and cancer [85]. Further work has confirmed these findings and confirmed the hypothesis that errors from MMR lead to errors in both the quality and frequency of chromosome pairing [86]. Thus, transcriptional mismatch repair errors in both germ line DNA and somatic cell DNA could stem from a single source and provide a biologic explanation for the link between male infertility and TGCTs.

CAG Repeats

Other etiologies for the link between TGCTs and infertility are CAG repeats in the androgen receptor (AR) and deletions and polymorphisms of the Y-chromosome [12, 61, 87, 88]. Previous studies have failed to demonstrate that longer CAG repeats in the androgen receptor gene confer a higher risk of malignancy. However, there is data that suggests that TGCTs with longer CAG repeats are more likely to metastasize [89]. AZF deletions on the Y-chromosome can cause infertility and may indicate a more global problem with MMR [57, 58]. Perhaps, longer CAG repeats when coupled with a faulty mismatch repair system provide an environment which allows embryologically aberrant CIS cells to transform into TGCTs with infertility being an early manifestation of this process. Alterations in testis stem cell regulation and DNA fidelity likely lead to infertility and TGCT. Chromosome 12, Hiwi, and environmental endocrine disruptors such as phthalates may account for the CIS to TGCT transition and possibly also confer infertility by altering germ line stem cell regulation. Intrinsic errors in MMR and possibly CAG repeats or deletions may play a critical role in conferring increased susceptibility for infertility and TGCT. Likely, the epidemiologic association can be explained by a multifactorial biological model in which multiple intrinsic genetic susceptibilities and environmental exposures confer an aggregate increase in risk for both abnormal spermatogenesis (male infertility) and TGCT.

Possible Mechanisms Underlying the Link Between Male Reproductive Health and Prostate Cancer

Testicular Dysgenesis Syndrome

As discussed previously, multiple Danish studies have culminated in the TDS [62–64]. TDS theory relates environmental modulators, genetics, hormonal function, and infertility in the development of testis cancer [64, 65]. This theory also has applicability to CaP due to the androgen sensitivity of the prostate gland. Because of abnormal gonadal function, androgen-sensitive organs such as the prostate may receive inadequate differentiating signals during critical stages of development thereby increasing their risk for malignancy [68–70].

Androgen Receptor CAG Repeats

Variations in the number of CAG repeats within the gene that codes for the AR are described in association with both male infertility and CaP. Mosaad and colleagues assessed AR CAG repeat expansion in Egyptian men evaluated for infertility and found differences between infertile and control groups. There was a negative correlation between CAG repeat length and sperm count thereby validating the concept that long stretches of CAG repeat may be associated with derangement of sperm production, presumably via decreased AR function [12, 61, 87, 88, 90]. Several studies have linked variation in CAG repeat length to clinically aggressive CaP but data associating AR CAG repeats with CaP have been inconsistent [91–95].

Prostasomes

Prostasomes are small membrane-bound vesicles produced within prostate acini that fuse with and transfer proteins to sperm affecting sperm motility and function. In the presence of prostasomes, sperm motility is increased, premature acrosome reactions are prevented, and sperm integrity is preserved during transit through the female reproductive tract. They therefore affect a couple's fertility [96]. Prostasomes are proposed as an etiologic factor in CaP, but the mechanism by which they contribute to a malignant transformation is unclear. Potential mechanisms linking prostasomes and CaP include the promotion of tumor angiogenesis, cell cycle dysregulation, and immunoprotection of malignantly transformed cells [97]. The mechanisms whereby prostasomes are associated and linked with CaP and fertility are unclear.

DNA Mismatch Repair

Similar genetic aberrations seen in infertility in MMR (described above) have also been described for CaP. Polymorphisms of the mismatch repair gene MSH3 and elevated levels of the mismatch repair protein PSM2 have been associated with CaP and with biochemical recurrence after radical prostatectomy [59, 79–86, 98, 99]. Thus, transcriptional mismatch repair errors in both germ line DNA and somatic cell DNA could stem from a single source and provide a biologic explanation for the link between male infertility and CaP.

Y-Chromosome

Abnormalities of the Y-chromosome have been proposed to underlie the association between infertility and CaP [48, 49, 57, 58, 60, 100]. The deletion of genes from the Y-chromosome is one of the most well-studied genetic causes of abnormal sperm production [101]. Y microdeletions occur in 6–8 % of severely oligozoospermic men and in 3–15 % of azoospermic men. Therefore, Y microdeletions are the most common molecularly defined cause of male infertility [102–104].

Positional cloning studies have identified most of the genes on the human Y-chromosome and have provided a resource for studying the expression of its genes in CaP. Lau and colleagues examined the expression of the Y-chromosome genes in a panel of prostate samples from men with benign prostatic hyperplasia (BPH), low- and/or high-grade carcinoma, and the prostatic cell line (LNCaP) stimulated by androgen treatment [105]. Results revealed heterogeneous and differential expression patterns of the Y-chromosome genes. This raises the possibility that some of these genes are either involved in or are affected by the oncogenic processes of the prostate. The variable regulation of several Y-chromosome genes by androgen stimulation suggests that they may play a role(s) in the hormonally stimulated proliferation of CaP cells.

Epigenetic Regulation

Epigenetics is the study of changes produced in gene expression caused by mechanisms other than changes in the underlying DNA sequence. DNA methylation and histone deacetylation are examples of this and both serve to suppress gene expression without altering the sequence of the silenced genes. These changes may remain for cell divisions during the remainder of the cell's life and may also last for multiple generations. However, there is no change in the underlying DNA sequence of the organism.

Data indicates that epigenetics may link infertility and CaP [106]. Multiple studies have reported the detrimental impact of epimutations on spermatogenesis. Rajender and colleagues reviewed the available literature and found strong evidence that epigenetic aberrations are associated with poor semen quality and may be significantly impacted by environmental factors [107].

Similar epigenetic mechanisms have been implicated in the development of multiple cancers, including CaP [1, 75, 76]. Aberrant DNA methylation (hypo- and hypermethylation) is the best-characterized alteration in CaP. This leads to genomic instability and inappropriate gene expression [108]. Global and locus-specific changes in chromatin remodeling are implicated in CaP. There is evidence suggesting a causative dysfunction of histone-modifying enzymes. MicroRNA deregulation also contributes to prostate carcinogenesis by its interference with androgen receptor signaling and apoptosis.

Importantly, environmental toxins/drugs may affect fertility and cancer risk via epigenetic modifications. This may account for the simultaneous impact of environment factors on both reproductive health and cancer risk. For example, 5-aza-2′-deoxycytidine (an anticancer agent) has been

shown to cause a decrease in global DNA methylation that leads to altered sperm morphology, decreased sperm motility, decreased fertilization capacity, and decreased embryo survival. Similarly, endocrine disruptors, such as methoxychlor (an estrogenic pesticide) and vinclozolin (an antiandrogenic fungicide) have been found in animal experiments to affect epigenetic modifications that may cause spermatogenic defects and poor prostate health in subsequent generations [109].

Environmental Exposures

While multiple environment toxicants have been implicated as causative factors for both poor spermatogenesis and CaP, perhaps the best described agents are those that mimic the effects of estrogens, the so-called phyto-estrogens and xeno-estrogens. A significant body of toxicology data suggests that exposure to certain endocrine disrupters is associated with reproductive toxicity including: [110]

1. Abnormalities of the male reproductive tract (cryptorchidism, hypospadias)
2. Reduced semen quality
3. Impaired fertility in the adult

Similarly, given the hormonal sensitivity of the prostate gland, there is increasing evidence both from epidemiology studies and animal models that specific endocrine-disrupting compounds may have impact on CaP risk. These effects may be linked to alterations with estrogen signaling and altered estrogen levels within the body. Epidemiologic evidence links increased CaP risk with specific pesticides, PCBs, and inorganic arsenic exposures. Animal studies demonstrate increased prostate carcinogenesis with several other environmental estrogenic compounds including cadmium, UV filters, and BPA. There is increased sensitivity of the prostate to these endocrine disruptors during development, such that individuals are particularly vulnerable during puberty, the neonatal period, and in utero [111]. Xu and colleagues reported that adult exposure also has impact. They found a significant association between serum levels of organochlorine pesticides and prevalent CaP [112].

Clinical Implications

Testicular Cancer

Male infertility confers a significant increased relative risk for TGCT on the order of 1.6–2.8 times that of age-matched controls [23, 25–27]. While the absolute risk of TGCT is low (US incidence is 5 per 100,000 person-years) [10], the increased RR has important implications for men evaluated for infertility. Infertile men merit a full history and physical examination by a urologist with expertise in male reproductive health because no screening test has been shown to be reliable in detecting serious underlying medical pathology such as TGCT. Future research will likely clarify the pathways that link male infertility to malignant transformation taking into account genetic predisposition and environmental exposure. Hopefully, with the elucidation of these pathways there will be evolution of appropriate tests that will identify susceptible men.

Prostate Cancer

CaP is the most common malignancy diagnosed in men and the second most common cause of cancer-related mortality in the USA. Epidemiologic data relating male infertility and CaP is mixed with relationship both to siring of children and offspring gender. Causality with respect to CaP is difficult due to an unclear etiology, but research does show some risk factors with possible mechanisms linking CaP to male infertility. There is a strong need for additional research to understand the etiology of both high- and low-grade CaP and how this is linked to possible mechanisms of male infertility and elucidate a common pathway. New research in epigenetics may allow further understanding of these mechanisms and lead to further understanding.

Conclusions

The sensitivity of male gametes may make infertility a harbinger of other medical diseases, including testicular germ cell and prostate cancer. Epidemiologic data relating male reproductive events to cancer risk is mixed but provides strong impetus for additional research. Current advances in molecular genetics and epigenetics may allow a deeper understanding of the mechanisms driving these linkages and ultimately lead to new interventions and predictive models for assessing cancer risk. The epidemiologic study of male infertility, its causes and outcomes, has been limited by difficulties in assembling large cohorts of infertile men for study and more research in this area is clearly warranted.

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Chemotherapy and Fertility

7

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Abbreviations

ABVD	Doxorubicin, bleomycin, vinblastine, dacarbazine
AFC	Antral follicle count
ALL	Acute lymphoblastic leukemia
AMH	Anti-Mullerian hormone
AML	Acute myeloid leukemia
BEACOPP	Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone
BEP	Bleomycin, etoposide, cisplatin
ChlVPP	Chlorambucil, vinblastine, procarbazine, prednisolone
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisone
COPP	Cyclophosphamide, vincristine, procarbazine, prednisone
CTX	Cyclophosphamide
DNA	Deoxyribonucleic acid
FSH	Follicle-stimulating hormone
HL	Hodgkin's lymphoma
MOGCT	Malignant ovarian germ cell tumor
MOPP	Nitrogen mustard, vincristine, procarbazine, prednisone
NHL	Non-Hodgkin's lymphoma
TGCT	Testicular germ cell tumor

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Introduction

As detection and treatment options for cancer patients improve, long-term toxicities of therapies become an important aspect of oncologic care, particularly in patients of reproductive age. Fertility preservation is exceedingly important to young patients and a discussion of options early on in the patient–physician relationship is very important. Duffy et al. reported that only 34 % of young women with breast cancer recalled a discussion with their oncologist about future fertility [15]. Some barriers to proper management of fertility concerns include incomplete knowledge of preservation options and risk by providers and shortage of specialists for referral for preservation [17]. All physicians and care providers who provide treatment to young cancer patients must be aware of toxicities associated with chemotherapy and options for fertility preservation in order to provide the best care for their patients. This chapter will cover the major classes of chemotherapy drugs and their impact on both male and

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female fertility followed by a focused review of the fertility impact of treatment for testicular germ cell tumors (TGCT), Hodgkin's lymphoma (HL), Non-Hodgkin's lymphoma (NHL), acute leukemia, and breast cancer.

Chemotherapeutic agents can reduce both male and female fertility, but the mechanism of impairment differs greatly between genders as a result of differing gonadal cell kinetics. Males have proliferating and continuously regenerating germ cells beginning at puberty whereas female germ cells proliferate in the prenatal period and arrest at the oocyte stage at the time of birth. In males, chemotherapeutic agents primarily affect the rapidly dividing spermatogonia with lesser impact on Sertoli cells and Leydig cells, the dormant cell populations of the testes [40, 41]. Irreversible azoospermia occurs when proliferating spermatogonia are unable to self-renew. Studies of gonadotoxicity of chemotherapeutic agents in male patients look at multiple endpoints including sperm count, serum inhibin B concentration, serum follicle-stimulating hormone (FSH) concentration, morphology of sperm, testicular weight/volume and most importantly, fatherhood.

Female patients exposed to chemotherapy may develop germ cell loss, subsequently causing follicular destruction. Follicular destruction then leads to inadequate estrogen production and consequent oligomenorrhea. Irreversible ovarian failure occurs if too few follicles remain to maintain menstrual cycling. Assessment of ovarian reserve is indirect in cancer survivors as compared to a semen analysis. Assessment of gonadal status in female patients involves hormonal analyses and a clinical evaluation. Different studies favor different hormones as best measure of ovarian reserve so it is more difficult in the female population to quantify fertility recovery after chemotherapy [62]. FSH, anti-Mullerian hormone (AMH), and antral follicle count (AFC) are the most sensitive predictors of ovarian reserve and are used in most studies on gonadotoxicity associated with chemotherapy in female patients [64]. The most important and clinically significant outcome for male and female patients is a successful pregnancy, but this is a long-term outcome affected by multiple confounders.

Agent-Specific Effects

Alkylating Agents

Alkylating agents induce deoxyribonucleic acid (DNA) damage by attaching an alkyl group to DNA molecules subsequently impairing DNA replication. Rapidly dividing cells, both malignant and nonmalignant, are thus most affected. Alkylating agents are not cell-cycle specific and are the most sterilizing of the chemotherapy drugs. Cyclophosphamide (CTX) and procarbazine have the highest rates of gonadotoxicity among the agents in this class. In patients who received either CTX or procarbazine, 68 % of patients were azoospermic between 1 and 20 years after completion of therapy [3]. CTX is the most well-studied alkylating agent regarding future gonadotoxicity; therefore, an algorithm was developed to calculate the CTX-equivalent dose for regimens containing other alkylating agents as a way to better predict potential gonadotoxicity [20, 21]. Another previously described metric often used to predict future fertility is the alkylating agent dose score [67].

Many authors have attempted to define a cumulative dose of CTX above which impaired spermatogenesis develops. Meistrich et al. found that permanent sterility is induced in male patients treated with a cumulative dose of CTX of greater than 7500 mg/m^2 for soft tissue sarcomas with CTX-containing regimens [39]. A subsequent study in adult male survivors of sarcoma also reported 7500 mg/m^2 as the cumulative dose above which impaired spermatogenesis was noted [28]. In contrast, Green et al. report a cumulative CTX equivalent dose of 4000 mg/m^2 as the cutoff above which impaired spermatogenesis is seen in adult male cancer survivors [20, 21]. Cumulative dose rather than dose rate appears to be the most important determinant of gonadal impairment in patients treated with alkylating agents. A universal cutoff for all patients does not exist as many cancer patients

have impaired spermatogenesis prior to initiation of treatment and other agents used may compound the effects of CTX [14].

The physiologic effects of CTX on the male reproductive system have been elucidated in animal studies. Oh *et al.* demonstrated that CTX-treated rats showed decreased testis weight, decreased epididymal sperm count, and decreased motility as compared to untreated rats [45]. A similar study in mice demonstrated decline in motility, increase in sperm head abnormality, and increase in sperm DNA damage [44].

The effect of CTX on premature ovarian failure in female patients has been well studied in patients with systemic lupus erythematosus (SLE). Pulsed CTX is given to many women with refractory SLE. CTX is metabolized into two active metabolites, phosphoramide mustard, and acrolein. Phosphoramide mustard causes follicular damage, particularly to the primordial follicles, by inducing apoptotic cell death of the oocytes and somatic granulosa cells [46, 47]. Warne et al. performed ovarian biopsies in female patients receiving CTX-based treatments for progressive glomerulonephritis or rheumatoid arthritis demonstrated abnormal follicular maturation. Only 2 of 17 patients in the cohort of women studied by Warne et al. demonstrated ova on biopsy [74]. The risk of ovarian failure in this population increases with age at which treatment is initiated as well as duration and dose of treatment [37].

The class of alkylating agents as a whole has been shown to be the most gonadotoxic but relative toxicities within this class of drugs vary greatly. Studying the individual effects on future fertility for specific drugs is difficult as most are given in combination and may have additive effects. Regimens containing a cumulative procarbazine dose above 4200 mg/m^2 decreased male patient's likelihood of siring a pregnancy as compared to regimens with lower cumulative procarbazine dose in the male childhood cancer survivor study [18, 19]. Female cancer survivors who had received lomustine or CTX showed a dose-related reduction in fertility in a similar study of female patients. As the adjusted alkylating dose increased, future fertility declined in female cancer survivors (Green at al. 2009). Busulfan has been shown to effect spermatogenesis at the early stages, primarily affecting the stem cell spermatogonia [38]. When CTX is combined with busulfan, an additional 45 % of patients showed impaired spermatogenesis as compared to CTX alone, suggesting either an additive effect or that busulfan exacerbates the gonadotoxicity caused by CTX [58]. Dacarbazine led to a transient reduction in intra-testicular testosterone and transient increase in severe oligospermia in mice testes [31]. Adult male survivors of childhood cancers who received ifosfamide as compared to CTX demonstrated lower prevalence of abnormal FSH as compared to patients treated with CTX, suggesting a lower risk of gonadal damage with ifosfamide-containing regimens. Delineating the individual effects of all drugs in this class is difficult due to the nature of cancer treatment, but existing data supports at minimum, a temporary gonadotoxic effect for all alkylating agents.

Platinum Agents

Platinum agents have a similar mechanism to alkylating agents and are often classified together as a result. The platinum agents are DNA-toxic, cell-cycle-specific agents that cause DNA cross-linking leading to impaired DNA repair and synthesis. Wallace et al. was the first to show gonadal dysfunction in survivors of child osteosarcoma who had received cisplatin and doxorubicin. Male patients demonstrated severe oligospermia and reduced testicular volumes but normal Leydig cell function. Three of the seven female patients were amenorrheic with evidence of ovarian damage [72]. The effect of cisplatin on male fertility is well studied in patients with TGCT as cisplatin is the cornerstone of medical therapy for TGCT. The gonadotoxicity of cisplatin is dose dependent and time to recovery increases as the total dose administered increases [49]. Some authors have cited 400 mg (or 4 cycles for TGCT) as the cutoff dose above which permanent sterility is observed [52]. All male patients who receive cisplatin will have temporary gonadotox-

icity with improvements to pretreatment baseline in 50 % of cases at 2 years and 80 % of cases at 5 years [51]. The treatment of malignant ovarian germ cell tumors (MOGCTs) almost always includes cisplatin-based regimens [62]. In multivariate analysis, history of receiving cisplatin-based therapy was the only statistically significant variable associated with reduced fertility. This study demonstrates that the gonadotoxicity of cisplatin-based therapy in female patients is dependent on the cumulative dose as seen with male patients [62]. Carboplatin does not carry the same risk of gonadotoxicity as cisplatin—the probability of recovery of spermatogenesis is higher in male TGCT patients treated with carboplatin as compared to cisplatin [32]. Carboplatin is used to treat stage 1 seminoma in patients who are not candidates for active surveillance however long-term survival is improved with cisplatin-based regimens for seminoma patients with stage II disease [30]. In patients with non-seminomatous germ cell tumors, relapse rates and death from disease are lower with cisplatin-based chemotherapy as compared to carboplatin-based regimens [7]. Despite reduced fertility impact of carboplatin, ultimately cancer-related outcomes drive regimen selection.

Microtubule-Targeting Agents

Vinca alkaloids are cell-cycle-specific chemotherapeutics that bind tubulin, preventing the formation of microtubules, which are necessary for cellular division. Vincristine is part of the chemotherapy regimens CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) for NHL as well as MOPP (mustargen, vincristine, procarbazine, prednisone), COPP (cyclophosphamide, vincristine, procarbazine, prednisone), and BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) for HL. All of these regimens contain an alkylating agent, which is the primary mediator of gonadotoxicity as discussed previously. Vincristine when given in combination with methotrexate caused temporary severe oligospermia and had no effect on female menstrual cycles in a small study of osteosarcoma patients [59]. A study in male mice revealed reduced testicular weights, abnormal sperm morphology, and increase in DNA damage when exposed to vincristine [13]. Vinblastine is included in ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) for the treatment of HL, a common malignancy in patients of childbearing age. ABVD causes transient gonadotoxicity, but the majority of patients recover to non-azoospermic state with rates of recovery ranging from 67 to 100 % [2, 71]. The cumulative risk of premature ovarian failure in female patients receiving ABVD was 3 % in a large cohort of HL patients [68]. Elucidating the drug-specific effects on male and female fertility for vinca alkaloids is difficult but based on existing research in hematologic malignancies, clinically significant gonadotoxicity from this drug class is unlikely.

Taxanes, another subclass of microtubule-targeting agents, are a key component in the treatment for breast cancer and gynecologic malignancies. Female rats exposed to paclitaxel demonstrate decrease number of antral follicles and an increase in follicular atresia; however, there was no difference in number of fetuses and implantations at 24 days posttreatment suggesting transient ovarian toxicity [65]. Male patients with solid tumors other than TGCT who received taxane-based chemotherapy in combination with carboplatin or gemcitabine demonstrated decreased inhibin B, elevated FSH, and decreased bilateral testicular volume ([9]). However, the impact of taxanes on human fertility remains poorly defined.

Topoisomerase I Inhibitors

Camptothecins inhibit topoisomerase I, an essential nuclear enzyme involved in DNA replication. Topotecan and Irinotecan are used clinically today primarily to treat gynecologic malignancies and colorectal cancer respectively. Very little data exists regarding the gonadotoxicity of these agents. Rat models have showed that treatment with camptothecins causes disruption of the endometrium and negatively impacts

cyclicity subsequently reducing implantation rate of embryos [33]. No human studies have replicated these results.

Topoisomerase II Inhibitors

This class of chemotherapeutics works by inhibiting topoisomerase II enzymes, which separate DNA strands for replication. Doxorubicin is a type II topoisomerase inhibitor used to treat a variety of cancer types. Female patients who had received doxorubicin-containing regimens showed increased likelihood of achieving pregnancy as compared to regimens that included an alkylating agent [18, 19]. In a study of premenopausal breast cancer patients receiving doxorubicin-based regimens, amenorrhea occurred in 33 % of patients between 30 and 39 years of age versus 96 % of patients between 40 and 49 years of age, confirming the relationship between age and risk of chemotherapy-induced amenorrhea [24]. Amenorrhea marks some degree of gonadal damage however many of these patients may show return of menstrual cycles and be able to achieve pregnancies. Male rats that received doxorubicin demonstrated decreased sperm counts and motility and increased teratospermia [54]. Limited data exists regarding gonadotoxicity of other agents in this class. A case report described amenorrhea due to mitoxantrone treatment in one patient [60]. Etoposide caused DNA damage in mouse spermatogonial cell line [34].

Antimetabolites and Antibiotics

Chemotherapeutic agents in these classes are not known to cause infertility [5, 59]. Additive effects with agents from other classes are possible.

Timing of Conception Following Chemotherapy

Upon completion of chemotherapy, patients may ask their oncologist when it is safe to try to conceive. There is a theoretical risk that chemotherapeutic agents may concentrate in the semen causing increased risk of genetic abnormalities in the embryo. This concern has not been directly answered by the literature. Klemmt and Sialli describe the concentration of various chemicals and medications in the semen in animal models and note that the concentration of most agents in the semen mirrors that of the plasma. It can thus be inferred that the drug is no longer present in the seminal fluid when it is no longer present in the plasma—this time frame can be calculated based on the half-life [29]. Hales et al. demonstrated the CTX-treated male rats transmitted the drug to females during treatment through the semen as evidenced by preimplantation loss of embryos [22]. There is also concern that treatment with chemotherapy can cause chromosomal changes in spermatozoa. Theoretically, these spermatozoa may result in early miscarriages or genetically abnormal offspring. De Mas et al. demonstrated higher rates of diploidy and disomy for chromosomes 16, 18, and XY in testicular cancer patients treated with BEP as compared to healthy controls 6–18 months following BEP [11]. A study in male rats demonstrated higher rates of DNA denaturation and strand breaks following treatment with BEP. Nine weeks following treatment, the mature spermatozoa were free of significant damage demonstrating repair may occur if a significant recovery period is granted. This group did note persistent effects on proteins in mature sperm heads at 9 weeks posttreatment suggesting all effects may not be mitigated in this time period [36]. Despite these concerns regarding long-lasting chromosomal effects of chemotherapy, Chow et al. found no difference in risk of congenital malformations in children of male cancer survivors. The risk of premature birth was also no higher [10]. Although existing data is not conclusive regarding a safe time period for conception following chemotherapy, it is reasonable to recommend that couples postpone attempting to conceive for the length of the life cycle of spermatozoa (74 days). Meistrich recommends waiting period of 6 months following completion of treatment but adds that no human studies are available to support this time period conclusively [40, 41].

Common Malignancies in Patients of Childbearing Age and Associated Chemotherapy-Related Fertility Impact

Testicular Germ Cell Tumor

TGCT is the most common solid tumor in young males with a peak incidence between the ages of 25–34. Five-year survival rates now exceed 95 % for all TGCT patients [6]. Given the age at which TGCT is commonly diagnosed and the excellent survival, future fertility is a principal concern for this patient population. Chemotherapy is an important component of the treatment of TGCT, largely for non-seminomatous germ cell tumors. The most widely used regimens are BEP (bleomycin, etoposide, cisplatin) or EP (etoposide, cisplatin). With the advent of these regimens, cure rates for TGCT became high, prompting a focus on long-term toxicity. Cisplatin is the agent most responsible for the excellent cure rate but is also known to cause gonadotoxicity as discussed previously. The gonadotoxicity and the time to recovery of spermatogenesis of cisplatin are dose dependent [49]. Pont et al. showed that men who receive cisplatin will have temporary gonadotoxicity with improvements to pretreatment baseline in 50 % of cases at 2 years and 80 % of cases at 5 years as mentioned previously in this chapter [51]. A similar study showed that among 89 TGCT patients with normospermia prior to chemotherapy, 16 % and 20 % developed oligospermia and azoospermia, respectively, at 1 year [32]. Some authors have cited 400 mg/m^2 as the dose above which irreversible azoospermia occurs [66]. A study on the male rat reproductive system revealed decreased testes and epididymal weights, decreased sperm motility and sperm counts after exposure to three cycles of BEP [4]. Studies in humans revealed decreased ejaculate volume and elevated numbers of DNA-damaged sperm in male TGCT patients post-chemotherapy [63]. Despite the known deleterious consequences of cisplatin-based therapy for TGCT patients, Huddart et al. showed that 71 % of male TGCT patients treated with chemotherapy successfully conceived [25].

A unique consideration for TGCT patients is that fertility is often impaired prior to the initiation of chemotherapy. Multiple explanations for impaired fertility prior to treatment exist including preexisting defect in germ cell lineage, history of cryptorchidism, baseline nutritional impairment, and local tumor effects [1, 63]. This patient population is at risk for impaired fertility prior to the initiation of treatment, highlighting the importance of early discussion about fertility implications of therapy and fertility preservation options as discussed in subsequent chapters.

Hodgkin's Lymphoma

Hodgkin's lymphoma has a bimodal distribution by age with a peak occurring in patients between the ages of 20 and 25 and again in late adulthood [6]. HL is treated primarily with ABVD in this era. Given that this regimen does not contain an alkylating agent or platinum agent, recovery of fertility is common in these patients. The Lymphoma group of the European Organization for Research and Treatment of Cancer reported that only 8 % of HL patients treated with non-alkylating regimens had an elevated FSH, an indirect marker for impaired spermatogenesis, at 32 months follow-up [69].

An older and less commonly utilized regimen for HL is MOPP (nitrogen mustard, vincristine, procarbazine, prednisone) which produced azoospermia in 97 % of males treated with this regimen for HL [71]. Both nitrogen mustard and procarbazine are alkylating agents with significant effect on future fertility as discussed previously. The use of ABVD instead of MOPP has markedly reduced the incidence of infertility in HL survivors without compromising cure. Children with HL in the United Kingdom are treated with alternating courses of ChlVPP (chlorambucil, vinblastine, procarbazine, prednisolone) and ABVD. Mackie et al. showed that about half of the female patients developed ovarian dysfunction after ChlVPP therapy alone, likely due to presence of chlorambucil, an alkylating agent [35]. Males were more likely to suffer gonadotoxicity after treatment for HL as

compared to females when the alkylating agent, mechlorethamine hydrochloride, was used [56]. When ABVD is used, recovery of fertility is common in this patient population. HL patients may have impaired fertility prior to treatment due to metabolic disturbances, malnutrition, fever, or hormonal down-regulation but many of these factors are transient [14]. A discussion regarding fertility preservation is crucial despite the move to ABVD from alkylating agent-based regiments.

Non-Hodgkin's Lymphoma

Non-Hodgkin's lymphoma is the fourth most common malignancy in patients between the ages of 20 and 40 [6]. CHOP is a commonly utilized regimen for the treatment of NHL. Pryzant et al. reported that 67 % of male NHL patients treated with CHOP were normospermic at 5 years posttreatment. A study in male rates showed increase in germ cell apoptosis, retained fertility but had a 50 % loss of live fetuses [70]. Female patients with NHL treated with CHOP demonstrate very low gonadal dysfunction, with 94 % of patients resuming normal menstrual cycles and 50 % of patients achieving pregnancies in their first remission [16]. Other treatment regimens and their associated toxicities are described in Table 7.1 [53].

Table 7.1 Treatment regimens used for non-Hodgkin's lymphoma and their associated gonadal toxicities

Regimen used for treatment of NHL	Male fertility impact	Female fertility impact
CHOP	67 % of men normospermic at 10.5 years post-treatment [53]	94 % of patients resumed normal menses, 50 % achieved pregnancy in first remission [16]
	Increase in germ cell apoptosis in rats [70]	
VAPEC-B	Motile sperm in 85 % of patients at 13.5 months post-treatment [55]	No data available
VACOP-B	Gonadal dysfunction in 0 of 15 patients at median follow-up of 28 months [43]	Gonadal dysfunction in 1 of 7 female patients at median follow-up of 28 months [43]
MACOP-B	Gonadal dysfunction in 0 of 15 patients at median follow-up of 28 months [43]	Gonadal dysfunction in 1 of 7 female patients at median follow-up of 28 months [43]
VEEP	Normal gonadal function in 92 % of patients [23]	Normal gonadal function in 100 % of patients [23]

CHOP cyclophosphamide, doxorubicin, vincristine, prednisolone, *VAPEC-B* vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, bleomycin, *VACOP-B* vinblastine, doxorubicin, prednisolone, vincristine, cyclophosphamide, bleomycin, *MACOP-B* mustine, doxorubicin, prednisolone, vincristine, cyclophosphamide, bleomycin, *VEEP* vincristine, etoposide, epirubicin, prednisolone

Acute Leukemia

Acute lymphoblastic leukemia (ALL) is the most common cancer of childhood and represents 6 % of cancers diagnosed in adults between the ages of 15–29. ALL affects males more commonly than females [6]. Treatment regimens involve the use of vincristine, corticosteroid, and an anthracycline. Some patients may also receive CTX, L-asparaginase, etoposide, methotrexate, or cytarabine. As with other malignancies, the use of CTX is the main determinant of future fertility in these patients. A study of 77 male long-term survivors of childhood ALL revealed that patients treated without CTX or testicular radiation had normal endocrine function. Semen analyses did not differ between survivors and controls when treated with a cumulative dose of 10 g/m^2 of CTX or less. Statistically significantly fewer survivors (14 %) compared to controls (43 %) fathered a child, with zero survivors who had received greater than 20 g/m^2 of cumulative CTX or testicular irradiation having fathered a child [26].

Acute myeloid leukemia (AML) is more common than ALL in adult patients of childbearing age [6]. Approximately 7 % of adult patients diagnosed with AML are of childbearing age, with 55 % surviving long term [12]. Cytarabine and anthracyclines are most commonly used to treat AML and are not known to be gonadotoxic.

Patients who go on to have hematopoietic stem cell transplantation generally have very poor fertility outcomes [75]. A study of Nordic survivors of AML revealed that 31 % of females and 9 % of males reported pregnancies at median follow-up of 11 years—these numbers were comparable to the pregnancies rates in their siblings who acted as the control group [42]. In general, AML survivors treated with chemotherapy alone generally retain fertility potential but the need for hematopoietic stem cell transplantation dramatically reducesfertility potential.

Breast Cancer

Breast cancer is the most common malignancy diagnosed in women. Approximately 20–25 % of breast cancers are diagnosed in women of reproductive age [27]. Women in this age group are often treated with adjuvant chemotherapy and/or hormonal therapy, as they have a worse prognosis than patients diagnosed later in life [48]. After treatment with chemotherapy, breast cancer patients suffer from amenorrhea at varied rates depending on the chemotherapy regimen used. Higher rates of amenorrhea are seen in patients older than 40 years of age [5]. The most commonly used chemotherapeutic agents in the treatment of breast cancer in the adjuvant setting are docetaxel, doxorubicin, CTX, and paclitaxel. Tamoxifen and trastuzumab are hormonal agents also used commonly in the treatment of breast cancer in premenopausal women. In premenopausal women treated with paclitaxel and trastuzumab, Ruddy et al. reported amenorrhea in 28 % of patients at median follow-up of 51 months [57]. For women less than 30 years of age, premature ovarian failure is uncommon. Women less than 40 years of age treated with 4 cycles of doxorubicin and CTX developed chemotherapy-related amenorrhea 10–15 % of the time [24]. The risk of premature ovarian failure rises with the use of CTX, epirubicin, and 5-fluorouracil, with 40 % of women less than 40 years of age experiencing premature menopause [8]. In general, data regarding the impact of tamoxifen and trastuzumab on chemotherapy-related amenorrhea is conflicting. In addition, a uniform definition of chemotherapy-related amenorrhea and premature ovarian failure does not exist across studies therefore predictions based on literature are difficult. Older age and the use of alkylating agents are consistent risk factors for chemotherapy-related amenorrhea across all studies [73]. Chemotherapy-related amenorrhea may not be permanent with one study showing that menses may resume 2 or 3 years posttreatment [50]. Although chemotherapy-related amenorrhea is the endpoint most commonly cited in studies of fertility after breast cancer treatment, transient loss of menses does not render a patient infertile. Further complicating the fertility issues surrounding breast cancer treatment lies in the idea that ovarian suppression induced by chemotherapy may have a therapeutic benefit in patients with hormone-sensitive disease [73]. There is consensus that the risk of chemotherapy-related amenorrhea increases with age, number of cycles, and the use of alkylating agents.

Offspring of Cancer Survivors

A theoretical risk of congenital anomalies and malignancy in offspring of cancer survivors exists based on the knowledge that cytotoxic therapies cause germ line mutations and DNA damage. The use of assisted reproductive techniques in this patient population also eliminates the natural selection process inherent to spontaneous conception. A retrospective cohort study within the Childhood Cancer Survivor Study found no association between treatment with alkylating agents and the presence of congenital anomaly in offspring. Similarly, testicular and ovarian radiation dose did not incur a higher risk of congenital anomalies in offspring of cancer survivors [61]. These results have been replicated in other studies, confirming that cancer survivors treated with radiotherapy and gonadotoxic chemotherapy regimens can safely conceive following treatment [76]. As discussed previously, the duration of time posttreatment after which it is safe to conceive has not been fully elucidated but a minimum of 6 months is often quoted.

Conclusion and Summary

The use of chemotherapeutic agents in patients with future childbearing potential requires a discussion of specific risks related to their fertility. It is well established that alkylating agents, particularly CTX and procarbazine, are the most gonadotoxic, followed by cisplatin, in both males and females. Determining the gonadotoxicity of individual chemotherapeutic agents is challenging as drugs are most commonly given in combination. As treatment regimens evolve, there is a time lag before the fertility impact can be well studied so predictions may need to be extrapolated from existing data at the risk of inaccuracy. Further confounding the determination of fertility impact lies in the fact that a patient's age and their malignancy may impair gonadal function prior to the initiation of chemotherapy. The most important marker of a survivor's fertility is achieving a pregnancy and subsequent live birth but these are late endpoints that are subject to confounding, as there are so many factors that contribute to achieving a pregnancy and ultimately having a live birth. Understanding the physiologic effects on the reproductive organs comes mainly from animal studies for many chemotherapeutics. It is imperative that care providers discuss future fertility potential and the available options for fertility preservation with their patients prior to initiating treatment.

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Radiation Therapy and Fertility

8

Jay P. Ciezki

Introduction

The therapeutic use of radiation was postulated within a few years of its discovery [1]. The therapeutic application of radiation has continued to this day and the patients who have been treated with it comprise the largest group at risk for infertility after radiation exposure. There are, of course, those who are the victims of accidental or wartime exposure, but their numbers are much smaller (and one hopes will remain so). Regardless, the lessons learned from the study of patients exposed in all instances provide data upon which one may draw to counsel patients about the effect of radiation on fertility. A full discussion of the ethical concerns with the methods suggested for fertility preservation are, of course, beyond the scope of this chapter but will be mentioned as appropriate for special circumstances.

Cancer Survivors

The high survival rate for patients treated for childhood and other cancers creates a significant group of patients with long-term sequelae from therapeutic radiation. The cancer treatment is usually multimodality in nature with radiotherapy being one of the modalities. Because radiotherapy is rarely used alone, it is difficult to get a picture of the pure effect of radiation on fertility of the patients who survive to reproductive age. The pure effect of radiotherapy may be of interest scientifically, but has little practical value because of the realities of cancer care. The reviews of these patients' experience after treatment, therefore, tend to have applicability because they are drawn from a population treated with multiple modalities that typically involve surgery, radiation, and chemotherapy. Fertility may be affected by damage to any part of the hypothalamic–pituitary–gonadal axis as well as the age at which this damage occurred. This portion of the chapter will focus on the effect of radiation with the understanding that it is impossible to completely separate the effect of each modality.

Male Cancer Survivors

Males treated for cancer express some of the effects on fertility in an anatomic-specific manner, others in an age-specific manner, and still other effects in a non-age-specific manner. Central nervous system malignancies often receive radiation to the brain. For those males getting radiotherapy to the pituitary region, 60 % will have

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some deficiency in gonadotropin levels while those getting radiotherapy to other regions of the brain show a 20 % rate of gonadotropin deficiency [2]. In long-term follow-up, the incidence of clinically significant gonadotropin deficiency in these patients is 20–50 % regardless of whether the radiation is administered in childhood or adulthood [2]. In a similar age non-specific manner, the dose of radiation to the testes, given with standard chemotherapy regimens, that will induce permanent azoospermia is similar for both boys and men [3]. In contrast, the degree of Leydig cell dysfunction is highly age dependent. Prepubertal males will express Leydig cell dysfunction with testicular radiation doses of about 20 Gy while this threshold is higher for men. Taking the effects of radiation on both spermatogenic and Leydig cells into consideration, the likelihood of fathering a child successfully decreases after a dose of about 7.5 Gy to the testes; however, this effect may be intermittent [4, 5].

The management of the known effects of radiation on fertility depends on the age and anatomic site at which exposure occurs. For those patients with hypogonadotropic hypogonadism, treatment with follicle-stimulating hormone, hCG (analog to luteinizing hormone), or gonadotropin-releasing hormone is appropriate [6]. For those with gonadal damage, the management is highly age dependent. Patients treated with radiation at a postpubertal age may be effectively managed with sperm banking prior to therapy [7] while those exposed at a prepubertal age have few options aside from experimental approaches involving cryopreservation of spermatogenic tissue.

Female Cancer Survivors

Female survivors of cancer suffer effects to the hypothalamic–pituitary–gonadal axis in a manner similar to males, but have the additional burden of potential damage to the uterus and other pelvic organs necessary to carry a pregnancy successfully. For prepubertal girls who receive radiation to the brain to a dose of 18–24 Gy in combination with chemotherapy, there are effects on their endocrinologic function in early adulthood. The data is sparse, but there is documentation of lower luteinizing hormone production and shorter luteal phases despite having achieved menarche and sexual development [8]. It is expected that there may be reproductive capacity in such patients, but that it would be compromised to some degree. This degree of compromise has yet to be fully documented. Similar to the spermatogenic cells, oogenic cells and oocytes are also extremely sensitive to radiation. Mathematical models have suggested that doses as low as 2 Gy may destroy 50 % of oogenic cells [9]. Uterine dysfunction after radiotherapy is closely tied to the age at which exposure occurred and may occur with doses as low as 14 Gy with younger patients being more susceptible to damage [10]. The dysfunction is related to changes in uterine size and physiology such as blood flow and myometrial fibrosis that leads to problems with carrying a pregnancy successfully [11].

Management options for female fertility preservation are variable [12]. Such options include ovariopexy to minimize dose to the ovaries by placing them out of the planned radiotherapy field, cryopreservation of oocytes and embryos, heterotopic transplantation and stimulation of previously cryopreserved ovaries, etc. The success rate is as variable as the number of options that are available. For patients with hypothalamic–pituitary–gonadal dysfunction, hormonal replacement therapy is required. A recently published trial of such regimens has shown that improvements are being made in this field with measurable positive effects on uterine anatomy, but the effect on pregnancy has not been examined [13]. Options for fertility preservation will be discussed in more detail in subsequent chapters.

Guidelines

Both the American Society for Reproductive Medicine (ASRM) and the American Society of Clinical Oncology (ASCO) have issued guidelines for fertility preservation to be used during cancer treatment [14, 15]. In each case, the recommendations are presented quite differently. ASCO has included suggestions for initiating a

discussion of fertility with patients and their families as well as more objective information. ASRM presents its opinions according to different clinical scenarios with an emphasis on the role of the fertility specialist. Of note, ASCO specifically recommends a referral to a fertility specialist and they state that the data do not support claims of delay in cancer treatment initiation because of a referral to a fertility specialist. This section of the chapter will present guidelines that borrow from each of the published professional society's opinions grouped according to a patient's sex and age.

Males

Reproductive Age

Options for males of reproductive age are the least controversial. Essentially, cryopreservation of spermatozoa is the method of choice. The spermatozoa may be sourced from ejaculation or surgical retrieval. It is important for the specimen for cryopreservation to be obtained prior to cancer therapy. Table 8.1 summarizes the recommendations of both professional societies in this patient group.

If circumstances do not allow for sperm banking, there is a potential for viable sperm post-therapy. Ignoring the effect of chemotherapy, protection of the testes during radiotherapy can reduce the dose to the testes significantly. Various devices have been tested, but the typical result is that the protection device results in the dose to the testes being about 1 % of the dose at mid-plane [16, 17]. This would typically result in a dose that is under the 7.5 Gy threshold mentioned earlier regarding fertility preservation, but these data were generated using older techniques that have a lower integral dose than modern intensity-modulated radiotherapy (IMRT) techniques. Importantly, for one to use these data, the radiotherapy technique chosen should be a non-IMRT method. Should a patient want to sire offspring after radiotherapy, there are no good data to use for guidance. The European Society for Medical Oncology has issued a statement recommending that a period of at least 1 year elapse after therapy before pregnancy is attempted [18]. In contrast, various investigators studying aneuploidy in sperm after radiation ± chemotherapy note that the aneuploidy rate does not decrease to pretreatment baseline levels until 18–24 months after the completion of cancer therapy [19].

Prepubertal

Managing fertility, or more precisely, future fertility, is very difficult for prepubertal patients. Regardless of the method chosen, the patient cannot give consent so the importance of counseling the entire family or guardian system responsible for the patient adds another level of complexity. Informed consent of the minor is not possible, but the patient may assent to a fertility preservation method if sufficiently mature to understand the nature of the experimental protocol. Unfortunately, the only methods available are experimental. Specifically, cryopreservation of testicular tissue obtained prior to radiotherapy. Because of the experimental nature of these

Table 8.1 Fertility preservation for males of reproductive age

Option	ASCO[a]	ASRM[b]
Cryopreservation of spermatozoa	Discussed as only established method of male fertility preservation; no detail about methodology	Note that this is the only established method of male fertility preservation; discusses the details of obtaining a viable sample for preservation
Hormonal therapy	Not recommended	Not discussed
Cryopreservation of testicular tissue	Experimental	Experimental

[a]American Society of Clinical Oncology

[b]American Society of Reproductive Medicine

methods, the patient's family and/or guardian must be involved to provide consent.

Females

Reproductive Age

Females, in general, have more options available for fertility preservation than males. Several methods with proven track records exist. A summary of these as well as some experimental options are presented in Table 8.2.

Prepubertal

As in boys, girls have few options for eventual fertility after radiotherapy. Again, the girl's family and/or guardian will need to be involved to give consent even if the girl can give assent. Currently, ovarian tissue cryopreservation is experimental but is the only way known for these girls to eventually have biologic offspring. The experimental nature of these procedures demands that they be carried out under the auspices of an Institutional Review Board (IRB). The problems associated with these techniques are being overcome and there have been successes reported [20]. Rarely, despite the damage done to the female reproductive system from cancer treatment at a young age, a spontaneous conception and successful full-term pregnancy can occur [21].

Mutagenesis

The risks associated with reproduction after radiation are not robust. In the examination of women who have spontaneously conceived after chemotherapy treatment, no significant increase in congenital abnormalities of the offspring was reported [22]. One assumes that some of these women, as girls, may have received radiation as part of their therapy. Environmental studies performed recently on radon exposure back up some of the poorly documented data from atomic bomb survivors that there is a potential impact of radiation on the production of mutations in offspring of those with prior radiation exposure [23]. A variety of methods exist, such as fluorescent in-situ hybridization (FISH), that can be employed to test the viability and potential for mutagenesis, but there are almost no clinical correlations between these tests and pregnancy outcome [19]. It is known, as stated earlier, that aneuploidy rates normalize after about 18–24 months post-therapy. Whether this is associated with healthy offspring is unknown.

Conclusion

The deleterious effect of radiation on human fertility is known to exist. It can reduce a patient's chances of conception and potentially cause mutations in any offspring eventually produced. Because of these facts, management revolves around preservation of tissue prior to exposure to radiation to

Table 8.2 Fertility preservation for females of reproductive age

Option	ASCO[a]	ASRM[b]
Cryopreservation of unfertilized oocytes	Recommended; does not require a male partner and bypasses ethical concerns about other methods	Recommended; while once experimental, newer techniques have enhanced the fertilization rate
Cryopreservation of embryos	The most established method, but some women may have ethical concerns that limit its use	Longest track record of success, but is limited by ethical concerns
Cryopreservation of ovarian cortical tissue	Experimental	Experimental with potential for re-implanting cancer cells when the tissue is implanted for reproductive use

[a]American Society of Clinical Oncology

[b]American Society of Reproductive Medicine

both maximize the availability of the tissue and minimize the potential for congenital defects in the offspring. The methodology employed can be both standard and experimental. Ethical considerations come into play with all minors whose parents/guardians wish to preserve the ability of the minor to eventually reproduce over and above those confronting adult patients from ethnoreligious backgrounds with objections to some of the procedures used to preserve fertility.

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Sperm Banking for Cancer Patients

9

Ashok Agarwal and Rakesh Sharma

Introduction

Although cancer is considered a disease of the elderly, approximately 9 % of men diagnosed with cancer are 44 years or younger [46]. The average age among patients with testicular cancer in one study was 29.9 years [134]. Survival rates exceeding 95 % have been reported for early-stage disease in reproductive-age males including testicular cancer and Hodgkin's disease [54, 105]. Long-term survival and cure rates for testicular germ cell tumors (TGCT) are excellent as well. However, the treatments for these cancers (chemotherapy, radiation therapy) can lead to temporary or permanent infertility. Therefore, the impact of cancer therapy on fertility is an important quality of life issue for these men and their partners [73]. Many cancer patients are young and single and will desire to have biological children in the future.

The American Society of Clinical Oncology (ASCO) advocates sperm cryopreservation as an effective method of fertility preservation in young men with cancer [70]. However, there is paucity in the number of patients utilizing sperm banking options for a variety of reasons. In this chapter, we describe the incidence of cancer among adults and adolescents seeking fertility preservation before treatment, sperm banking techniques, and the challenges that limit the use of this technology among cancer survivors.

Common Male Cancers and Treatment Effects

The most common cancers that affect males of reproductive age are testicular cancer, Hodgkin's disease, acute leukemia, non-Hodgkin's lymphoma, and soft tissue tumors such as sarcomas [5, 7, 39, 51, 77, 90, 99]. Male infertility and testicular cancer may coexist in males with undescended testes and those with a history of in utero exposure to xenoestrogens [20, 38, 98, 132]. The quality of spermatozoa in men diagnosed with cancer is often suboptimal, even prior to the initiation of chemotherapy or radiotherapy [5, 13, 68, 91, 94]. Sperm quality is poor in testicular cancer patients and in those with Hodgkin's lymphoma prior to therapy [15, 16, 28, 54, 122, 134]; although other studies have failed to confirm this finding [3, 28].

A few reports have found poor sperm quality to be significantly correlated with malignancy type [23, 76, 87, 135]. Pre-existing germ cell

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defects that lead to cancer also impair spermatogenesis. As such, poor semen quality is observed among referred men with asthenospermia being the most common finding in 64.2–86.3 % of pretreatment cancer patients and teratozoospermia in 93.2 % [8, 27]. At diagnosis of testicular cancer or Hodgkin's disease, 50–70 % of patients present with oligozoospermia [28, 55, 68, 129], severe oligozoospermia (<1 million sperm/mL) (22.6 %) [28], azoospermia (9.7–21 %) [28, 68, 129], and abnormal sperm morphology (22.8 %) [27]. In one study, men with testicular and Hodgkin's disease had increased chromosomal aneuploidy rates in germ cells prior to the start of cancer therapy [120]. Hodgkin's disease may activate cytokine secretion, which contributes to oxidative stress and impaired fertility [25, 135].

In addition to the cancer itself, modern oncologic treatments are also highly toxic to reproductive health. They affect normal testicular function and decrease semen quality [1, 14, 19, 65, 85, 113, 119, 131]. The more commonly used treatment is the less toxic ABVD, which causes significantly less damage to reproductive organs. A common side effect of toxic treatment is temporary or permanent azoospermia and severe oligozoospermia [84, 92, 113]. Radiotherapy is harmful to spermatogenesis [114, 133]. Fewer than 50 % of men reported successful conception without assisted technologies after treatment of TGCT with surgery and/or chemotherapy [21, 73].

It is not possible to predict which patients will be affected permanently after treatment [113]. In general, spermatogenesis and semen parameters are expected to return to normal levels in 50 % of patients after 2 years and in 85 % of patients after 5 years of treatment [56]. However, between 15 and 30 % of patients are permanently affected by gonadotoxic treatment and do not recover their reproductive ability [88]. Impaired fertility after treatment, whether temporary or permanent, results in reproductive concerns for a large number of cancer survivors [72, 109]. Advances in early diagnosis and treatment have made testicular cancer one of the most curable cancers [99].

Cancer and Sperm Banking

Sperm banking involves collecting and freezing sperm for potential future use. It is a simple non-invasive way for cancer patients to preserve their ability to have biological children. It is an important aspect of pretreatment oncologic management, especially in young men given the negative impact specific treatments have on semen quality [71, 80]. Sperm banking before cancer treatment is an effective method of fertility preservation endorsed by professional societies in the United States [70]. However, surveys in the United Kingdom [44], Australia and New Zealand [50], Canada [82], and the United States [111] reveal that many patients do not receive adequate and timely information regarding sperm banking. The American Society of Clinical Oncology (ASCO) advocates sperm cryopreservation as an effective method of fertility preservation in young men with cancer [54, 70, 71]. It is, therefore, important that oncologists become familiar with fertility preservation options.

Cancer patients of reproductive age are most commonly referred to sperm banks. In the United States alone, they comprise about 44 % of all referrals [121]. Most fertility preservation options require the application of Assisted Reproductive Technologies (ARTs). Although many cancer patients have poor pretreatment semen quality, most have sperm that are suitable for freezing with good chances of survival and subsequent ART use by either intrauterine insemination (IUI) or intracytoplasmic sperm injection (ICSI). All males of reproductive age—even men undergoing radical prostatectomy for prostate cancer—should cryopreserve their sperm [83, 103]. All other cancer patients should consider banking semen samples

before undergoing any type of chemotherapy or radiation therapy, and physicians should always provide them with the education they need to decide for or against cryopreservation.

Sperm Banking in Young Adolescents

Over the past quarter century, the incidence of cancer in adolescents and young adults has increased, and several countries have reported improvement in survival rates in this patient population [30]. Cure rates for pediatric cancers have dramatically improved in recent years and are currently approaching 80 %.

Sperm banking is a viable option for many adolescent cancer patients whose fertile years are still ahead of them at the time of diagnosis. As a result, adolescent and pediatric cancer patients are increasingly being provided with opportunities for sperm cryopreservation. The American Society of Reproductive Medicine (ASRM) and the American Society of Clinical Oncology (ASCO) support the practice of sperm banking for adolescent cancer patients [136] and represent the standard of care for these individuals. Failure to offer this option may remove the patient's only reproductive option [54]. Patients' parents are also quite receptive to sperm banking.

However, sperm banking is not universally practiced in pediatric oncology centers, and very few adolescent-friendly facilities are available [10, 111]. In a recent large multicenter survey composed of 23 centers in a French national network of sperm banks [30], the mean percentage of cancer patients who were 11–14 years of age increased from 1 % in 1986 to 9 % in 2006. In their report, 4314 patients attempted to produce a semen sample, 4004 succeeded, and sperm was banked in 3616 young children. The youngest age of the patient who provided a semen sample was 12.4 years.

All young males 12 years of age or older should be offered the opportunity to bank their sperm before the start of any treatment [14]. Adolescents who have already achieved sexual maturity (at least Tanner stage 2 and a testicular volume of 5 mL) can provide a semen sample by masturbation [47]. If masturbation is not feasible, other methods can be offered such as penile vibratory stimulation and electroejaculation under general anesthesia [44, 47, 52, 108].

In prepubertal males, there are no haploid sperm or even spermatids in testicular tissue. Therefore, fertility preservation is a challenging situation. In these cases, testicular tissue freezing, stem cell isolation and transplantation, in vitro maturation, and induced spermatogenesis are options [125, 126]. Although testicular biopsy cryopreservation is offered in some centers, evidence is lacking as to whether it can restore fertility in prepubertal boys. Therefore, this protocol should be offered with ethics and IRB involvement.

One study reported an increase in the proportion of adolescents and young adult males who banked sperm in a 12-month period (from 8 to 68 %) by implementing awareness programs. These programs included workshops that reviewed the existing literature on the topic, discussions on the barriers discouraging providers from proposing sperm banking, presentation of patients' and parents' experiences, and information on sperm banking facilities, including out-of-pocket costs [116]. It is important that full collaboration between multidisciplinary teams be encouraged so that this specific population of young patients can be appropriately informed before they start cancer treatment [78].

In a survey, Ginsberg and colleagues [43] reported that 55 % of adolescents and 88 % of their parents had favorable initial impression of sperm banking. The patients also reported that the timing of sperm banking communications had been acceptable and well worth the wait before the initiation of the cancer treatment. Adolescents newly diagnosed with cancer were more likely than their parents to prioritize fertility as a "top 3" life goal [64].

Cryopreservation Techniques

The most commonly used method of cryopreservation involves manual sperm storage in liquid nitrogen. This can be done using fast freezing or slow freezing methods or with a programmable freezer. With all three methods, a low molecular weight cryoprotectant is added to a processed semen sample to prevent ice formation in sperm cells [34]. All cryoprotectants optimize osmotic pressure and pH and provide extracellular energy to sperm. They also include antibiotics to prevent bacterial contamination.

Advantages and Disadvantages of Cryoprotectants

The freezing and thawing process of cryopreservation has detrimental effects on spermatozoa—it can impair sperm motility and vitality and reduce acrosome integrity [35, 36]. Various cryoprotectants and cryopreservation methods are used to maintain sperm viability after thawing [60].

Glycerol

Glycerol is a commonly used cryoprotectant [49]. It is often supplemented with citrate or egg yolk, which acts as cryobuffer because they contain macromolecules that do not permeate the cell membranes of the sperm. Sperm cells are highly permeable to glycerol, which serves as an energy source for spermatozoa and also maintains osmotic pressure by forming hydrogen bonds with membrane phospholipids and sugars [137]. This increases membrane stability and reduces the overall damage to the membrane [137]. The egg yolk/glycerol mixture results in post-thaw outcomes that are superior to those of glycerol alone [95].

TEST-Yolk Buffer

A combination of TES [*N*-Tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid, pK_a 7.5] and Tris[(hydroxymethyl)aminomethane] is combined with fresh egg yolk, dextrose, and penicillin–streptomycin to form the cryobuffer known as TEST(TES and Tris)-yolk buffer (TYB) [79]. TYB instead of glycerol is the preferred cryoprotectant for normal and subnormal semen samples.

Slow Freezing

Manual slow freezing usually takes 2–4 h to complete. Cleveland Clinic's method of controlled, slow freezing involves the complete liquefaction of the semen sample by placing the specimen in an incubator at 37 °C (Fig. 9.1). Cryovials are labeled and color coded. Freezing medium equal to 25 % of the original specimen volume is gradually added to the centrifuge tube with a sterile pipette, and the specimen with the freezing medium is gently rocked for 5 min on a test tube rocker (Fig. 9.2). This is repeated until the added freezing medium equals that of the original specimen volume (Fig. 9.3). The cryodiluted patient sample is added to pre-labeled cryovials using sterile serological pipettes (Fig. 9.4). The labeled vials are placed in a labeled cryocane and covered with a cryosleeve (Fig. 9.5). The cryocane with two cryovials is placed upright in a freezer at −20 °C for 8 min. Following this, the canes are removed from the −20 °C freezer and placed upright in LN_2 vapor tank (−80 °C) for a minimum of 2 h. The vials are exposed to LN_2 vapors only. Therefore, the level of LN_2 in the tank must not exceed 12 cm. The canes are flipped after 24 h and plunged in LN_2 (−196 °C) (Fig. 9.6).

After 24 h, the test vial is removed; the cap is loosened and placed in the incubator at 37 °C. The sample is mixed and analyzed using the Computer Assisted Semen Analyzer for count, motility, curvilinear velocity, linearity, and amplitude of lateral head movement. Sperm cryosurvival is calculated examining the percentage motility of post-thaw specimen to that of the pre-freeze specimen. The number of inseminations possible from the frozen specimen is calculated based on the fact that 15–20 million motile sperm are required for one insemination [66, 79].

The major drawback with this technique is that ice crystals can form within cells if the

Fig. 9.1 Incubator set at 37 °C and depiction of sample undergoing liquefaction

Fig. 9.2 Sample placed on a test tube rocker for 5 min after the addition of Test-Yolk Buffer

cooling rate is too fast. Additionally, slow cooling can shrink the cells due to water osmosis. Hence, it is important to control the cooling rate [102]. Slow, staged freezing using automated, computerized methods has been reported to limit cryodamage of low-quality sperm [93]. However, automated freezers are time-consuming and expensive, requiring up to five times more liquid nitrogen [89].

Rapid Freezing

Rapid freezing protocols are commonly used for sperm cryopreservation and provide better post-thaw motility and cryosurvival than slow freezing protocols in non-oncologic controls [130]. The Irvine Scientific method is a fast and convenient cryopreservation method that can be used

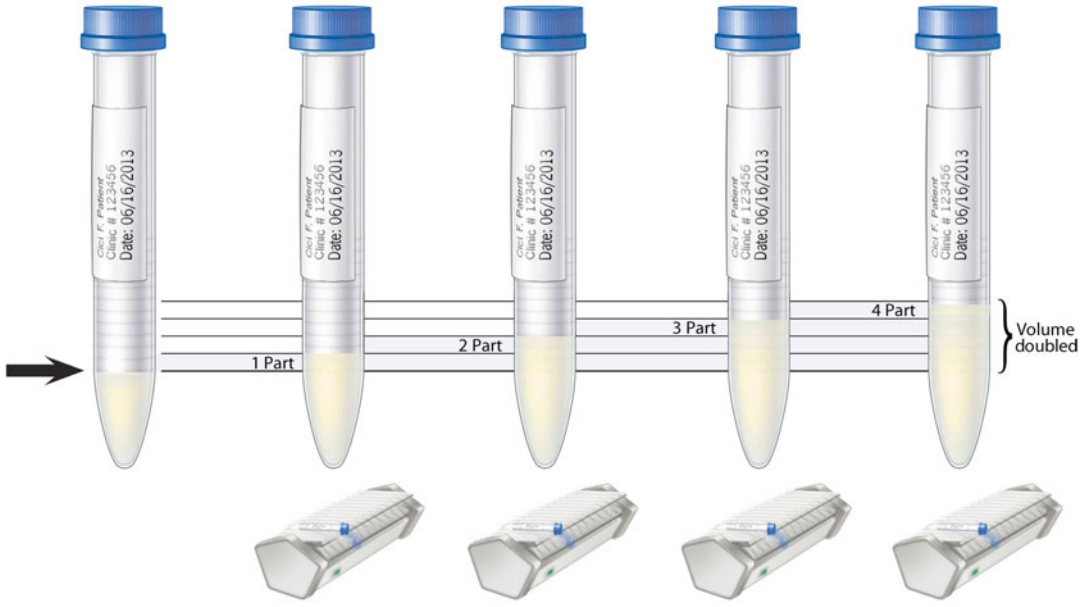

Fig. 9.3 Stepwise addition of Test-Yolk buffer to patient sample. Volume of Test-Yolk Buffer equal to 25 % volume of patient sample—added 4 times, or until total volume in test tube has doubled

Fig. 9.4 Even distribution of cryodiluted patient sample into cryovials using a sterile serological pipette

Fig. 9.5 Cryotank canister containing cryocanes and cryovials added slowly, upright into cryotank

Fig. 9.6 Long-term storage of semen sample in liquid nitrogen tank

to rapidly freeze and store sperm long term. With this method, the entire volume of freezing medium is added at one time, and the specimens are immediately immersed in liquid nitrogen [66, 79].

Vitrification

Vitrification, an ultrarapid freezing method, may offer improved results compared to rapid freezing protocols [58, 101, 104, 117]. The vitrification technique is advantageous in that it requires no equipment and is straightforward, quick, and inexpensive. It is more commonly used to freeze oocytes and embryos. Spermatozoa are osmotically fragile, and the use of high concentrations of permeable cryoprotectants is cytotoxic, drastically reduces spermatozoal motility, and causes genetic damage in sperm cells. Cooling can be achieved using either liquid nitrogen or liquid nitrogen vapor phase. Vitrification—either with no cryoprotectant or very low levels of cryoprotectant—has been

reported [22, 67]. In normospermic samples, no significant difference was shown in the sperm recovery rate and motility rate between spermatozoa cryopreserved without any cryoprotective agent and those preserved with sucrose [22]. Furthermore, the authors reported a higher viability and lower DNA damage than those cryopreserved with sucrose using a cryotop carrier. Lack of cryoprotectant is compensated by the use of high cooling rates achieved by directly plunging samples into liquid nitrogen (~720,000 K/min), and the use of an extremely small sample volume increases the surface area for exchange of heat [81].

Post-Thaw Effects of Freezing

Freezing and thawing of cryopreserved sperm samples has a negative impact on sperm quality, which affects ART outcomes. Men with testicular cancer, in particular, have worse sperm quality than fertile men and men with other common cancers [32, 53]. In a recent report, men with TGCT had a sperm survival rate of only 44.8 % and had the lowest odds of a post-thaw total motile cell count (TMC) above 5 million compared with controls and men with other cancers; they also had the lowest odds of successful IUI [53]. Men with seminoma demonstrated higher sperm concentration, TMC, and percentage motility than those with NSGCT [15, 16, 40]. On the other hand, NSGCT histology was associated with a higher post-thaw TMC (OR: 4.3) than seminoma. The association between TGCT histology and cryosurvival is not clear and may be related to testicular development, Sertoli cell function, or gene and protein expression [42, 74].

Similarly, previous studies have found conflicting evidence for an association between cancer stage and semen parameters [2, 48]. No association was reported between cancer stages and improved post-thaw TMC [53]. Identifying optimal cryopreservation procedures and predictors of post-thaw semen quality is therefore important.

Use of Motility Enhancers in Cryopreservation

In healthy, non-oncologic controls, motility enhancers such as pentoxifylline—an inhibitor of cyclic adenosine monophosphate (cAMP) phosphodiesterases—and deoxyadenosine—an adenosine analogue—have produced variable improvements in sperm motility [11, 35, 36, 57, 115, 118]. Incubation of semen samples with pentoxifylline before freezing has been shown to significantly enhance sperm motility, amplitude of the lateral displacement of the spermatozoa head, and the ability of spermatozoa to undergo the acrosome reaction [35–37, 107]. The positive effects of pentoxifylline stem from its ability to remove reactive oxygen species (ROS) and increase levels of intracellular cAMP [35, 36, 115]. There is limited evidence regarding the impact rapid freezing protocols, motility enhancers, and density gradient purification have on post-thaw sperm quality in men with testicular cancer [32, 53].

Several factors associated with improved post-thaw TMC in preserved specimens among men with TGCT have been reported: NSGCT histology, use of density gradient purification, and greater TMC in fresh specimens. Interestingly, in this same model, age, advanced cancer stage (II or III), rapid freezing protocol, and use of motility enhancer were not associated with changes in post-thaw TMC. Optimizing postcryopreservation sperm recovery through various techniques including freezing methods, motility enhancers, and media is important as it has implications for the level of reproductive interventions needed and their associated costs [80]. There are no predictors of optimal post-thaw semen parameters among men with testicular cancer. Density gradient centrifugation has been shown to improve cryosurvival specifically in oligozoospermic, non-oncologic controls [18, 26]. Use of a density gradient has been shown to significantly improve the odds of post-thaw TMC (OR: 8.2) in men with testicular cancer [53].

Home Sperm Banking Kit

An attractive alternative option for patients who are unable to travel or for those who find providing a semen sample at a sperm bank emotionally challenging is the introduction of a home sperm banking kit called NextGen. With this kit, patients can collect a semen sample in the privacy and comfort of their home and ship it overnight to a sperm banking facility. Cryosurvival rates were examined in sperm collections from patients with and without cancer—both on-site and off-site—using remote collections with the NextGen kit [6]. Pre-freeze and post-thaw sperm motility, total motile sperm, and percent cryosurvival rates were compared. Cryosurvival rates were similar between the NextGen and on-site collection samples in both infertile men (53.14 ± 28.9 % vs. 61.90 6 ± 20.46 %; $p = 0.51$) and men with cancer (52.71 ± 20.37 % vs. 58.90 ± 22.68 %; $p = 0.46$). Cancer patients can bank sperm effectively using a home banking system.

The Effect of Cryopreservation on Sperm Characteristics

DNA stability, acrosomal integrity, motility, and viability are necessary in order for sperm to fertilize an egg [86]. These sperm functions must be present in pre-freeze specimens and conserved throughout cryopreservation and the post-thaw period for fertilization to be possible with IUI and IVF. Both viability and motility tend to decrease by the same percentage after cryopreservation in healthy individuals and testicular cancer patients.

Bonetti et al. found a mean post-thaw recovery rate just under 30 % in cancer and healthy patients [14]. In general, cancer patients have a lower pre-freeze quality sperm than healthy patients [134], and, as a result, their semen tends to have poor post-thaw quality. Furthermore, preexisting defects in germ cells or spermatogenesis, including a possible history of cryptorchidism or intraepithelial germ cell neoplasia, are common in patients with cancer, and this can further reduce sperm quality [134]. Other factors can also lower sperm quality: local endocrine effects of a tumor, systemic endocrine disturbances, autoimmune effects resulting in antisperm antibodies, and stressors resulting from illness [48]. Repeated ejaculates from cancer patients do not vary substantially in terms of semen quality [62]. Optimized sperm cryopreservation protocols are necessary to compensate for the decrease in cryopreservation-thawing tolerance of spermatozoa in cancer patients.

Clinically, post-thaw TMC and cryosurvival have important implications for ARTs in couples desiring pregnancy. Several studies have found that a post-thaw TMC >5–10 million is predictive of successful IUI [75, 127]. It is suggested that men with TGCT cryopreserve a minimum of 15 vials before oncologic treatment. Each vial yields approximately a TMC of 1 million. Preserving 15 vials would offer a couple desiring fertility two attempts at IUI and also ensure viable sperm for IVF if both IUI attempts fail [53]. Optimizing post-thaw TMC and cryosurvival through density gradient purification may also avoid the need for IVF and offer a significant cost benefit for couples [80].

A recent study found that only 4.5 % patients who banked semen samples used the samples in ART in 10 years [122]. While post-thaw semen quality is often not optimal for IUI, even sperm of very poor quality can be used for ICSI. The only male factor that determines successful fertilization by ICSI is the production of a single motile sperm—the outcome is independent of other basic semen parameters, not including DNA integrity [48]. If a patient is unable to produce a sample, minimally invasive procedures are available to recover sperm from the testis and epididymis. These are reviewed in detail in Chap. 13 in the text.

Success rates of IVF and ICSI treatments using cryopreserved semen currently are almost as high as those using fresh semen [128]. The average pregnancy rate using cryopreserved semen is 54 %, and that can range between 33 and 73 % [128].

Advantages of ICSI and Use of Cryopreserved Spermatozoa

Developments in IVF and ICSI have revolutionized the treatment of male-factor infertility and have made sperm cryopreservation cost-effective and the most successful treatment option for men who have viable sperm [88]. ICSI reduces the need to store many samples and increases the chances for future reproductive success. This is especially helpful in patients who have the opportunity to preserve only one or two specimens before initiating cancer treatment [54]. Other investigators report significantly higher pregnancy rates and better results using ICSI compared with IVF or IUI [4, 61, 96, 107].

Certainly, patients with good post-thaw semen quality and sufficient samples can be treated initially by IUI before attempting ICSI-IVF cycles, although success rates (live birth rates) may be low [4]. In a study by Agarwal et al., the success rate (pregnancies) with ICSI using cryopreserved sperm was 37 % [4]. A recent study from Copenhagen reported a total of 151 ART cycles (55 IUI cycles, 82 ICSI, and 14 ICSI-frozen embryo replacement) in which the clinical pregnancy rate per cycle was 14.8 % after IUI and 38.6 % after ICSI.

ART in Cancer Patients

In one report, the ART outcomes of 118 male cancer survivors undergoing 169 IVF-ICSI cycles with a female partner were studied; these couples chose to undergo IVF-ICSI using cryopreserved sperm that had been stored before cancer therapy. The clinical pregnancy rate was 56.8 %, which was comparable to the average pregnancy rate achieved with other male-factor patients. The pregnancy outcome in such cases after conventional IVF, before the use of ICSI, was significantly lower. Fertilization failures with IVF were seen in 11 % of patients as compared with 0.6 % after the introduction of ICSI. In another study, 258 patients cryopreserved their semen before chemotherapy, and only 18 of these returned for treatment. Six pregnancies were achieved [12]. In a report by Lass et al., 231 men were referred for cryopreservation for malignant diseases [68]. Of the six couples who returned for infertility treatment after chemotherapy, two couples achieved pregnancy after IUI, one couple after IVF, and two couples with ICSI. Given the superior success rates of ICSI over IUI, it is recommended that ICSI be performed with cryopreserved sperm to avoid the risk of failed fertilization and depleting a limited sperm supply.

In the past, semen with poor pre-freeze or post-thaw quality from patients with testicular cancer resulted in low rates of successful pregnancies using IUI [14]. However, with ICSI, semen samples with the poorest semen parameters can be used to achieve pregnancy because the male patient is required to produce only a single motile sperm. Even though cancer patients have lower quality sperm and the quality is further lowered by cryopreservation, ICSI circumvents this issue, making it worthwhile to cryopreserve semen samples with even the worst classical sperm parameters [33].

ICSI is an option for utilizing cryopreserved sperm retrieved with TESE and micro-TESE in patients with obstructive as well as nonobstructive azoospermia [59]. The use of ICSI in these patients has significantly increased and will continue to so [31]. Although the overall number of embryos transferred and the pregnancy rate per cycle have been reported to be lower in nonobstructive patients, the pregnancy rate per cycle is not different.

Although fertility preservation options are growing, as noted in the 2013 update to the ASCO guidelines for fertility preservation for patients with cancer, there is a paucity of well-designed studies and outcome data on the application, success, and effects of fertility preservation in patients with cancer [71]. To date, there are limited data regarding the outcomes of ART treatment using cryopreserved sperm from male cancer survivors [124]. Several groups have published reviews to help facilitate fertility preservation in patients with cancer. These include International Society for Fertility Preservation, fertiPROTEKT—a collaboration of centers in

Germany, Switzerland and Austria, and individual centers with vast experience in fertility preservation for cancer patients. These documents provide guidance but lack outcomes on fertility preservation. A recent survey of Reproductive Endocrinologists [97] reported that 83 % of the participants offered semen cryopreservation services to male patients with cancer. Additionally, 22 % of those offering sperm banking responded that they did not recommend banking to men with cancer who had already been exposed to chemotherapy. Finally, 33 % reported offering electroejaculation services, and 84 % reported offering emergent testicular sperm extraction (TESE) options for men who are unable to provide a semen specimen through masturbation.

Challenges and Risks Associated with Cryopreservation

When cryopreserved samples leak in liquid nitrogen, there is a potential for cross-contamination to occur [24]. Regulatory bodies have issued current good tissue practice (CGTP) guidelines to prevent any adverse events resulting from these risks. All facilities offering sperm banking are regulated by the FDA and American Association of Tissue Banks (AATB) guidelines and are required to be registered with these bodies. The FDA has issued guidelines for businesses that provide human cells, tissues, and cellular- and tissue-based products; these establishments must follow the requirements in the CGTP regulations.

Crawshaw et al. reported five main challenges of sperm banking in young cancer patients: attributes of professionals (professional manner vs. "matter of fact" approach when dealing with young patient, stressing the fact that cryopreservation is a part of the routine procedure in order to "play it down", or embarrassment of the professional during their encounter with the patient); gender of the professional involved; age of the professional involved compared to the young patient); skills of professionals (difficulty of developing and maintaining both their knowledge and skill base both in pediatric oncology and ART) when dealing with young patients; infrequent nature of work attached to fertility preservation services), consent issues (amount and complexity of the information although not extensive, but issues such as these young men having to masturbate; or the level of maturity of the young men; consent requirement in ART units), issues relating to the effects of the process on the young men (concern if they were very ill, unable to produce sperm or of the sample produced was not good enough to bank), and follow-up services (annual review of storage; ongoing support or information services, posthumous services). This study outlined the difficulties in building and maintaining an adequate knowledge and skills base in this field and lack of appropriate training [29]. Challenges also arise about what is to be done with stored materials in the event of the patient's death.

The utilization of frozen samples remains low [31]. It is difficult to know the fertility status of the patients who do not come forward for follow-up testing, those conceiving naturally, those with no intention of conceiving, and those who may have psychological reasons for not participating. Because utilization of banked specimens is low, sperm banks should be carefully managed to ensure that resources are targeted to the patients in most need.

Factors Preventing Individuals from Banking

While options for fertility preservation or sperm banking are available, and more patients continue to be referred by their oncologists to discuss their fertility options, knowledge regarding the medical application of fertility preservation is still lacking. Even when patients were properly informed regarding infertility risk and cryopreservation, 42–54 % did not use sperm banking [63]. Despite the well-established link between antineoplastic therapy and infertility, only 18–24 % of young men with cancer were reported to have banked their semen prior to treatment [1, 111]. Studies have shown that only 5–10 % of patients who bank their semen prior to treatment return

for IVF treatment using their cryopreserved specimens [4].

The psychological consequences of banking/not banking among patients in need of fertility preservation, particularly in the context of oncofertility, have been reviewed recently [17]. In another report, a significant variability was reported in the practice of fertility preservation for patients with cancer [97].

Effective promotion of sperm banking involves adequate communication regarding the severity and personal risk for infertility, assessment of the importance of having children, emphasis on the benefits of banking and addressing possible obstacles such as cost, misperceptions or cultural and other factors [1].

There are various factors for underutilization of sperm banking among young men. Some of the common factors cited were:

1. *Priority*

   Sperm banking is not usually a priority for patients who have already completed their family and for those that do not want to have children or for patients who are too young to understand the impact [1].
2. *Cost*

   Presumed high cost by health care professionals is a major factor in sperm banking for patients [14]. In most instances, banking is not covered or is only partially covered by insurance agencies. As cancer, itself, may already have a profound financial impact, many patients have concerns regarding the cost of sperm banking and continued long-term storage of specimens [1]. A survey of patients revealed that financial constraints were a major obstacle for 7 % of cancer survivors who chose not to bank sperm [111]. Cost may play an even larger role in younger patients with limited or no income [1].
3. *Time interval*

   The urgency to start therapy as soon as possible is also a major factor that prevents young patients from sperm banking [52]. Patients with leukemia have a relatively short time interval between initial diagnosis and the initiation of gonadotoxic therapy [134].
4. *Lack of information*

   Very few men diagnosed with cancer bank their specimens and the most common reasons in one study were lack of information or the attitude of the oncologist and practices regarding banking of sperm before cancer treatment [112]. There is a lack of education/counseling services by health care professionals [48] and limited use of cryopreservation by urologists and gynecologists in most fertility programs due to a lack of information regarding the effectiveness of gamete cryopreservation and a lack of agreement on the best universal method.
5. *Psychosocial issues with sperm banking—anxiety and emotional stress*

   A diagnosis of cancer in a young person can provoke a life-altering crisis. The diagnosis itself as well as the threat of infertility both can cause a tremendous stress on these individuals [123]. Schover et al. [111, 112] noted some interesting psychological aspects in cancer survivors: (1) they may experience a higher level of infertility distress than healthy controls (2) adolescents being more distressed than adults, (3) women are more often distressed than men, (4) those with inheritable cancers are more frequently distressed than those with non-inheritable cancers (5) a lower quality of life might be associated with less concern with regard to infertility (6) cancer survivors might view their relationship with children more positively and (7) be more likely to prefer adoption or third-party donation and (8) overall, they may lack accurate risk knowledge [110, 123]. These factors need to be acknowledged by health care professionals and utilized in proper care and treatment of these patients.

   Surveys show that failure of physicians to provide patients with sufficient information in a timely manner is one of the main reasons patients fail to utilize cryopreservation [33, 69]. This could include situations in which the option was not presented entirely [111], the actual risk of infertility was downplayed by the physician [1], or patients who expressed interest failed to receive counseling and

referral to a sperm bank [111]. These investigators reported that although more than 90 % of oncologists felt that male patients at risk for infertility should be offered sperm banking [111], only 52 % discussed the option with their patients [33].

Providing the patient with accurate information may help restore the patient's perception of the benefits of sperm banking. Rates of cryopreservation might be further improved by presenting sperm banking as a standard practice to patients and their families.

Counseling and Ethical Considerations

Counseling patients on fertility preservation, especially at the time of an initial oncologic diagnosis, can be challenging. The counseling should be offered by the oncologist or the physician who is delivering the diagnosis [128]. The important task of the oncologist is to clearly explain, in a compassionate manner, the disease, possible lines of therapy, and probable implications of the disease and its effects on male infertility. It is important to highlight the role health care practitioners' play in patient decision-making with regard to fertility preservation [136]. Early and open communication with patients and implementation of a multidisciplinary oncofertility team are vital.

Because cancer and cancer treatment both adversely affect spermatogenesis, the onus is on the oncologist to discuss the effects on fertility and fertility preservation options and stress the importance of cryopreservation. It is the responsibility of the reproductive medicine specialist to ensure that other physicians are aware of the relatively good pregnancy outcomes that can be achieved with cryopreserved semen [128]. Educating physicians outside reproductive medicine as to when to refer a patient to a fertility specialist and discuss sperm banking and subsequent future fertility options is vital [128]. Oncologists should also be aware of current ART procedures available such as ICSI that require single healthy sperm for fertilization.

There are a variety of instructional resources available on the internet. One such organization, SaveMyFertility.org, is dedicated to increasing awareness of fertility preservation options among both providers and patients by providing informative materials that facilitate and stimulate discussions on the importance of sperm cryobanking (save my fertility.org). It is recommended that physicians initiate and guide sessions on fertility preservation with their patients. An informed nurse and other health care provider staff, however, may be helpful in providing continuing support throughout oncologic treatment [41].

There are a number of barriers that can prevent patients from receiving fertility preservation counseling: lack of time during a patient visit, anxiety at the time of diagnosis, conflicting cultural or religious views, loss to follow-up during referral to a specialist, an inadequately communicated sense of seriousness regarding fertility loss, and a physician who is poorly informed about fertility preservation options [91]. Naturally, discussing potential fertility loss can cause patients to become very anxious—58 % of patients felt that their levels of anxiety had affected their ability to think about fertility. It may be beneficial instead for physicians to discuss fertility preservation options initially at the time of diagnosis and again perhaps a week later but still before the start of treatment. Cultural or religious views that oppose masturbation or artificial insemination, for example, may also prevent fertility discussions from occurring productively [136].

Physicians who do not work in reproductive medicine may have a suboptimal knowledge of the effects of cancer and treatment on fertility, and this may be the limiting reason why many patients do not receive fertility counseling [9, 43]. The American Society of Clinical Oncology (ASCO) in their 2006 guidelines recommended sperm banking as a standard part of care, but it is still not implemented as often as it should be. Educating both physicians and other health care providers to integrate sperm banking awareness among the difficult discussions with their patients is vital [41].

Counseling adolescent patients poses an additional challenge to health care providers [9]. Doctors must work to alleviate patient anxiety by

considering biological issues and acknowledging the psychological needs and individual situation when counseling young cancer patients [45]. Uncertainty on how to navigate the legal and ethical issues may discourage them from appropriately counseling these patients.

Many patients and their families make use of open and spontaneous discussion about fertility [9]. Such discussions can encourage patients to look to the future and reassure them that the aim of cancer therapy is cure. Sperm banking options must be on a case-by-case basis, especially in instances where a patient may be too sick with a very poor prognosis to produce a semen sample and—in this case, sperm cryobanking may not be worth the effort.

Future infertility is not a problem of the individual, but of the couple, and a product of a family. As a result, decisions regarding fertility preservation can impact others besides the patient. This is true in case of minors, where these patients have little autonomy to decide sperm banking without their parents' approval [43]. In other instances, some families may not be comfortable discussing masturbation, sexuality, or reproduction issues with their son. The boys themselves may feel particularly uncomfortable talking openly about these issues or providing a sample via masturbation or they may not fully understand the future implications of a more immediate loss of fertility if they are too young to be thinking about having children. Asking them to produce a sample when accompanied by a parent may unduly embarrass them.

Another serious ethical issue is the postmortem period in cases where the patient did not use his semen sample. Who does the sample belong to? Each clinical circumstance is unique, and each individual patient's diagnosis, prognosis, current desires and future hopes, relationship status, and (specifically in adolescents) maturity level must be taken into account when discussing fertility preservation options.

Conclusion

Cryopreservation is recognized as a therapy to alleviate the reproductive morbidity associated with cancer treatment. Chemotherapy, radiation, and/or surgical therapy all have gonadotoxic effects, leading to impairment of sperm quality resulting in infertility. Fertility preservation options should be discussed at an early stage during treatment planning for cancer. Efforts continue to optimize sperm freezing methods including recent research efforts in providing cryoprotectant-free sperm vitrification and refining the cooling and warming protocols to obtain optimal outcomes with vitrification. Assisted reproductive techniques such as ICSI require the use of only a single healthy sperm for a successful pregnancy. This makes sperm freezing a very viable option for cancer patients. Consistency in future practice is important as the cancer population served by family practitioners is young and focused on survival. This can be accomplished with collaboration and continued communication among clinicians providing fertility preservation services and oncologists and other health care providers. By all indications, the role for fertility preservation is only likely to increase over the next several decades. Providing fertility preservation options to these survivors is therefore imperative.

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Fertility Preservation Strategies in the Male Cancer Patient

10

Kelly A. Chiles and Peter N. Schlegel

Introduction

Despite the advances that have been made in improving the quality of life of cancer survivors, some surmountable obstacles remain. Of paramount importance is for healthcare providers to appreciate the role that fertility preservation plays in relieving the anxiety associated with a cancer diagnosis. Current American Society of Clinical Oncology (ASCO) and American Society for Reproductive Medicine (ASRM) guidelines unequivocally state that all men of reproductive age considering gonadotoxic therapy should have a discussion regarding fertility preservation, and that sperm cryopreservation (banking) is the gold standard for achieving this [1, 2]. Unfortunately, less than half of oncologists routinely refer their patients for fertility preservation, and even fewer men participate in fertility preservation programs [3–5].

Almost half of all adult male survivors of childhood cancers will suffer from infertility [6]. Several aspects of cancer treatment can affect reproductive health, and any kind of cancer can be associated with loss of reproductive function. Alkylating and platinum agents are notoriously toxic, and multiagent chemotherapy regimens compound the damage. Because there is considerable overlap in semen parameters, from azoospermia to normal counts, of men who received comparable doses of chemotherapy agents, there is no minimum dose below which fertility can be guaranteed [7]. Radiation to the testis for testicular cancer or as part of a total body irradiation conditioning protocol for stem cell transplant clearly has significant ramifications for gonadal function. Importantly, cranial radiation also poses a high risk of infertility because the high dose warranted for intracranial malignancy can permanently damage the hypothalamus and pituitary, thereby compromising testicular function [8]. Furthermore, men who require retroperitoneal surgery risk damage to their sympathetic nervous system that can cause retrograde ejaculation or anejaculation. Pelvic surgery poses further risk of nerve damage as well as risk to reproductive organs such as the vas deferens. Clearly the diagnosis of any cancer warrants a discussion regarding fertility preservation.

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Barriers to Fertility Preservation

The reasons behind the lack of referral of patients for fertility preservation counseling are complicated. Understandably, oncologists feel a strong sense of urgency regarding starting chemoradiation [9]. This sense of time urgency, however, needs to be tempered with an appreciation for the greater damage that can be done to a patient's long-term quality of life. Once treatment efficacy for a cancer has been established, a critical guiding principle is to limit the toxicity and/or complications from such treatment. Fertility preservation is an important effort to limit complications of treatment. The patient's understanding of the outcomes of chemoradiation and the opportunity to attenuate the possibility of future infertility must not be compromised because of haste to begin treatment. Working closely with reproductive health specialists should allow oncologists and patients to have expedited conversations about fertility preservation strategies and minimize any delay in treatment.

Studies have demonstrated that lack of time for this sensitive discussion as well as the logistics of the actual donation influence whether healthcare providers introduce the subject of fertility preservation to their patients. Oncologists at cancer centers were queried regarding their fertility preservation practices, and although 91 % felt that sperm banking should be offered to all eligible men, only 10 % always did so [10]. The most commonly cited reasons for not offering this option were a general lack of awareness of the resources available for fertility preservation and lack of time to discuss fertility preservation. Additional structured interviews with medical and surgical oncologists continually demonstrate these same concerns as the obstacles which prevent a discussion regarding fertility preservation [11]. A team-centered approach is crucial because oncologists should be able to direct their patients to reproductive specialists as a means of attenuating time constraints during their oncologic visit and logistical concerns.

In addition, referral to specialists will circumvent any discomfort oncologists may have discussing sensitive topics. Conversations with a male cancer patient regarding fertility preservation should include a discussion regarding his comfort with masturbation. The oncologic team should have a support network in place of providers who can, on relatively short notice, speak comfortably and frankly with patients (and when necessary parents) about providing semen specimens for cryopreservation. This conversation can be difficult to have with adult males, and can become even more complicated when it pertains to adolescent patients. Despite this difficulty, studies demonstrate that adolescent patients almost always want to be informed and involved in discussions of their care even if they are not making the primary decisions [12]. Parents of adolescents are equally eager to be involved in the conversation regarding fertility preservation, and parents of boys as young as 12 embrace the opportunity even when the risk of infertility is low [13].

Misconceptions about patients' cultural or religious beliefs can inhibit frank discussions about fertility preservation [14]. In fact, language and cultural barriers are considered common when trying to breach the subject of fertility preservation [15]. It is important to recognize that assumptions cannot be made about a man's personal feelings regarding masturbation or assisted reproduction regardless of his religion or culture. In addition, providers should recognize that although candid discussion seems more challenging when it must take place through an interpreter, the patient would benefit nonetheless.

Fertility Preservation Strategies

Ejaculated Specimen Collection

Sperm cryopreservation of at least one adequate masturbated specimen prior to beginning cancer treatment is the standard for fertility preservation in postpubertal males [16]. Optimally, several samples, each provided after a 48–72 h period of abstinence, would be preferred, but time is obviously a limiting factor. All specimens will undergo a semen analysis to ensure adequacy. Importantly, healthcare providers and the patient

should impress upon the sperm bank the need for aliquoting the specimen into multiple samples. Because at least 50 % of the sperm will not survive the freeze-thaw process, and many couples desire multiple children or require multiple treatment attempts to conceive, it behooves the patient to ensure an adequate number of vials for his future reproductive goals. In addition, because men with cancer may have poorer quality ejaculates before treatment even begins, multiple specimens or aliquots could be beneficial [17, 18].

The patient should be alerted to the fact that only specialized lubricants specifically approved for cryopreservation, such as mineral oil, can be used for collection. Many common over-the-counter lubricants affect sperm motility and can be spermicidal, while other lubricants, such as saliva, pose the risk of contaminating the specimen with bacteria. Although this is not optimal, men who feel that masturbation is not acceptable can still provide an ejaculated specimen by using a specialized condom in order to collect ejaculate during intercourse. These sperm collection reservoirs can then be emptied into a collection cup and delivered to the sperm bank.

In addition to moral objection, there are a number of circumstances when it is not possible to obtain a masturbated specimen. The diagnosis of cancer is attended with an understandable amount of anxiety, and can also be associated with significant physical discomfort and/or hospital admission. A large number of circumstances might render a man unable to produce a masturbated specimen and alternative collection is necessary. In addition, up to 12 % of men who provide a semen specimen before cancer treatment begins will be azoospermic or severely oligospermic with nonmotile sperm, mandating alternative means to acquiring sperm sufficient for cryopreservation [8].

It is quite possible that a male who normally has no issue with self-stimulation may be unable to ejaculate because of external circumstances. In these cases, penile vibratory stimulation (PVS) may be able to provide the necessary stimulus to allow production of a specimen. Traditionally, PVS is reserved for men with neurologic impairment of sensation but who still have an intact ejaculatory reflex arc, such as men with peripheral neuropathy from diabetes or certain spinal cord injuries. The same principle of increased stimulation, however, can be applied to men having difficulty with ejaculation on demand for oncofertility purposes. PVS involves the application of a vibrator to the frenulum of a penis, and can be performed by the patient without the presence of healthcare providers or need for anesthesia. Notably, only a handful of cases have been published which document success of PVS for fertility preservation [19, 20]. It is highly likely, however, that the success rate should be at least as high as when used for neurologic anejaculation, and anywhere from 66 to 83 % of men with spinal cord injury will ejaculate within 2 min of commencing PVS [21, 22]. Furthermore, males who utilize PVS unsuccessfully can be salvaged with additional approaches to sperm retrieval [23].

In cases where a masturbated specimen is unable to be produced, electroejaculation (EEJ) can be utilized. EEJ is a relatively noninvasive procedure that can be performed on postpubertal adolescents or adults. Although classically utilized for men who have suffered a spinal cord injury and subsequent nerve injury that damages their ejaculatory reflex, EEJ can be used in men with intact spinal cords as long as it is performed under general anesthesia. As with all patients who undergo EEJ, a thorough rectal vault examination prior to insertion of the transrectal probe is warranted; the presence of a rectal tumor that comprises the mucosa or placement of the probe will preclude using EEJ. Patients who suffer from a perineal mass may not be able to undergo EEJ if the mass prevents sufficient contact between the electrodes and the seminal vesicles and vas. Because the efficacy of EEJ relies solely on the ability of the electrode to induce smooth muscle contraction of the vas and seminal vesicle, it is suggested that smooth muscle relaxants and paralytics be minimized during the procedure. The success rate for acquiring sperm via EEJ in boys aged 10–19 is almost 50 % based on review of the published literature; success rates increase with increasing sexual maturity [24]. The reasons for EEJ failure include lack of production of ejac-

ulate during the procedure as well as lack of sperm in any ejaculate produced [25]. Clearly a third option for sperm retrieval is warranted despite the success of EEJ.

Testicular Specimen Collection

Surgeons who provide fertility preservation need to be prepared for the possibility that sperm must be collected directly from the epididymis or testis in the event of EEJ failure. Epididymal collection should only be utilized when there is evidence suggesting that the etiology of the azoospermia is obstruction, and EEJ could therefore be omitted. Suspicion of obstruction in a cancer patient is the same as for an azoospermic patient without cancer. A medical history that predisposes a patient to ejaculatory organ obstruction, such as bilateral hernia repairs, in the context of normal sized testis would raise suspicion for obstruction, as could the presence of large pelvic tumor. If time permits, a follicle stimulating hormone level should be obtained to verify normal spermatogenesis; however, this lab result should not delay intervention. Patients should be informed that when the suspicion of obstruction is present, confirmation will take place in the operating room upon visualization of the reproductive system. Patients with dilated tubules can undergo epididymal aspiration, whereas patients who do not demonstrate evidence of obstruction at the epididymis should preferentially undergo testicular sperm retrieval. When obstructed, microsurgical epididymal sperm aspiration (MESA) should primarily be utilized in order to preclude injury to the testicular vessels that can occur if blind percutaneous epididymal biopsy is attempted. The vast majority of azoospermic men will have nonobstructive azoospermia, however, and microdissection testicular sperm extraction (microTESE) is the most effective means for acquiring sperm under these circumstances. The utility of microTESE in a cancer patient is underscored by the observation that having any kind of cancer may compromise their germ cell reserve. Being azoospermic prior to treatment absolutely mandates the gold standard in sperm retrieval in order to best optimize what might be the patient's only opportunity to find sperm. Fortunately, up to 1/3 of young males will have successful recovery of sperm directly from the testis when masturbation or EEJ fails to provide adequate sperm. Importantly, requiring operative intervention is not associated with delaying the onset of cancer treatment, and patients can often begin their regimen the day of surgery [26].

Ex vivo Sperm Retrieval

Men who are unable to produce a masturbated specimen or are azoospermic, and who are also undergoing an orchiectomy, present a unique opportunity for fertility preservation. Typically, once the specimen is removed by the oncologist, the microsurgeon can bivalve the testis on a back table away from the operative field [27]. Care must be taken to ensure that the pathologist is well aware of intended disruption to the capsule so that staging remains accurate. The parenchyma should be carefully inspected to appreciate the location of the tumor as well as the location of optimal seminiferous tubules. The tubules that do not encroach upon the testicular mass can be preferentially chosen in order to maintain macroscopic integrity of the tumor. Because of the need to maintain anatomical boundaries and because men with testicular cancer often have the most severely affected spermatogenesis, use of the operating microscope and a formal microdissection testicular sperm extraction (microTESE) is encouraged when ex vivo sperm retrieval is attempted. This surgical technique is reviewed in more detail in Chap. 13. As with all operative specimens, including the ejaculate from an EEJ and aspirate from a MESA, the processed specimens should be examined in the operating room in order to identify whether sperm (and any motility) is appreciated. The microTESE should continue until sperm is identified or until all tubules have been interrogated.

It should be emphasized that the processing and freezing of ejaculated specimens are markedly different from those specimens obtained

from the epididymis or testis. The sperm bank which will be receiving any specimens acquired from MESA or microTESE should confirm that they have the facilities to harvest and preserve sperm from non-ejaculated, tissue-based specimens.

Special Considerations for the Pediatric Patient

All discussions of sperm retrieval technique thus far have pertained to postpubertal males, including adolescents. As mentioned previously, involving pediatric patients in fertility preservation discussions is important for their psychosocial well-being, and it allows these patients to provide assent for a procedure for which they are not legally qualified to provide consent. Any cases which involve a conflict of pediatric assent and parental consent warrant swift inclusion of ethical board review and the oncologic team to reach a satisfactory resolution.

Prepubertal males can only participate in fertility preservation protocols that are experimental because they have no mature sperm that can be isolated regardless of approach [28]. Any cryopreservation procedure from a prepubertal boy should do so under an Institutional Review Board approved experimental protocol per current fertility preservation guidelines. Often the goal of these experimental protocols focuses on isolating germ cells from a testis biopsy with the intention of finding a way to develop mature sperm capable of fertilization. Similarly, techniques which would allow germ cell preservation and subsequent testicular transplant are being investigated [29, 30]. There are multiple cancer centers which have active oncofertility programs that are able to offer participation in these clinical trials, and parents of boys as young as 3 months old are participating [31].

Pretreatment Suppression Therapy

Sperm production after toxic therapy is profoundly diminished because rapidly dividing cells are particularly sensitive to antineoplastic agents. It has been hypothesized that inhibiting the division of germ cells will therefore decrease their sensitivity to these agents. The hypothalamus–pituitary–gonadal (HPG) axis has been exploited for decreasing germ cell division, and studies have used various regimens of hormones such as GnRH agonists (or potentially antagonistic agents) or exogenous testosterone to temporarily arrest sperm production. The majority of research that tests this theory has been performed in animals with conflicting results, and it is not surprising that the human trials in males performed to date have not provided uniform answers [32, 33]. Because of the conflicting data, current guidelines do not recommend spermatogenic suppression in males undergoing toxic therapies. Suppressive approaches should instead be incorporated into well-designed clinical trials because of the uncertainty of efficacy and the need for clearly defined regimens.

Future Considerations for the Male Patient Who Cryopreserved

Once fertility preservation has been pursued, an entirely new set of questions besets the cancer patient as he transitions to cancer survivor [34]. Looming questions include not only what the effect cryopreservation might have on the integrity of his sperm, but also what effect his chemoradiation may have had if he cryopreserved after beginning treatment [35, 36]. More importantly, the survivor may want to know whether there is any increased risk to children he may have from using this sperm. It is accepted that although the cryosurvival of sperm is only about 50 %, there is no association with increased morbidity of children born from sperm that was banked prior to treatment [37, 38].

It is well established that men with cancer, and testicular cancer in particular, have compromised sperm DNA integrity and semen analysis parameters prior to any treatment [39, 40]. Challenging the testis with chemoradiation certainly exacerbates any issues with sperm structure and function, and for this reason it is

recommended that cryopreservation takes place prior to any treatment. In cases where it is not possible, additional counseling to men who cryopreserve after the commencement of treatment is warranted. Serial investigation of semen specimens produced after chemoradiation demonstrates that DNA damage to sperm is most apparent immediately during and following treatment; however, this does begin to improve between 12 and 24 months after treatment and continues over time [41]. While sperm DNA tests themselves are not entirely definitive of pregnancy outcomes, there is evidence that increased DNA damage is associated with spontaneous abortions regardless of etiology [42, 43].

Patients will understandably want to know whether there is greater risk to offspring using cryopreserved sperm obtained prior to treatment or from natural conception that is attempted after having received treatment for cancer. There has been no evidence of differences in live birth rates or increased major congenital anomaly in the offspring of male cancer patients; however, one study suggested a possible tendency towards lower birth weight (<2500 g) in the offspring of men who were treated at a very young age [44]. Further studies have demonstrated no difference in the birth weight of offspring of males treated with cancer, and have reaffirmed there is no increase in perinatal morbidity or birth defects compared to children born to men not treated for cancer [45]. Finally, men who suffer from highly heritable genetic cancer syndromes often require specialized counseling because their offspring do have a higher risk of developing cancer and because preimplantation genetic diagnosis is a potential, albeit complicated, option [46].

An important question which survivors will eventually ask is how long sperm should be stored. The rate of utilization of banked sperm is generally low at less than 10 %, and this underscores the importance of discussing when to discard [47, 48]. Fortunately, the most common reason for non-utilization is lack of need; however, patients should be counseled to consider what should happen to their stored specimens in the event of their death any time that advanced directives are discussed. Although it may present a financial burden given the years of commitment it requires to store banked sperm, men should be counseled to only discard specimens when they feel that their families are complete in much the same way that men considering sterilization procedures such as a vasectomy are counseled.

Conclusions

Every male of reproductive age who is facing gonadotoxic treatment should be offered fertility preservation. Although an ejaculated specimen from a postpubertal male is preferred, alternative sperm retrieval techniques for anejaculatory or azoospermic men include PVS, EEJ, MESA, microTESE, and ex vivo sperm retrieval. The team-centered approach of fertility specialists, oncologists, and physician extenders will enable men with cancer to survive their cure with the greatest quality of life.

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Fertility Preservation in the Female Cancer Patient

11

Rebecca Flyckt and Tommaso Falcone

Introduction

For a woman in her reproductive years, a cancer diagnosis is not a rare event. According to the National Cancer Institute, there are hundreds of thousands of women each year in the United States who are diagnosed with invasive cancer, and approximately 10 % of these cases are in women less than 45 years old [1]. Although this diagnosis is not infrequent, it is life changing. Among the many concerns of young female cancer patients is whether they will be able to conceive and deliver a healthy baby once their cancer treatments are complete. Reproductive-aged women are at risk of developing amenorrhea, subfertility or infertility, and primary ovarian failure (POF). Women are increasingly aware of the deleterious reproductive effects of chemotherapy and radiation, and yet, discussions of fertility preservation are still far from universal. A recent study of breast cancer patients <40 years old indicated that only 55 % of women had counseling regarding fertility consequences and options documented in the medical record [2]. In addition, this report identified that women >35 years old and women with prior parity were at particular risk of not receiving fertility preservation counseling. Interestingly, only half of participants correctly recalled whether fertility discussions were a part of their initial counseling, implicating that the highly emotional context of a cancer diagnosis challenges clinicians' abilities to have fully informed dialogues on fertility preservation. A further consideration is the fact that a minority of women who receive adequate counseling actually pursue fertility preservation, mainly due to time constraints and financial burden. When comparing fertility preservation in male vs. female subjects with cancer, a marked gender gap (60 % vs. 2 %) is noted [3].

The types of cancers that affect young women are diverse. By far, the most common malignancy in reproductive-aged patients is breast cancer, which affects over 200,000 patients a year [1]. Although most of these cases of breast cancer are in menopausal women, it is estimated that 11 % of cases are in women younger than 45 [4]. The second and third most common malignancies in females in the United States are colon and lung cancer, respectively, although lung cancer is primarily a disease of older women. In young women, the highest prevalence invasive malignancies are

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Table 11.1 Probability of a woman developing invasive cancer from birth—age 49 years, United States, 2009–2011

Site	Probability	%
Breast	1 in 53	1.9
Colorectal	1 in 326	0.3
Kidney and renal pelvis	1 in 752	0.1
Leukemia	1 in 516	0.2
Lung and bronchus	1 in 541	0.2
Non-Hodgkin lymphoma	1 in 543	0.2
Thyroid	1 in 135	0.7
Cervical	1 in 358	0.3
Uterine	1 in 367	0.3

Adapted from: Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015 Jan-Feb; 65(1):5–29

Table 11.2 Cancer treatments and risk of ovarian toxicity

Risk level	Treatment
High	Bone marrow transplant
	Total body irradiation or pelvic radiation
	Cyclophosphamide (alkylating agents), nitrogen mustards, melphalan, busulfan, procarbazine, chlorambucil
Medium	Platinums (cisplatin, carboplatin), nitrosureas, adriamycin, etoposide
Low	6-mercaptopurine, bleomycin, actinomycin, vincristine, methotrexate, 5-fluorouracil, mitomycin
Uncertain	Taxanes (taxol and taxotere), monoclonal antibodies

breast, thyroid, colon, uterus, cervix, and leukemia/lymphomas. Table 11.1 identifies the frequencies of different kinds of cancer in women <49 years old based on recent national statistics [1]. Although the cancers afflicting young women are diverse, the overall death rates have steadily declined for almost all types of cancer over the past two decades. For these increasing numbers of women who survive their disease, the ability to bear children has a significant impact on post-cancer quality of life.

The choice of chemotherapeutic treatment directly impacts subsequent reproductive function. It is well known that alkylating agents such as cyclophosphamide are the most harmful to a woman's ovarian reserve (i.e., the number of fertilizable oocytes in the resting pool of the ovary) in a manner that is both dose- and duration-dependent [5]. The ovarian reserve, once depleted, cannot be restored. This impact is greatest for women who are of advanced maternal age at the time of chemotherapy administration [6]. Multiple markers of ovarian reserve have been proposed including anti-mullerian hormone (AMH), follicle stimulating hormone (FSH), and inhibin B. Of these, AMH has emerged as a particularly promising predictor of ovarian function after cancer treatment [7]. Table 11.2 lists commonly used agents and the risk of ovarian toxicity.

In addition to chemotherapy, radiation and surgery can also lessen women's reproductive capacity. The ovarian follicle is extremely sensitive to radiation damage. As an example, the radiation dose at which half of oocytes are lost in humans (LD50) was previously estimated at 4 Gy [8]; newer data has further reduced this threshold to <2 Gy [9]. As with chemotherapy, the dose and extent of radiation as well at the patient's age at the time of radiation exposure are critical factors in determining the magnitude of the effect on fertility [10]. Radiotherapy can also impact the ability of the uterus to function normally, especially in younger patients [11]. Newer radiation techniques such as IMRT (intensity-modulated radiation therapy) and proton radiotherapy may be able to more precisely target malignancies while minimizing the affect on nearby pelvic organs [12].

There are currently only a few strategies available to reduce the impact of chemotherapy, radiation, bone marrow transplantation, and surgery on reproductive function. It is well known that many of the best treatments for cancers in young women will negatively affect the ovaries and/or uterus. Therefore, fertility preservation prior to treatment remains our most powerful tool in helping young women maintain future options for childbearing. Both medical and surgical management should be considered in treatment planning for cancer patients. Medical treatment consists mainly of injected extended-release preparations of gonadotropin releasing hormone (GnRH) agonists to lessen the impact of chemotherapy on ovarian reserve. Surgical management features ovarian transposition when pelvic radiation is anticipated to avoid direct radiation of the gonads. These two

Table 11.3 Summary of fertility preservation options for women

Approach	Strengths	Weaknesses
Medical		
GnRH agonist	Inexpensive, noninvasive, eliminates menses in pancytopenic patients	Side effects of hypoestrogenemia, unclear efficacy
High dose progestins in early stage endometrial cancer	Increasing support for the safety of the approach	Concern for treatment failure
Surgical		
Ovarian transposition	High efficacy in well-selected patients	Surgical risk, requires experienced surgeon, risk of ovaries drifting back into radiation field
Fertility sparing surgical approaches	High efficacy in well-selected patients	Concern for recurrence if undetected residual disease
Cryopreservation		
Embryo freezing	Gold standard with longest history of successful pregnancy	Requires partner or option for sperm donation, expense, time commitment, IVF procedure
Oocyte freezing	Increasing success using vitrification techniques, does not require male partner, greatest flexibility	Newer and less established technique, expense, time commitment, IVF procedure
Ovarian tissue freezing	Only option for women requiring immediate treatment, no partner required	Surgical risk, experimental technology with limited number of live births to date
In vitro maturation	Does not require stimulation for IVF, can be used after egg retrieval or with frozen ovarian tissue	Experimental technology with limited number of live births to date

options will be discussed in some detail in the first two sections. The gold standard for fertility preservation is oocyte and embryo cryopreservation prior to treatment, which will be reviewed in the following two sections. Standard techniques for in vitro fertilization will be discussed in more detail in a subsequent chapter. In the final sections of this chapter, two developing technologies, ovarian tissue cryopreservation and in vitro maturation (IVM), will be discussed. A summary of these options is presented in Table 11.3.

Medical Management

Protection of the gonads from chemotherapy-induced ovarian damage using adjuvant medical therapy is a desirable aim. For this reason, the administration of GnRH agonists has been proposed as a method of suppressing pituitary gonadotropins that stimulate FSH-dependent maturation of ovarian follicles. Quiescence of the reproductive axis could theoretically lessen the incidence of POF in women exposed to chemotherapy. However, it is known that the primordial ovarian follicles (those that are depleted with gonadotoxic treatments) are *not* FSH-dependent, therefore the biologic plausibility of protective effects of GnRH agonists has been questioned. Accordingly, the last several decades have shown the utility of GnRH agonists for ovarian protection in female cancer patients to be controversial. The use of GnRH agonists for preservation of ovarian function during chemotherapy remains off-label.

Two recent systematic reviews with meta-analyses have shown higher rates of spontaneous menstruation in women co-treated with GnRH agonists [13, 14]. The first review examined six eligible randomized studies to determine the overall incidence of POF, resumption of ovulatory cycles, and occurrence of pregnancy. Although there was no statistically significant difference in

subsequent pregnancy rates, there was a benefit to GnRH co-treatment in resumption of ovulation/menses and a decreased risk of POF [13]. The second review analyzed seven studies with a total of 677 women meeting inclusion criteria. This study similarly found that women who received GnRH agonists had a significantly higher rate of spontaneous menstruation after completion of chemotherapy [14]. However, results from meta-analyses are not uniform. An additional meta-analysis of randomized controlled trials published in 2014 showed no difference in rates of spontaneous menstruation between women with breast cancer who did not receive tamoxifen and were treated with chemotherapy plus GnRH agonists vs. chemotherapy alone [15]. Some randomized trials using AMH as a measure of ovarian reserve do not show a difference after GnRH analog co-treatment [16, 17], although again these findings are not uniform [18]. Despite these conflicting results, a Cochrane Database review on this topic has stated that the use of GnRH agonists "should be considered in women of reproductive age receiving chemotherapy" due to a demonstrated protective effect on ovarian function, although no difference in pregnancy rates was again cited [19].

Several concerns regarding the available literature exist. Most prospective studies in humans have been small and uncontrolled and the results from randomized trials are variable. The available trials have not consistently stratified by age or chemotherapy regimen and the follow-up intervals have been relatively short. In addition, the end points of many studies are resumption of ovulation and/or rates of amenorrhea. These outcome measures are indirect assessments at best of true fertility, which would include rates of subsequent conception, pregnancy, and live birth. And lastly, although the proposed mechanisms are many [20], no single theory has emerged.

One notable study not included in the above meta-analyses is a 2015 publication in the New England Journal of Medicine [21]. This multicenter controlled trial on the use of the GnRH agonist goserelin randomized 257 premenopausal receptor-negative breast cancer patients into a co-treatment group vs. a chemotherapy alone group. Those who received goserelin had an ovarian failure rate of 22 % vs. 8 % in the chemotherapy alone group. Among 218 women available for evaluation, pregnancy rates were statistically significantly higher in the goserelin group (21 % vs. 11 % in the chemotherapy alone group). Although the debate continues, this well-designed prospective trial may lend additional support to the use of GnRH agonists for ovarian protection in reproductive-aged women undergoing chemotherapy. These results should not, however, cause clinicians to overlook more established modalities of fertility preservation.

Surgical Management

Many solid tumors are managed surgically prior to planned chemotherapy and/or radiation. For these tumors, fertility sparing procedures can be performed. For example, in very early stage cervical cancer patients, radical trachelectomy (removal of cervix with uterine preservation) can be performed when appropriate to preserve the uterus for future pregnancy. For patients who will be receiving pelvic radiation, the ovaries can be transposed to higher regions of the pelvis where radiation exposure will be minimized (Fig. 11.1). This procedure can be completed laparoscopically, usually just prior to planned radiation so that the ovaries remain in position during treatment. It can also be completed via laparotomy during primary surgical treatment of the patient's malignancy. Complications of this procedure include fallopian tube or ovarian infarction, ovarian cyst formation, and pelvic pain [5]. The success of this procedure is highly variable and may depend on patient selection, surgical expertise, drifting of the ovaries into the field of radiation, and radiotherapy regimen. In one study, the success rate was as high as 90 % in cervical cancer patients receiving postoperative vaginal brachytherapy [22]. In the same study, patients receiving external radiation therapy and vaginal brachytherapy had a success rate of 60 %. Following treatment, the ovaries do not generally require surgical repositioning for preg-

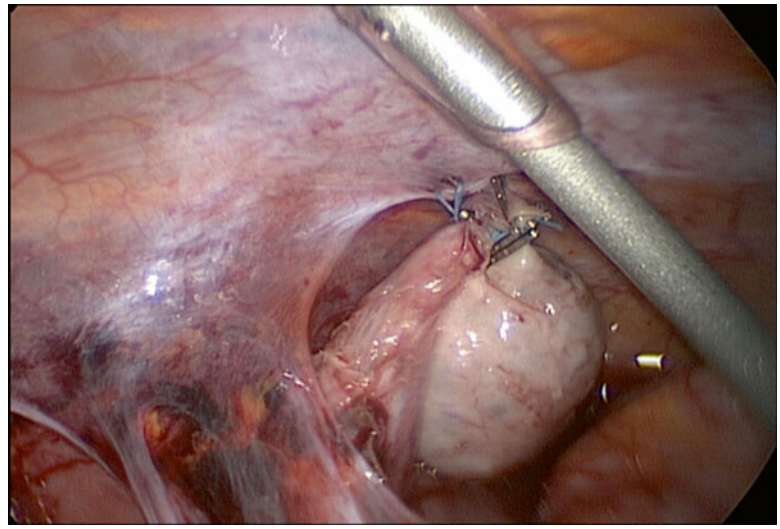

Fig. 11.1 Laparoscopic ovarian transposition with preservation of vascular pedicle in a patient prior to planned pelvic radiotherapy. Location is lateral and above the pelvic brim

nancy to occur. However in some cases of documented subfertility, additional surgery may be required to restore the ovaries to their normal anatomic location or alternatively in vitro fertilization may be required. In these cases, egg retrieval can be complicated by the high position of the ovaries, and alternative (i.e., laparoscopic or transabdominal ultrasound-guided) egg retrieval techniques may be necessary.

Patients with endometrial cancer as well as ovarian cancer and borderline ovarian tumors are also candidates for fertility preserving approaches. Appropriately selected patients with stage 1 borderline ovarian tumors and ovarian cancers may be counseled regarding fertility sparing surgery at the time of their staging, with preservation of the uterus and/or unaffected ovary [23]. Endometrial cancer patients may elect medical therapy rather than surgical management of their disease. Women of reproductive age represent ¼ of new cases of endometrial cancer [24]. In these women, medical therapy can sometimes be pursued in patients desiring fertility preservation with early stage disease. Increasing data supports the safety of treatment with high dose progestins in this patient population in lieu of gold standard hysterectomy, bilateral salpingo-oophorectomy, and lymph node dissection [24].

Embryo Cryopreservation

For postpubertal women who have a source of sperm (either an established male partner or a sperm donor), embryo freezing offers one of the best options for fertility preservation. Embryo cryopreservation has been performed since the 1980s, offering three decades of experience and reassurance regarding efficacy and safety. Although the data from patients preserving embryos due to a cancer diagnosis are limited, patients are often counseled using pregnancy rates from the considerable data available from donor oocyte cycles (Table 11.4, ASRM). The chance of success will mainly be determined by the number and quality of frozen embryos. Cryopreserved embryos have pregnancy rates that may be slightly lower than fresh embryos and, as might be predicted from data on ovarian aging, also vary in quality based on the age of the female partner (ASRM). Embryo cryopreservation was, until quite recently, the only technique for fertility preservation recommended by the American Society for Reproductive Medicine.

Determining which patients are eligible for embryo freezing cycles prior to cancer treatment is complex, and requires close discussions

Table 11.4 Pregnancy rates from donor oocyte cycles and fresh IVF embryo transfer cycles, 2010

	Oocyte donors all ages	<35	35–37	38–40	41–42	>42
Fresh cycle, live birth/ET	55.6	47.8	38.4	28.1	16.8	6.3
Thawed, live birth/ET	34.8	38.7	35.1	28.5	21.4	15.3
Average number embryos transferred	2.0	1.9	1.9	2.1	2.2	2.1

From: ASRM Practice Committee: Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy. Fertil Steril 2013

between the patient and/or her partner, the oncologist, the radiation oncologist (if involved), and the fertility specialist. The patient should be medically stable for controlled ovarian stimulation (COS) and healthy enough to undergo oocyte retrieval. One major consideration is the degree of anemia and/or thrombocytopenia and the presence of underlying platelet dysfunction, given that some degree of bleeding risk is inherent in the egg retrieval process. Other factors include patient age, risk of POF, ovarian reserve, and prior parity, which can predict the success of such cycles and factor into risk/benefit calculations. For example, given the low ovarian reserve and overall poor success rates in women >42, some of these patients may not pursue embryo freezing (ASRM). A final major consideration in the discussion of embryo freezing is the timing of recommended cancer treatments. Unlike with male subjects, female COS requires several weeks to complete. When treatments must be started urgently, this time frame may simply not be feasible.

The stimulation techniques for embryo freezing IVF cycles and oocyte freezing cycles (discussed below) are very similar. One of the major concerns until recently was coordination with the phase of the patient's menstrual cycle. In traditional IVF protocols, stimulation begins shortly after menstruation. One study of breast cancer patients indicated that IVF for fertility preservation did not significantly delay onset of chemotherapy, with a median of 71 days from diagnosis to chemotherapy in the fertility preservation group vs. 67 days in the non-fertility preservation group [26]. Although the average delay in therapy is 2–3 weeks for IVF with embryo cryopreservation, patients receiving consultation in the mid-follicular phase might have to wait an additional 3 weeks or more to begin ovarian stimulation. This timing issue can amount to a significant delay in necessary cancer care. Newer protocols involving "random start" IVF, where ovarian stimulation can be initiated at any point in the menstrual cycle, have been reported to be successful and are increasingly used to limit the amount of time from fertility preservation consultation to initiation of chemotherapy [27]. Protocols involving GnRH antagonists allow aggressive stimulation to obtain as many mature eggs as possible in a single cycle, while still limiting the risk of ovarian hyperstimulation syndrome (OHSS).

OHSS is a condition of excessive ovarian response to FSH stimulations that features ovarian enlargement, intravascular fluid depletion with excess extravascular fluid accumulation, and electrolyte abnormalities [28]. Serious complications of severe OHSS include renal failure, thrombosis, coma, and even death. For patients with cancer, the occurrence of OHSS can further delay cancer treatments. Therefore, the importance of limiting OHSS in already ill cancer patients is clear. GnRH antagonist protocols combined with GnRH agonists to trigger the final maturation of oocytes virtually eliminate the risk of OHSS [28] and should be considered in cancer patients whenever possible. In patients with hormone sensitive tumors, antagonists in combination with aromatase inhibitors (such as letrozole or tamoxifen) can reduce exposure to endogenous ovarian estrogen production, although there is some data that the number of immature eggs and requirements for FSH may be higher in such cycles [29].

There is conflicting evidence as to whether COS in cancer patients leads to fewer oocytes and embryos vs. healthy controls. Whereas some studies show no difference in number of oocytes retrieved, number of mature oocytes, rate of maturity, or number of fertilized embryos [29], others do show a significant difference [30, 31]. The retrospective study by Domingo et al. included 223 women with malignancy undergoing COS for fertility preservation prior to chemotherapy, and determined that there were significantly fewer oocytes retrieved in this group vs. a control group consisting of male factor infertility patients undergoing COS [30]. One possible explanation may be that different cancer subtypes (including whether the breast cancer patient is BRCA1 positive) may confer higher risk of poor ovarian response to COS [32]. Fertility preservation consultations should include a discussion of possible lower numbers of retrieved eggs vs. what would be predicted in healthy age-matched controls.

Oocyte Cryopreservation

In 2013, the American Society for Reproductive Medicine lifted the "experimental" label from oocyte cryopreservation [25]. Although prior to this point the technique was considered investigational, the practice of egg freezing for fertility preservation in cancer patients has a long history owing to several notable benefits. First, not all young women have an established partner or are desiring sperm donation to create embryos. Second, although some young women do have a partner, oocyte cryopreservation offers maximum flexibility for the eventual disposition of these gametes. Embryos that are created by a woman and her partner become shared property, a situation which can become legally, ethically, and emotionally complex in the years that follow. Third, recent developments in the vitrification of human oocytes have significantly increased the success of this procedure.

Previously, most oocytes were cryopreserved using a slow-freeze method that has several technical limitations when applied to oocytes. Vitrification was introduced in the 2000s and has become, at many centers, the gold standard for embryo cryopreservation. Vitrification rapidly induces a "glasslike" state and reduces the chance for ice crystal formation. More recently, it has been applied to oocyte cryopreservation and has been shown to increase rates of frozen oocyte survival when thawed [34]. A recent study demonstrated that success with vitrified oocytes appears equivalent to that using fresh oocytes and superior to oocytes cryopreserved using slow-freeze techniques [35]. In fact, age-specific calculators can now be used to assist in counseling patients regarding their specific chances achieving a live birth using oocyte cryopreservation depending on the method of freezing and number of oocytes frozen [36]. This calculator can be accessed by clinicians and patients online at http://www.fertilitypreservation.org/index.php/probability-calc. Most researchers agree that approximately 20 vitrified eggs are desirable to have a strong chance for live birth, given a live birth rate per oocyte of 5.7 % [37].

The data regarding children born from oocyte freezing cycles are reassuring. A 2009 review of almost 1000 infants born from oocyte cryopreservation showed no increase in anomalies vs. children resulting from spontaneous conception [38]. The ASRM underscores the safety of oocyte cryopreservation by citing "no increase in chromosomal abnormalities, birth defects, and developmental deficits" in pregnancies conceived using this technology in their Practice Committee document [33]. Caution should be used in interpreting data on the success of fertility preservation using oocyte freezing, as most of the available studies are of women without an underlying malignancy. Only a handful of live births have been reported after oocyte vitrification in cancer survivors [39].

Ovarian Tissue Cryopreservation

For prepubertal girls and for adult women who cannot delay treatment to undergo egg or embryo freezing, OTC may be the only feasible fertility preservation option. Women with hematologic

malignancies such as leukemia and lymphoma represent the highest proportion of adult cancer patients pursuing OTC [40]. OTC is, however, still considered investigational by the ASRM and can therefore only be offered under IRB approved experimental protocols.

Somewhere between 30 and 40 live births have been reported using OTC and the techniques employed by different groups are varied [37]. OTC can be accomplished via laparoscopy or minilaparotomy, and can involve a portion of the ovary or an entire ovary. A large number of primordial follicles can be contained in a single biopsy, which can then be reimplanted once cancer treatments are complete, or can be used for subsequent IVM if/when the technology becomes available. Reimplantation can be performed using either orthotopic (ovarian pedicle or nearby peritoneal window) or heterotopic sites, such as the abdominal wall, forearm, or breast. Reimplantation within the pelvic cavity is preferred, as only one clinical pregnancy has been reported with heterotopic transplantation [42]. Either larger strips of ovarian tissue or smaller cubes can be used, both of which have been shown to be effective options [37, 43]. The ovarian tissue can be frozen using either vitrification or a slow-freeze method, although to date all live births have been from tissue cryopreserved using slow-freeze methods. We prefer to use small cortical strips as described by Donnez et al. [41]. Figures 11.2, 11.3, and 11.4 demonstrate a laparoscopic OTC procedure from our institution prior to vitrification.

Age margins for patients desiring OTC are not clear. Due to concerns for reproductive aging, some groups recommend offering this to women only under the age of 35, and there is no lower threshold for patients <18 years old [37]. Very limited data is available for OTC in children [44]. Due to the small size of the ovaries in prepubertal children, the OTC procedure may necessitate the removal of an entire ovary rather than simple biopsies, as can be performed in adults. A discussion of the risk of POF given the patient's planned treatment regimen is very important before undertaking OTC in a pediatric population.

Reimplantation should occur when the patient is disease free and ready to conceive. The ovarian tissue, once restored, becomes hormonally active within a few months and is functional for a mean of approximately 5 years [37]. In some cases, the graft can be tested via immunohistochemistry or PCR before reimplantation to rule out the presence of malignant cells. The question of malignant cell contamination of the graft and potential reintroduction of the primary malignancy with reimplantation is significant; a 2010 publication using PCR demonstrated malignant cells in the ovarian tissue of more than 50 % of leukemic patients [45]. A recent review examined the possibility of malignant cells in ovarian specimens for a variety of cancers [46]. Based on this review, the risk appears greatest with leukemia, neuroblastoma, and Burkitt lymphoma; however, there is at least moderate risk (0.2–11 %) of ovarian metastasis with other more common cancers such as colon cancer, advanced breast cancer, cervical adenocarcinoma, Ewing sarcoma, and Non-Hodgkin lymphoma [46].

In Vitro Maturation

An area of ongoing interest and investigation is the in vitro culture and maturation of immature human oocytes. Antral follicles can be seen by transvaginal ultrasound, and represent a cohort of immature oocytes prior to recruitment. Transvaginal aspiration can be accomplished in a manner similar to that used in traditional IVF cycles for much larger follicles, and this population of immature oocytes can then be matured in the embryology lab. For OTC, the ability to culture oocytes from preserved tissue would obviate the need for reimplantation with any attendant risk. In fact, the first live birth from a patient who had undergone oophorectomy for ovarian cancer using this technique was recently reported [49]. In this case, the immature oocytes from the ovarian specimen were fertilized following IVM. The resulting embryos were cryopreserved and used later for successful pregnancy, providing proof of concept for this technique.

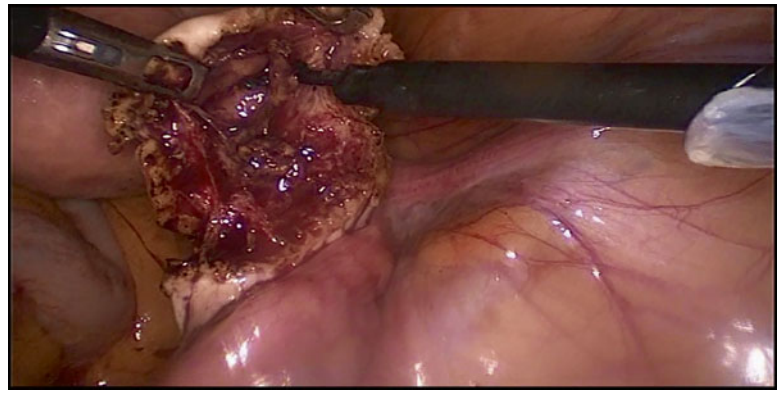

Fig. 11.2 Laparoscopic ovarian tissue cryopreservation in a premenopausal female breast cancer patient prior to gonadotoxic chemotherapy. Dissection of cortical layer

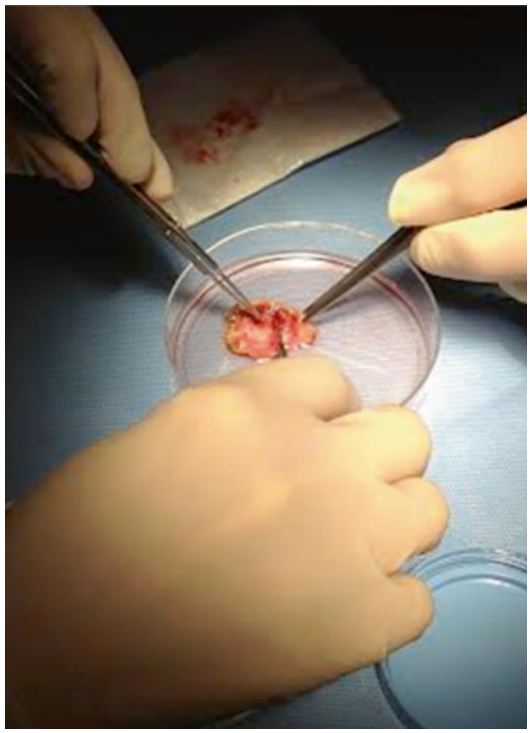

Fig. 11.3 Preparation of ovarian tissue for cryopreservation

Fig. 11.4 Strips of ovarian cortical tissue measuring 5–10 × 5 × 1–2 mm in thickness

For egg freezing fertility preservation cycles, IVM could potentially eliminate the need for COS using gonadotropins prior to oocyte retrieval and would help to further reduce delays in planned chemotherapy or radiation. Unfortunately, current applications of IVM have yielded disappointing implantation, clinical pregnancy, and live birth rates and relatively few labs are able to offer this technique. Clinical outcomes after IVM in the most experienced hands yield live birth rates of 16.5 % vs. 44.3 % in the IVF control group [47]. A few thousand children have been born from IVM and fertilization of immature oocytes in fresh IVF cycles; however, very few births have been reported from frozen–thawed IVM oocytes [48].

Conclusions

Due to advances in cancer treatments, reproductive-aged women with cancer are increasingly becoming survivors who wish to conceive. Alongside this evolution, fertility preservation options have similarly expanded. GnRH agonists may preserve ovarian function during gonadotoxic chemotherapy. Fertility sparing surgeries and ovarian transposition represent surgical options for fertility preservation. For women who require imminent treatment and prepubertal girls, IVM of immature oocytes and OTC are investigational techniques with significant promise. For reproductive-aged adult women with sufficient time before treatment, established techniques of egg and embryo cryopreservation become more successful each year as laboratory techniques improve. At the time of diagnosis, the fertility specialist must work closely with the patient and their oncology team to develop an individualized plan of care. As the options for fertility preservation increase, all clinicians must become aware of the technologies that are available, the relative strengths and limitations of each approach, and the chance for success for an individual patient in achieving a healthy baby once their disease has been treated.

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Cancer Treatment in Pregnancy

12

Jeff Chapa

Introduction

Cancer and pregnancy are words that elicit very different emotional responses from both patients and their caregivers. Thus, when these seemingly polar occurrences coincide, the result is a very complex situation, which can be extremely difficult for all parties involved and requires careful consideration of therapeutic options. Fortunately, cancer remains a relatively rare diagnosis during gestation, complicating approximately 1 in 1000 pregnancies in the United States [1]. Although with delayed childbearing being the current trend, this number is likely to be higher going forward, as the underlying risk for malignancy increases with age.

This chapter focuses initially on the development of a multidisciplinary approach for treating the pregnant patient with cancer. While treatment must ultimately be individualized to the patient and her cancer, collaboration across numerous specialties is required to optimize both maternal and neonatal outcomes. Once this framework is established, specific treatment modalities, including surgery, chemotherapy, and radiation therapy, will be discussed in the context of pregnancy. Finally, management of some of the more common neoplasms that complicate pregnancy will be discussed individually.

Developing a Framework for Treatment of Cancer in Pregnancy

The treatment of cancer during pregnancy is an extremely challenging undertaking and requires a collaborative effort with participation of obstetricians, medical and radiation oncologists, surgeons, anesthesiologists, neonatologists, and possibly other subspecialists. Obstetricians and neonatologists offer a perspective on maternal physiology and fetal development that complements the knowledge oncologists have for treating cancer. Formal, multidisciplinary discussions involving the entire medical team are often necessary and may be needed periodically, as these cases are frequently characterized by their fluid nature.

Along with bringing together the necessary medical expertise, it is also extremely important not to overlook the wishes and beliefs of the patient and her family. In this setting, a nonphysician care coordinator can help facilitate communication, which should be direct and frequent, between different medical services and the patient. These individuals can also play a critical role in advocating for the patients and their

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families throughout the decision-making process. Also, pregnancy or a new cancer diagnosis, individually, can be significant stressors for patients and their loved ones. When combined, the effect can be even more profound, and a myriad of social and economic issues are often present. Access to individuals, such as social workers and financial counselors, can provide patients with information regarding available resources to help navigate these difficult situations. Ultimately, this may mean the difference for a patient in maintaining compliance with a proposed treatment plan and having a desirable outcome.

Once assembled, the task of the multidisciplinary team is to consider and formulate diagnostic and therapeutic options based on the underlying malignancy, its prognosis, and the gestational age. In most cases, there is little or no evidence to suggest that pregnancy adversely affects the patient's prognosis [2]. Pregnancy can delay the diagnosis of a malignancy due to overlap of presenting symptoms with common complaints during gestation. It can also lead to delay or withholding of necessary treatment due to concern for the developing fetus, and this can be deleterious for both the mother and her unborn child. Preserving maternal health should remain paramount, and timely discussion and implementation of the plan of care is essential for optimizing outcomes. Treatment plans should be developed collaboratively with open discussion of implications for the mother and her developing fetus, which may lead to some modification of diagnostic and therapeutic approaches. Pregnancy termination is an option that must be presented when cancer is diagnosed early in gestation, particularly for those cases identified in the first trimester. The decision should ultimately be left to the patient and her family, as in most cases, some form of treatment can be initiated during pregnancy without incurring excessive maternal or fetal risk. In the United States, pregnancy termination can be performed up to 24 weeks gestational age in most states.

As with the management of any condition during pregnancy, gestational age plays a critical role in medical decision-making for the gravida with an underlying malignancy. Treatment, whether surgical or medical, including both chemotherapy and radiation therapy, has the potential for adverse fetal effects, which can vary depending on the gestational age. During the implantation period, which spans the first week following conception, most insults will either have no effect or lead to spontaneous abortion. During the period of organogenesis, which is generally 1–8 weeks postconception, exposures have the potential to lead to congenital malformations or miscarriage. Later exposures can lead to growth restriction and developmental delays. There is also concern for postnatal effects, including increased risk for infertility and pediatric malignancy. These effects will be further discussed when addressing specific therapeutic modalities.

Treatment Modalities for Cancer During Pregnancy

Surgery

The safety of surgery during pregnancy with modern anesthetic and operative precautions appears to be well established. Approximately 0.5–2.0 % of pregnant women in North America undergo surgery for indications unrelated to their pregnancy [3]. While the majority of these procedures were not for malignancy, rates of miscarriage, maternal death, birth defects, and adverse neurodevelopmental outcomes in offspring were not increased with surgery during gestation [4]. Commonly used anesthetic agents have a long history of use and appear to be safe during all stages of pregnancy.

In general, surgical intervention in the first trimester is performed primarily for emergent indications, as elective procedures are often postponed due to concerns for miscarriage and teratogenicity with anesthetic agents, although these risks again seem to be more theoretical than actual. The second trimester is the preferred time for surgery during pregnancy, as the fetus is beyond the period of organogenesis and is less susceptible to physiologic alterations in the mother. Surgical intervention during the third

trimester is a riskier proposition due to increased sensitivity of the gestation to changes in maternal hemodynamic status. Intraoperative monitoring for fetal distress and uterine contractions may be necessary. Thus, many clinicians, whenever possible, may choose to delay surgery in the third trimester and allow for sufficient fetal maturity, at which time they proceed with delivery and then perform the indicated procedure.

When surgery is performed during gestation, avoiding hypotension and hypoxia is of paramount concern, as decreased uterine perfusion and oxygen delivery can have a negative impact on developing fetus. Maternal positioning is extremely important as pregnancy advances, as long-term maternal supine positioning can lead to compression of the vena cava by the gravid uterus and significantly decrease cardiac output and blood flow to the uterus. Left lateral displacement is preferred whenever possible, particularly in the latter half of gestation. Clinicians must be aware of the increased aspiration risk in the gravida due to delayed gastric emptying. It is also important to distinguish between intraabdominal malignancies and those cancers located outside the abdomen. A laparotomy performed for an abdominal or pelvic malignancy may be more difficult as the uterus enlarges, impairing the ability to perform an optimal tumor resection. Surgeons need to be aware that aggressive retraction of the gravid uterus can compromise perfusion and may precipitate preterm labor or placental abruption. Finally, postoperative complications such as fever, gastrointestinal problems, and thromboembolic disease must be addressed promptly. Prophylactic anticoagulation should be administered postoperatively, as both pregnancy and cancer are considered hypercoagulable states. Appropriate postoperative pain management is indicated, with acetaminophen and opiates being primarily used.

Chemotherapy

The decision to administer chemotherapy during pregnancy is a difficult one due to conflicting maternal and fetal interests. Most chemotherapy agents are pregnancy category D (Fig. 12.1) and have teratogenic potential with greatest risk during the period of organogenesis [5]. Thus, administration of these drugs during the first trimester is generally avoided, if delaying treatment does not adversely affect maternal outcome. If treatment cannot be delayed, the patient should be counseled about the risks to the fetus, and pregnancy termination should be offered.

Although teratogenicity is often the primary concern with administering chemotherapy during pregnancy, miscarriage, stillbirth, intrauter-

FDA Category	Pregnancy Category Definition
A	Adequate, well-controlled studies in pregnant women have not shown any risk to the fetus
B	No adequate, well-controlled studies in women, but studies in animals have not found any risk to the fetus
C	No adequate, well-controlled studies in women, but studies in animals have shown some harmful effect on the fetus, or no adequate studies of the drug
D	There is clear evidence of risk to the human fetus, but benefits of treating mother may outweigh risks to fetus
X	Clear evidence that the medication causes abnormalities in the fetus; risks to fetus do not justify use during pregnancy

Fig. 12.1 Pregnancy drug categories

ine growth restriction, and predisposition to cancer later in life are significant concerns with administration of these drugs during the second and third trimesters [6]. Serial assessment of fetal growth is indicated for any pregnant woman undergoing chemotherapy, with tests of fetal well-being being performed when indicated. Side effects from chemotherapy can occur, and should be treated appropriately in a timely manner. Most anti-emetics are pregnancy category B, while corticosteroids and granulocyte colony-stimulating factor are considered safe during pregnancy [7]. Delivery should be planned and performed 2–3 weeks after the last chemotherapy dose to allow for sufficient recovery of fetal and maternal bone marrow and decreasing the risk for infectious complications in both mother and infant.

To date, the most experience with chemotherapy during pregnancy has been with hematologic and breast cancers, and studies have failed to identify long-term neurologic or developmental sequelae in children with in utero exposure [8–10]. Unfortunately, data and experience is limited for many chemotherapeutic agents, and this should be addressed clearly with patients prior to initiating treatment. A recent series of patients evaluated obstetrical outcomes in patients exposed to chemotherapy during gestation, and the risks for hypertensive disorders, gestational diabetes, and spontaneous preterm labor were not significantly increased in this group compared to those without such treatment [11]. Overall rate of preterm delivery was increased with chemotherapy, but the majority of these births were iatrogenic due to oncologic indications.

Radiation Therapy

While it is the least frequently employed therapeutic modality during gestation, radiation therapy is used to treat approximately 4000 pregnant women with cancer annually in the United States. In general, radiotherapy is avoided for abdominal and pelvic malignancies during pregnancy due to risks with fetal exposure. Shielding can be used when treating malignancies outside the abdomen to decrease exposure to the fetus, but scatter still occurs, with its effect depending upon the radiation source, as well as the size and proximity to the fetus of the radiated field. The most common indications for radiotherapy in pregnancy are breast cancer, cervical cancer, lymphoma, melanoma, and brain tumors, either primary or metastatic [12].

When considering radiation effects on pregnancy, the amount and timing of exposure is extremely important (Fig. 12.2). In the preimplantation period, radiation exposure more than 0.1 Gy can lead to miscarriage in animal studies, with 50 % lethality at a dose of 1 Gy [13]. During organogenesis, data from animal studies and sur-

Time period (weeks post-conception)	Impact of Radiation on Fetus
Preimplantation (0-1)	Lethal result
Organogenesis (1-8)	Multiorgan malformation; mental retardation; microcephaly; growth restriction
Early Fetal (8-15)	Mental retardation; microcephaly; eye, skeletal, and genital abnormalities; growth restriction
Mid Fetal (15-25)	Mild microcephaly; mental retardation; growth restriction
Late Fetal (25-term)	Increased cancer risks; impairment of fertility; Growth restriction

Fig. 12.2 Effects of radiation on pregnancy

vivors of nuclear exposure, the risk for malformations increases above a threshold dose of 0.1–0.2 Gy. Thus, most diagnostic procedures can safely be performed during pregnancy and should not be delayed. From 8 to 15 weeks postconception, the central nervous system appears to be especially sensitive to radiation. During this period, a fetal dose of 0.1 Gy can result in decrease in intelligence quotient (IQ). With doses >1 Gy the risk for severe mental retardation is approximately 40 %. With significant radiation exposure between 15 and 25 weeks following conception, malformations are rare but microcephaly and mental retardation occur, particularly with doses >1 Gy. Growth restriction can be seen with lower doses with a threshold of 0.2 Gy at this gestational age. Interestingly, growth restriction associated exposure during embryogenesis appears to be reversible in the postnatal period, while growth restriction during the fetal period appears to be more long lasting. With exposure occurring greater than 25 weeks postconception, concerns with neurological development are less striking, and growth restriction, infertility, and increased cancer risk later in life are the main concerns [14, 15]. The risk for childhood cancer following in utero radiation exposure may be increased by a factor of 1.5–2 [16].

Breast Cancer

Breast cancer is the most commonly diagnosed malignancy during gestation, occurring in approximately 1 in 3000 pregnancies [17]. Most cases present as a painless lump palpated by the patient, although physiologic changes such as engorgement, hypertrophy, and nipple discharge may hinder detection during pregnancy. Delay in diagnosis is common ranging from 2 to 6 months, and this has led to a greater proportion of breast cancers diagnosed in pregnancy being advanced (stage II or greater) [18]. Ultrasound is the imaging modality of choice to initially evaluate a breast lump during pregnancy, as it can accurately distinguish between cystic and solid masses. Mammography can safely be performed during gestation with minimal radiation exposure to the fetus, but a younger population, along with physiologic changes in the breast during pregnancy, makes it less effective as a primary screening tool [19]. If the mass is solid, core biopsy is the preferred diagnostic procedure, as fine needle aspiration is less reliable and technically more challenging during pregnancy [20].

If the core biopsy confirms a malignancy, appropriate staging should be performed to plan treatment. Mammography with abdominal shielding should be performed to look for multifocal disease in the affected breast and evidence of bilateral disease in the contralateral breast. A chest X-ray with abdominal shielding can also be performed to evaluate for pulmonary metastases. Screening for liver metastases can be accomplished with abdominal ultrasound. If the patient is symptomatic and bony metastases are suspected, an MRI based skeletal survey or modified bone scan is warranted [21]. Finally, in patients with suspected central nervous system involvement, MRI of the brain should be performed.

The approach to treating breast cancer in pregnancy should be similar to that taken in a patient who is not pregnant, yet a major challenge remains the assimilation of recent advances in the treatment of breast cancer into the care of pregnant women with the same disease. Data suggests that any form of treatment during pregnancy offers a survival advantage relative to deferring treatment until after delivery [22]. The impact of pregnancy on outcomes for breast cancer has been the subject of some debate. The negative impact of pregnancy cited by some studies may likely have been due to authors including breast cancer diagnosed up to 1 year postpartum into the pregnancy group, despite the fact that the literature consistently shows women diagnosed 2–3 years following pregnancy have a worse prognosis [23, 24]. Amant et al. excluded postpartum cases and showed that there was no significant difference between disease-free survival, recurrence, or overall survival between pregnant women with breast cancer and a matched nonpregnant cohort [25].

Surgical management of breast cancer during pregnancy does not differ from that in the nonpregnant patient. There does not appear to be a

significant advantage to mastectomy over breast conserving surgery during pregnancy, after controlling for age, stage, tumor size, race, and hormone receptor status [26]. Following breast conserving surgery, radiation therapy is recommended to prevent local recurrence of disease. This is generally deferred until after delivery, due to concerns with fetal exposure. Although, if radiation therapy is administered in the first and second trimesters with appropriate abdominal shielding, the calculated dose to the fetus appears to be below the threshold values for organ malformation [27].

Adjuvant chemotherapy has been shown to improve survival in women diagnosed with breast cancer, and this treatment should be offered during pregnancy after completion of the first trimester. Anthracycline based regimens with cyclophosphamide and possibly 5-fluorouracil are increasingly being used in the setting of pregnancy [28]. Taxanes can be added or started following four courses of anthracycline based regimens, with some improvement in outcomes being reported [29]. More recently, dose dense chemotherapy regimens, which decrease the interval between cycles, have been shown to possibly be more effective than conventional schedules and shorten the time to complete chemotherapy. Experience in pregnancy is limited, but dose dense regimens do not appear to increase maternal or fetal complications [30].

Gynecologic Cancer

Gynecologic cancers are relatively common malignancies diagnosed during pregnancy. Neoplasms of cervical and ovarian origin will be our primary focus here, as they are the most frequently encountered cancers from this group during gestation [31]. Endometrial cancer in association with pregnancy is rare, with most cases being reported at the time of uterine curettage following pregnancy loss or delivery and having a good prognosis [32]. Vulvar cancer is also a rare diagnosis during gestation and should be managed for the most part as in the nonpregnant patient [33].

Cervical Cancer

Cervical cancer is the most common of gynecologic cancers diagnosed during gestation, with an incidence of 1.5–12 cases per 100,000 pregnancies [34]. For many women of reproductive age, pregnancy is a reason to seek medical care and undergo routine health maintenance tests, such as a Pap smear and pelvic examination. While most patients with cervical cancer are asymptomatic and diagnosed by cytology, postcoital bleeding and vaginal discharge, both of which are extremely common complaints during pregnancy, have been noted by some. Those with more advanced disease can have pelvic or flank pain and a mass noted on pelvic exam.

Diagnostic procedures after abnormal cervical cytology should be the same as in nonpregnant women, with colposcopy and biopsy of suspicious lesions. Endocervical curettage is generally not recommended during pregnancy. If there are no signs of invasive disease, management of cervical dysplasia can generally be delayed until the postpartum period, although serial follow-up with colposcopy during pregnancy can be performed, if a lesion is particularly worrisome. When biopsy suggests invasive cervical cancer, referral to a center with appropriate personnel, including specialists in gynecologic oncology and maternal–fetal medicine is warranted. Treatment planning can be complex and relies on a number of factors, including the clinical stage, the gestational age at time of diagnosis, and the patient's desires and beliefs regarding the pregnancy and termination. We will focus here on squamous cell cancer of the cervix, as this is the predominant histological type. Adenocarcinoma, clear cell, small cell, and glossy cell cancers of the cervix are reported in pregnancy, tend to be more aggressive, and may necessitate a different approach to management.

Staging of cervical cancer is performed using the International Federation of Gynecology and Obstetrics (FIGO) system (Fig. 12.3) and is based on clinical examination and biopsy results. For lesions that are not visible, cold-knife conization is warranted and can be done safely during pregnancy, although there may be some increased risk

Stage 0	Carcinoma *in situ*
Stage IA	Invasive carcinoma only diagnosed by microscopy; invasion < 5 mm and largest extension <7 mm
Stage 1B	Visible Lesions limited to the cervix
Stage II	Invades beyond the cervix but not to the pelvic sidewall or lower 3rd of vagina
Stage III	Invades the pelvic side wall or lower 3rd of vagina
Stage IV	Growth to adjacent organs or distant spread

Fig. 12.3 Cervical cancer staging

for bleeding [35]. MRI can be useful in evaluating parametrial and vaginal invasion as well as lymph node status for pregnant patients with cervical cancer, and spares the fetus from radiation exposure associated with other imaging modalities [36, 37].

If the cone biopsy result confirms focal, microinvasive disease with negative margins and absence of lymphovascular space invasion (stage IA1) and the pregnancy is desired or the gestational age is beyond viability, then the patient can be managed expectantly with vaginal delivery at term, as the cone biopsy is curative. The risk for lymphatic spread is extremely low in these cases. Alternatively, if the patient is previable, desires pregnancy termination, and is not concerned with loss of future fertility, a simple hysterectomy with the fetus in situ can be performed.

For early disease (Stage IA2, IB1, and IIA), treatment is guided by the gestational age and the patient's desire to continue the pregnancy. If the gestation has not yet reached viability and the patient decides to end the pregnancy, radical hysterectomy with pelvic lymphadenectomy with fetus in situ is recommended [38, 39]. If the pregnancy is desired or the gestational age is advanced, early stage cervical cancer can be followed conservatively until a point in gestation where the risk for complications from prematurity are lower, when classical cesarean section and concomitant radical hysterectomy with pelvic lymphadenectomy should be performed [40]. Radiation therapy is also an option for patients with early stage cervical cancer, although a surgical approach is often preferred to preserve ovarian function younger women.

Significant gains in fetal outcome can be achieved up to 30–32 weeks gestational age, and this seems to be a reasonable point at which to proceed with delivery in these cases. Consultation with a neonatologist and administration of corticosteroids to enhance fetal lung maturity are recommended. Added fetal benefit diminishes, particularly after 34 weeks gestational age; thus, further delay is not warranted. Delaying treatment in early stage cervical cancer to gain fetal maturity in this manner does not appear to lead to progression of disease [41]. Adjuvant treatment is then based on risk factors and results of the pathological examination of the surgical specimens.

For bulky (stage 1B2) or advanced (stage IIB–IV) cervical cancer, external beam pelvic radiation with concurrent chemotherapy has become the standard of care. When bulky or advanced cervical cancer is diagnosed during pregnancy, treatment options are again based on the patient's desire to continue the pregnancy and the gestational age. If the pregnancy has not yet reached viability and the patient desires to end the pregnancy, administration of appropriate radiotherapy with chemosensitization is recommended with the fetus left in situ [42]. Spontaneous abortion will generally occur

within a few weeks after initiation of radiation. Misoprostol or surgical evacuation of the uterus may be indicated following fetal demise. Alternatively, some advocate routine hysterotomy in the second trimester to remove the fetus prior to initiating therapy, as this approach may be more palatable for the patient.

If gestation is advanced beyond viability, conservative management with delay of treatment for 6–8 weeks will help fetal maturity and not impact maternal prognosis [43]. If the gestation is previable and the pregnancy is strongly desired, neoadjuvant chemotherapy has been used in patients with locally advanced cervical cancer during the second and third trimesters to allow the fetus to mature [44]. Most of these regimens involve cisplatin, either alone or combined with other drugs, including bleomycin, 5-fluorouracil, paclitaxel, vincristine, and bleomycin. A recent review and meta-analysis of case reports, while limited, shows complete or partial response in most patients without significant adverse fetal outcomes [45]. Cesarean delivery seems to be preferred mode of delivery in these cases, with a vertical uterine incision away from the lower uterine segment. Although the data is not definitive, some studies reveal markedly higher recurrence rates among patients who delivered vaginally compared to those undergoing cesarean delivery, and there are reports recurrence of cervical cancer at an episiotomy site [46].

Ovarian Cancer

With ultrasound becoming a routine part of prenatal care, the identification of adnexal masses during pregnancy has become commonplace. While the vast majority of these are physiologic ovarian cysts, which will resolve spontaneously, the underlying risk for cancer is 0.9–3 % [47]. Deciding which patients require surgical intervention versus expectant management can be challenging. Radiologic signs, which raise suspicion for malignancy, are size >6 cm, enlargement of the mass over time, presence of extra-ovarian disease, including ascites and omental thickening, and morphologic characteristics, such as having solid components, thick septations, and papillary excrescences [48]. As pelvic CT is not recommended during pregnancy, MRI can be useful as an additional imaging modality in differentiating between benign and malignant tumors. Tumor markers have a very limited role during pregnancy, as CA-125 levels are typically increased during pregnancy [49].

Fortunately, the prognosis for ovarian cancer diagnosed during pregnancy is much better than in the general population. In sharp contrast to the nonpregnant population, the majority of ovarian malignancies diagnosed during gestation are low grade and limited to the ovary (stage I) [50]. In most cases, the malignancy can be treated and the pregnancy continued to term, without adverse maternal or fetal outcomes. Also, the incidence of low malignant potential or borderline tumors during gestation is high, with these tumors being nearly as common as a true malignancy. Nonepithelial cancers, particularly germ cell tumors, are also common given the younger age of the obstetrical population. Epithelial ovarian cancers, which have the worst prognosis, may be more prevalent during pregnancy than would be expected based on age at diagnosis; however, many of these neoplasms are early stage, which again is quite different than what is seen outside of pregnancy [51].

When there is sufficient concern for malignancy or the patient is symptomatic from the mass, surgical intervention should be performed. It is preferable that a surgeon capable of performing appropriate surgical staging for ovarian cancer be present or immediately available. In general, when disease appears limited to a single ovary, a conservative surgical approach is warranted with unilateral salpingo-oophorectomy. Laparotomy is preferred over laparoscopy, particularly if the mass is large and suspicion for malignancy is high. The mass should be removed intact, as intraperitoneal rupture of a malignant tumor or cyst has potential for adverse effect on maternal outcomes. Frozen section pathology should be performed at the time of surgery, and the histologic diagnosis should guide further intervention [47]. Diagnosis of a low-malignant potential tumor or

an invasive ovarian malignancy calls for immediate surgical staging, including pelvic washings, peritoneal biopsies, omentectomy, and pelvic and paraaortic lymphadenectomy. If metastatic disease is evident, surgical debulking of disease should be attempted, although it may not be optimally performed due to desire to preserve the pregnancy. With advanced disease very early in gestation, pregnancy termination can be considered to allow for optimal debulking and timely initiation of appropriate adjuvant chemotherapy.

Following surgery, treatment of ovarian cancer during gestation should try to mirror what is done outside of pregnancy, waiting until the second trimester to initiate treatment. For nonepithelial ovarian tumors, indications for adjuvant chemotherapy are the same as for nonpregnant patients. Treatment with bleomycin-etoposide-cisplatin is generally accepted outside of pregnancy, and there is limited experience that this can be done effectively and safely during gestation [52]. Alternatively, paclitaxel-carboplatin, for which there is more experience during pregnancy, can be used. For invasive early-stage epithelial ovarian cancer, platinum-based chemotherapy can be administered. With more advanced stage tumors, optimal debulking is not possible when trying to preserve the pregnancy. In this setting, neoadjuvant chemotherapy with paclitaxel and carboplatin can be given during pregnancy until fetal maturity, and complete cytoreduction surgery can be performed after delivery [53].

Hematologic Malignancy

As a group, hematologic cancers (Fig. 12.4) pose a significant challenge for clinicians when diagnosed during pregnancy, as a discord between maternal and fetal interests often arises. This conflict is often characterized by the urgent need to treat with chemotherapeutic agents, which have significant potential for fetal harm, in order to preserve maternal health. Lymphomas have an estimated prevalence of 1 in 6000 pregnancies, while leukemia is less common, with an incidence of 1 per 100,000 gestations [54]. In this section, we will focus on those malignancies most likely to occur in women of reproductive age, while management of rarer conditions in pregnancy is reviewed elsewhere.

The diagnosis of hematologic malignancies may be more difficult during gestation, as presenting symptoms, such as fatigue and dyspnea, are common in pregnancy. Also, pregnancy related physiologic changes, such as anemia and thrombocytopenia, can delay appropriate evaluation. Lymph node biopsy is often performed to diagnose lymphoma and can be done safely in pregnancy. Bone marrow biopsy, which is important in diagnosing leukemia, can also be performed during pregnancy. Ultrasound and MRI are useful for further staging. Positron emission tomography (PET) combined with CT scan is typically used outside of pregnancy to stage lymphoma, but because of concerns regarding fetal radiation exposure, use of this modality should be delayed until after delivery.

Common Hematologic Malignancies During Pregnancy
Lymphoma
Hodgkin's disease
Non-Hodgkin's lymphoma (indolent, aggressive, very aggressive)
Leukemia
Acute Myeloid Leukemia
Acute Promyelocytic Leukemia
Chronic Myeloid Leukemia

Fig. 12.4 Hematologic malignancies in pregnancy

Lymphoma

Approximately 3 % of all cases of Hodgkin's lymphoma are diagnosed during pregnancy, making it the most common hematologic malignancy noted during gestation [55]. If the diagnosis is made in the first trimester, treatment can usually be deferred until the second trimester, when teratogenic risk is less. An exception to this is the patient with advanced disease in whom delaying therapy may adversely affect maternal prognosis. In this setting, treatment should be started immediately and pregnancy termination be offered. For cases diagnosed in the second trimester or beyond, chemotherapy should be initiated immediately. Most commonly, the ABVD protocol (Adriamycin, bleomycin, vinblastine, and dacarbazine) is used, and more aggressive protocols such as BEACOPP (bleomycin, etoposide, Adriamycin, cyclophosphamide, vincristine, prednisone, and procarbazine) reserved for advanced stage disease. Based on 20-year survival data from a case control study of 48 pregnant women diagnosed with Hodgkin lymphoma, prognosis appears to be similar to that for matched, nonpregnant subjects [56].

Non-Hodgkin lymphomas are the next most common group of hematologic malignancies, and their management is based on their aggressiveness. With indolent lymphomas, treatment can often be delayed until after delivery or later in gestation. Rituximab, a monoclonal antibody against leukocyte antigen CD20, can be combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) or with cyclophosphamide, vincristine, and prednisone (R-CVP) to treat these cases. Alternatively, for more aggressive forms of non-Hodgkin lymphoma, treatment should not be delayed, and diagnosis in the first trimester should prompt consideration for pregnancy termination. For aggressive lymphoma, including large B-cell lymphoma, treatment with R-CHOP should be initiated. Finally, with highly aggressive lymphomas, such as Burkitt lymphoma, treatment includes high-dose methotrexate, a highly teratogenic antimetabolite with a known embryopathy [57]. Thus, in these cases, consideration for pregnancy termination should extend to the point of fetal viability, and early delivery is warranted in those undergoing treatment in the third trimester.

Leukemia

Acute myeloid leukemia (AML) represents the majority of cases of leukemia presenting during gestation. These malignancies can progress rapidly and be fatal unless treated promptly and aggressively with chemotherapy. Pregnancy termination should be offered and considered when the diagnosis of AML is made in the first trimester. Treatment consists of remission induction with cytarabine and an anthracycline. Daunorubicin has been preferred due to increased placental transfer and concerns with fetal cardiotoxicity with idarubicin [58, 59]. This regimen is also associated with significant maternal toxicity including mucositis and prolonged neutropenia, with risk for systemic bacterial and fungal infections. Postremission therapy consists of high-dose cytarabine or allogenic stem cell transplantation, which is not an option during pregnancy. In general, once recovery of bone marrow is achieved in near-term pregnancies following remission, early delivery is warranted, so that further therapy can be initiated. With treatment of AML with appropriate regimens during pregnancy, outcomes appear similar to that for cases diagnosed in nonpregnant women [60].

Acute promyeolocytic leukemia (APL) is a subtype of AML with unique characteristics that warrant special attention here, as it tends to be more common in patients of reproductive age. The clinical presentation of APL is usually a bleeding diathesis due to disseminated intravascular coagulation, which necessitates immediate treatment with all-trans retinoic acid (ATRA) and anthracycline based chemotherapy [61]. ATRA, like other retinoids, is associated with significant teratogenicity when used in the first trimester, specifically cardiac and CNS defects [62]. Thus, a diagnosis of APL in the first trimester warrants consideration for pregnancy termination. ATRA can be used successfully during pregnancy after

the first trimester, with delivery planned after resolution of coagulopathy, recovery of bone marrow suppression, and reasonable fetal maturity.

Chronic myelogenous leukemia (CML) accounts for 15–20 % of all cases of leukemia in adults, with the median age at diagnosis being 50 years and 10 % of patients being of childbearing age [63]. Due to advances in the understanding of the biology of this disease, long-term survival is now possible with the advent of tyrosine kinase inhibitor therapy with Imatinib. Human and animal studies of imatinib during pregnancy have revealed a pattern of fetal malformation, including skeletal malformations and omphalocele [64, 65]. Thus, the drug is contraindicated during pregnancy. For the rare patient diagnosed with chronic phase CML during pregnancy and requires treatment, interferon-α or hydroxyurea, which can be used after the first trimester, may be initiated. If the gravida happens to be in an accelerated phase of CML, pregnancy termination should be offered and tyrosine kinase inhibitor therapy initiated.

A more common scenario is the patient, who has stable chronic CML managed with imatinib and wishes to conceive. Concerns regarding disease progression and development of drug resistance have been brought up with discontinuation of tyrosine kinase inhibitor therapy. To date, evidence suggests that discontinuing imatinib following a prolonged major or complete response appears to be safe [66, 67]. Many of these patients can be treated with interferon-α during their pregnancy and return to imatinib following delivery. Patients who relapse have for the most part successfully been treated with imatinib [68].

Melanoma

Malignant melanoma is one of the most common cancers in women of reproductive age, and its incidence appears to be increasing [69]. As the risk for melanoma increases with age, current trends of delayed childbearing, along with sun exposure and tanning practices, will lead to more cases being diagnosed in relation to pregnancy [70]. Thus, clinicians need to be suspicious of any pigmented, cutaneous lesion during pregnancy, particularly if it is growing, rapidly changing in appearance, or symptomatic with itching or ulceration. Discovery of such a lesion should warrant prompt referral to an appropriate specialist and subsequent biopsy or wide local excision using local anesthesia [71].

If melanoma is diagnosed, histologic findings, including presence of clear margins and Breslow depth, should be used to guide further management. Regional lymph nodes should be assessed by physical examination; if lymph nodes are palpable, they should be biopsied [72]. Sentinel lymph node status is the most significant prognostic factor for patients with localized disease as far as recurrence and survival [73]. Thus, if disease appears to be localized, sentinel lymph node mapping and biopsy may be indicated, depending upon the thickness and other tumor characteristics. This is most commonly done with 99mTechnetium-sulfur colloid and can be done safely during gestation with fetal radiation exposure <5 mGy [74]. Isosulfan blue dye, should not be used during pregnancy due to the risk for allergic reactions, including anaphylaxis, which can be catastrophic during gestation [75]. Alternatively, based on patient or provider preferences, women with an apparently localized lesion can be followed closely through gestation, and sentinel node biopsy can be delayed until after delivery. If lymph node involvement is confirmed during gestation, further evaluation for distant metastasis, including a chest X-ray with shielding of the gravid uterus and ultrasound or MRI of the abdomen, is warranted.

With localized disease treated with excisional biopsy, pregnancy can be managed expectantly with delivery at term. However, the management of advanced disease (stage III and IV) during pregnancy can be quite a dilemma, as the prognosis for these patients is poor. Traditional chemotherapeutic agents have not been shown to significantly increase in survival for these patients, and their use during pregnancy needs to be considered carefully due to potential for adverse fetal effect [76]. Interferon has also been used in this setting, although the potential for

severe toxicity limits its use during pregnancy. If the gestation is previable, pregnancy termination can be considered to facilitate maternal treatment, but this has not been shown to improve maternal outcomes [77]. Thus, this decision to end the pregnancy rests with the patient and her beliefs and values. For more advanced gestations, delivery may be performed at 32–34 weeks gestational age, when morbidity from prematurity is less, to expedite maternal treatment.

Melanoma is the most likely malignancy during pregnancy to metastasize to the pregnancy, accounting for nearly one-third of cases reported in the literature [78]. In addition, there is significant risk for fetal metastasis, particularly if placental disease is confirmed. Regardless of disease stage, a thorough histologic evaluation of the placenta needs to be performed following delivery in a patient with a history of melanoma, including appropriate immunohistochemical staining. Additionally, the infant needs to be evaluated at birth and followed closely for signs of metastatic disease. Fetal metastases of melanoma most commonly manifest as skin lesions or abdominal swelling. No case of presentation of metastatic disease after 11 months of age has been reported, but this does not ensure later metastases cannot occur, as seen with adults [79]. Because of this risk for placental and fetal metastases, as well as the limited options for treatment of metastatic melanoma, patients with localized melanoma should be disease free for some period of time, depending on their age and risk for recurrence, before attempting to conceive [80].

Although there has been much conjecture regarding impact of pregnancy and its hormonal milieu on melanoma, existing data suggest that this effect may be minimal. While pigmentary changes such as melasma and linea nigra suggest that pregnancy induces increased pigment production from melanocytes, the impact on existing nevi remains questionable. Pennoyer et al. prospectively followed women with nevi through pregnancy and showed little change in existing nevi and no evidence of transformation to melanoma [81]. Furthermore, there is no evidence that women taking oral contraceptives or hormone replacement therapy have an increased risk for melanoma [82, 83]. Still others caution that women predisposed to melanoma and dysplastic nevi may be negatively impacted by pregnancy [84].

Diagnosis during pregnancy does not appear to have an adverse effect on the course of melanoma. Daryanani et al. evaluated patients with early stage melanoma (Stage I and II) and showed that 10-year disease-free survival and 10-year overall survival were not different among women with melanoma diagnosed and treated during pregnancy and an age-matched, nonpregnant control group [85]. O'Meara et al. performed a population-based study of 412 women with melanoma diagnosed in relation to pregnancy, which includes cases diagnosed with a year following delivery, comparing them to age-matched controls. There were no differences in stage, tumor thickness, lymph node metastasis, and survival between the two groups [86].

Malignant Brain Tumors

Malignant brain tumors are rare during pregnancy, but their management poses a significant challenge. These cases often may present acutely and are associated with significant maternal morbidity and mortality, along with adverse neonatal outcomes. Physiologic changes during gestation, including water retention and engorgement of blood vessels, may exacerbate symptoms due to brain tumors, yet diagnosis is often delayed, as presenting symptoms, such as headaches, nausea, and vomiting, are common complaints during pregnancy. Brain tumors can also manifest as seizure activity or intracranial hemorrhage [87]. Sex hormones, particularly progesterone, seem to play a role in the behavior of these neoplasms during gestation. The two most frequent brain malignancies encountered during pregnancy are meningiomas and gliomas [88]. The former have been shown to frequently express progesterone receptors, which could provide a mechanism for their emergence and growth often seen during gestation, while there are reports of growth and dedifferentiation with the latter during pregnancy [89–91].

The primary focus of treatment should be preservation of maternal health with fetal concerns of secondary importance. Diagnosis early in pregnancy warrants a discussion regarding pregnancy termination, as the prognosis for the mother is often poor, but this is ultimately the patient's decision to make. Brain edema often contributes to symptoms related to these tumors and can be treated with corticosteroids. Anticonvulsant therapy is often indicated, as seizure activity can be catastrophic for mother and fetus, and newer agents, such as lamictal, seem to be safe and are preferred during pregnancy [92].

Neurosurgical intervention should be based on the severity of neurological symptoms, the gestational age, and histology of the tumor. Craniotomy with dissection of the tumor can be done successfully during pregnancy without deleterious fetal effects [93]. Radiation and chemotherapy have also been used successfully for the treatment of malignant brain tumors during pregnancy, and limited data suggests neonatal outcomes can be good. Delivery should be performed at term or as close to it as possible. Vaginal delivery can be allowed in most cases and is preferred; however, cesarean section is indicated in the setting of increased intracranial pressure.

Colorectal Cancer

Colorectal cancer is a relatively rare occurrence during pregnancy with an estimated incidence of 1 per 13,000 live births, yet it warrants special consideration due to diagnostic and therapeutic challenges in the gravida [94]. Interestingly, the epidemiology of colorectal cancer in pregnancy is quite different than in the general population. Cases noted in pregnancy tend to be in younger patients, and these individuals may be more likely to have predisposing factors, including underlying familial cancer syndromes. There is also preponderance of rectal cancers among cases diagnosed during pregnancy, which is in direct contrast to what is seen among nonpregnant patients [95]. Metastatic disease to the ovaries is also far more common with colorectal tumors diagnosed during pregnancy [96].

Diagnosis of colorectal cancer is difficult during pregnancy for multiple reasons, and often, it is not made until late in gestation. Common presenting signs of colorectal cancer, including abdominal pain, nausea, vomiting, and changes in bowel habits, are common in normal pregnancy. Additionally, weight loss, presence of an abdominal mass, and anemia associated with colorectal cancer can be masked by normal physiologic changes that occur during gestation [97]. This is further compounded by hesitancy on the part of clinicians to perform diagnostic procedures such as colonoscopy on pregnant women. As some colorectal cancers have been shown to demonstrate estrogen and progesterone receptors, there is a suggestion that pregnancy hormones could promote the growth and spread of these tumors, although data supporting this theory is limited and conflicting [98].

Once a diagnosis of colorectal cancer is made based on biopsy results, staging should be performed. While a CT scan of the abdomen and pelvis is typically performed for this purpose in most nonpregnant patients, this is not always acceptable during gestation due to the risk for fetal radiation exposure. Ultrasound or an MRI of the abdomen and pelvis can be done safely during pregnancy and is comparable to CT scan in terms of their adequacy of evaluation for metastatic disease. CEA level is also indicated as a means of tracking response to treatment or progression of disease, although levels may be slightly elevated in pregnancy [99].

While patients presenting in pregnancy tend to have delayed diagnosis and be of more advanced stage, the long-term outcomes are no different than in stage matched nonpregnant patients. Factors to consider in planning treatment include location of the cancer, gestational age, acuity of symptoms, stage of tumor, and the patient's desires. If diagnosed prior to viability, pregnancy termination is an option that must be discussed.

Surgery can be performed during pregnancy if the tumor is deemed amenable to resection, particularly during the first half of gestation; however, many cancers are not diagnosed until the third trimester, and in these circumstances, it may be prudent to proceed with delivery at 32–34

weeks gestational age, when reasonable fetal maturity has been reached, and then go forward with standard treatment [100]. If the tumor is not resectable or causes obstruction during pregnancy, a colostomy can be performed to allow for maturation of the fetus. Adjuvant chemotherapy is indicated with advanced stage colon cancer and can be administered during pregnancy after the first trimester. 5-Fluorouracil is the most often prescribed agent in this setting, and there are some reports of safety with use of this drug during the last two trimesters [101]. Adjuvant radiotherapy is used for rectal cancers, but not recommended during pregnancy due to potential for fetal harm. In general, vaginal delivery is preferred, although, with some distal tumors such as anterior rectal cancers, cesarean delivery may be indicated due to obstruction of the birth canal.

Thyroid Cancer

Thyroid cancer is known to occur commonly in women of reproductive age, and thus an overlap with pregnancy is expectedly seen. The incidence of thyroid cancer during pregnancy has been reported to be as high as 14 per 100,000 live births, making it the second most common type of malignancy detected during pregnancy in one series [102]. About 10 % of thyroid cancers occurring during reproductive years are diagnosed during pregnancy or in the postpartum period [103]. Fortunately, most of these cancers are histologically favorable, early stage at diagnosis, and carry a good prognosis. While there are some concerns regarding hormonal factors during pregnancy impacting the growth and progression of thyroid malignancies, these effects appear to be more noted in vitro and not seen clinically. A large retrospective study on nearly 600 pregnancy-associated thyroid cancers revealed no significant differences in outcome, disease-free survival, and morbidity, when compared to age-matched nonpregnant women [104].

Most thyroid malignancies diagnosed in and around pregnancy present as a thyroid nodule, which may not be immediately recognized due to the physiological increase in the size of the thyroid gland that occurs during gestation. Once a nodule has been confirmed either with clinical exam or ultrasound, fine needle aspiration is safe and diagnostically reliable during gestation [105]. While the presence of a thyroid nodule can lead to great anxiety in both the patient and her medical team, it should be noted that the majority of thyroid nodules noted during pregnancy end up being benign [106, 107]. To this end, some authors have advocated only performing biopsy on those nodules found early in pregnancy and waiting until the postpartum period to investigate nodules noted in the latter half of gestation [108, 109].

Once a diagnosis of thyroid cancer is established during pregnancy, it is important to consider the histology of the tumor, the gestational age, and the patient's wishes. The two most important histologic subtypes are differentiated thyroid cancer, which includes follicular and papillary cancer, and medullary thyroid cancer. The former is much more common and carries a favorable prognosis for disease-free long-term survival, as the overwhelming majority of tumors in reproductive age women are early stage at the time of diagnosis. The latter arise from the parafollicular cells in the thyroid gland and account for approximately 5–10 % of thyroid cancers. They tend to be more aggressive than differentiated tumors and are particularly important because of the association with multiple endocrine neoplasia syndromes (MEN IIA and IIB) and the possibility of other underlying neoplasms, such as pheochromocytoma, which can be catastrophic if undiagnosed at the time of thyroid surgery or delivery [103].

Surgery, in the form of thyroidectomy, can safely be performed during the mid-trimester with no increase in adverse maternal or neonatal outcomes [110]. Alternatively, if a differentiated cancer is noted later in gestation or the patient declines therapy during her pregnancy, surgery can be postponed until after delivery without impacting the long-term prognosis. There is no documented risk for metastases to the placenta or fetus with thyroid malignancy. If thyroidectomy is performed during pregnancy, supplementation with thyroxin needs to be immediately initiated to prevent any adverse effects on fetal growth and

cognitive development. Regular assessment of maternal thyroid hormone status with TSH and Free T4 levels is indicated. Thyroxin dose should be adjusted to maintain Free T4 in the higher part of the normal range for the duration of pregnancy. Calcium and vitamin D supplementation may also be indicated following thyroidectomy. Adjuvant treatment with radioiodine (I-131) may be indicated in some cases, but this must be postponed until after delivery. For patients who receive I-131, pregnancy should be postponed 12 months after completion of treatment to avoid a potential increased risk for miscarriage and assure that any residual disease has been adequately treated [111].

Lung Cancer

Although it is increasingly becoming one of the more common malignancies among women, lung cancer during pregnancy has been a rare occurrence. However, we can expect the number of these cases to rise due to delayed childbearing and increasing cigarette smoking by women. Non-small cell lung cancer, mostly adenocarcinoma, is primarily reported during pregnancy, although small cell lung cancer is also seen. Most cases of lung cancer are discovered at advanced stages, and often become evident due to symptoms from metastatic disease. In pregnancy, nonspecific symptoms, low clinical suspicion, and reluctance to perform radiologic testing contribute further to delayed diagnosis [112].

Treatment intent for lung cancer during pregnancy is mainly palliative, often consisting of platinum-based chemotherapy given after the first trimester. Radiation therapy can be given safely during gestation for metastatic disease, particularly involving the brain and cervical spine. For cases presenting early in gestation, pregnancy termination should be offered, but this decision rests solely with the patient, as there is no evidence that ending the pregnancy will improve maternal outcomes. Neonatal outcomes have been good with the main source of morbidity coming from iatrogenic prematurity, often related to disease progression in the mother. Pathological examination of the placenta is indicated following delivery, and the infant must be followed closely, as there are multiple reports of lung cancer metastasizing to the placenta and fetus [113]. Maternal postpartum outcomes are generally poor, but consistent with what is reported for nonpregnant women presenting with the same stage of disease [114]. In a recent case series, 28 out of 37 patients were known to be dead within 1 year of delivery [115].

Conclusion

The task of treating cancer during pregnancy is daunting from the perspective of any single clinician. Lack of familiarity with pregnancy can be a major obstacle for non-obstetricians in their approach to these patients. A collaborative, multidisciplinary approach is required to enhance the chance for successful maternal and fetal outcomes. Also, the patient's beliefs and desires must not be overlooked when formulating a management plan. While large prospective randomized trials are not available, there are a number of large case series for many types of cancers in pregnancy that provide useful insight. Furthermore, experience with common diagnostic and treatment modalities in the setting of pregnancy has increased greatly in recent years. With timely administration of appropriate therapy, many pregnant patients diagnosed with cancer can have successful pregnancy outcomes and long-term prognosis similar to their nonpregnant counterparts.

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Surgical Approaches for Sperm Harvest in the Azoospermic Cancer Patient

13

James Wren and Robert E. Brannigan

Cancer, Cancer Therapy, and Fertility Impairment

The advancements in multimodal chemotherapeutic regimes for numerous malignancies have resulted in a significant improvement in overall patient survival; however, certain chemotherapeutic agents can lead to sub and infertility [1–5]. One in two men will develop a malignancy in their life, of which 4 % are under the age of 35 years old [6]. Patients diagnosed with a malignancy who are under 19 years of age have an 85 % 5-year survival rate, while those 0–44 years of age have a 75.6 % 5-year survival rate [7, 8]. This improved survival into young adulthood and beyond has given rise to patient concerns regarding future fertility which may have been compromised as a result of the curative chemotherapy and radiation therapy [9]. Platinum based agents (cisplatin and carboplatin) and alkylating agents (cyclophosphamide, ifosphamide and chlorambucil), which have been effectively employed to successfully treat testicular cancer, Hodgkin's, non-Hodgkin's lymphoma and leukemia, can cause post-chemotherapy azoospermia as a result of damage done to spermatogonia type B, and more importantly, spermatogonia type A cells [2–4, 10, 11]. The time from induction of chemotherapy to the point where patients are typically rendered azoospermic is 7–8 weeks [12]. However, the post-chemotherapy recovery period, which can vary from 6 months to 5 years, is unpredictable with up to 50 % of patients having severe, permanent oligospermia or azoospermia in the long term [13–15]. The specific chemotherapeutic agents used, along with the duration of therapy and dosing interval have been shown to be associated with likelihood of post-chemotherapy azoospermia [13–15]. This is discussed in more detail in Chap. 7. Currently, there is no accurate predictor of which patients will develop temporary or permanent azoospermia [16].

Given the uncertainty in return of post-chemotherapy spermatogenesis, it is strongly recommended that patients undergo sperm cryopreservation prior to chemotherapy and/or radiation therapy [17]. However, not all patients undergo cryopreservation, with up to 70 % of testicular cancer patients not banking sperm before chemotherapy treatment [18, 19]. This high percentage is multifactorial, including

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patient/oncologist lack of awareness of future fertility compromise, urgency of initiation of chemotherapy, cost of sperm cryopreservation, and some patients' limited interest in future fertility [18–20]. Additionally, a subset of patients may already have developed underlying malignancy-associated azoospermia, rendering cryopreservation untenable [2, 21]. The prepubescent patient population is also unable to benefit from sperm cryopreservation due to the absence of spermatogenesis [22]. Over the last several decades, a demographic shift has occurred, whereby some patients are delaying the pursuit of fatherhood until later in life. This shift is due to many factors, including occupational and lifestyle choices, and a desire for second families following divorce or the death of a spouse. Collectively, these issues highlight the importance of post-cancer treatment fertility options. This is especially relevant given the high number of patients who do not undergo pre-treatment sperm cryopreservation [23]. One approach to facilitate post-cancer reproductive health has been the establishment of formalized male fertility preservation programs. Such programs have been shown to result in a 2.7-fold increase in the number of males who undergo fertility preservation consultation and pretreatment sperm cryopreservation [6, 24].

Testicular cancer has been associated with underling severe oligospermia and azoospermia prior to radical orchiectomy in some males [25]. Interestingly, many patients have been noted to have a post-orchiectomy improvement in semen parameters for as of yet unidentified reasons. In other patients, the pre-orchiectomy azoospermia can persist following radical orchiectomy, even in those who do not undergo chemotherapy or radiation therapy [25].

For many years, the prevailing dogma in reproductive medicine was that chronic post-chemotherapy azoospermia was synonymous with sterility. However, advances in the past two decades have revealed this not to be the case, with numerous studies demonstrating successful term pregnancies with healthy children being born through the use of the assisted reproductive techniques in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [26–31]. Absence of sperm in the ejaculate was historically felt to reflect a complete failure to produce sperm at the testicular level. However, we now know that a lack of sperm in the ejaculated semen (azoospermia) is not necessarily synonymous with complete absence of spermatogenesis [31, 32]. Patients with azoospermia may still have viable spermatogenesis, within certain seminiferous tubules amenable to sperm retrieval techniques to facilitate sperm isolation, cryopreservation, and subsequent use in IVF/ICSI.

Numerous studies have shown the success of microdissection testicular sperm extraction (micro-TESE), which has emerged as the gold standard sperm retrieval technique in persistently azoospermic, post-chemotherapy patients. Chan et al. showed in 17 patients with post-chemotherapy azoospermia who underwent a total of 20 micro-TESE procedures, successful sperm retrieval in 9 of the 20 (45 %) procedures [26]. Four of those 9 (44 %) had biochemical pregnancies, with 3 of the 9 (33 %) having clinical pregnancies that ultimately resulted in 2 (22 %) live deliveries. These results were equivalent to patients who had undergone TESE for nonobstructive azoospermia without a background of malignancy or chemotherapy [33]. Damani et al. found that 15 of 23 (65 %) patients with post-chemotherapy azoospermia had successful sperm extraction [27]. Meseguer et al. in a retrospective study of 12 patients found that 5 (41 %) males who underwent micro-TESE had motile spermatozoa for cryopreservation and IVF/ICSI [28]. Among these 5 couples, a 68 % fertilization rate occurred, with one healthy term delivery occurring.

Although the application of different forms of sperm retrieval techniques with the use of sperm in IVF/ICSI has been shown to be successful, questions regarding potential genetic risk associated with the use of ART in cancer survivors have arisen [34–38]. Whether from paternal hereditary transmission or from the nonhereditary mutagenic effects of chemotherapy on the germ cells, there has been concern about potential pediatric oncologic occurrences [39]. Some studies have shown an increased incidence of sperm chromosomal abnormalities following chemotherapy,

but these changes appear to be transient and usually resolve 6–18 months following therapy [40, 41]. However, given that sperm retrieval and IVF/ICSI circumvent biological checkpoints, there is a theoretical possibility of fertilization with spermatozoa containing chromosomal abnormalities that could result in miscarriages or birth defects [42]. Although these concerns have been raised, studies to date assessing congenital defects or childhood cancer in offspring of cancer survivors have not revealed an increased risk, with offspring of cancers survivors being found to have a 0.3 % chance of malignancy, which is consistent with controls [43, 44].

Surgical Techniques for Sperm Harvest

The following sperm extraction techniques are available for men who have two separate semen analyses showing post-cancer therapy azoospermia:

1. Fine needle aspiration (FNA)
2. Percutaneous testicular biopsy (PTB)
3. Conventional testicular sperm extraction (TESE)
4. Microdissection testicular sperm extraction (micro-TESE)

micro-TESE has emerged as the gold standard sperm retrieval technique, being superior to the other approaches in regards to better sperm retrieval rates (SRR) and reduced complications [45, 46]. Fine needle aspiration (FNA) and percutaneous testicular biopsy (PTB) are relatively inexpensive, fast, minimally invasive, and they can both sometimes be performed under local anesthesia. However, these approaches have a lower SRR than conventional TESE or micro-TESE, whilst also having a higher risk of associated vascular injury. Micro-TESE's major advantage is the microscopic magnification, which assists in visualizing and preserving the subtunical arteries as well as identifying the most appropriate (larger and more opaque) seminiferous tubules for sperm retrieval to optimize yield [45, 47, 48].

Fine Needle Aspiration (Fig. 13.1)

FNA for nonobstructive azoospermia was first reported in 1996 [29]. The technique, which enables the surgeon able to obtain blind, deep testicular samples, has been altered since its inception with variations in needle size (from 19 gauge up to 23 gauge) and number of puncture sites (single puncture up to 15 punctures). Although this procedure facilitates sperm retrieval, the success rate is less than TESE [49].

Positioning: The patient is placed in the supine position, and the scrotum is shaved and sterilely prepared. FNA can be performed under local, regional, or general anesthesia.

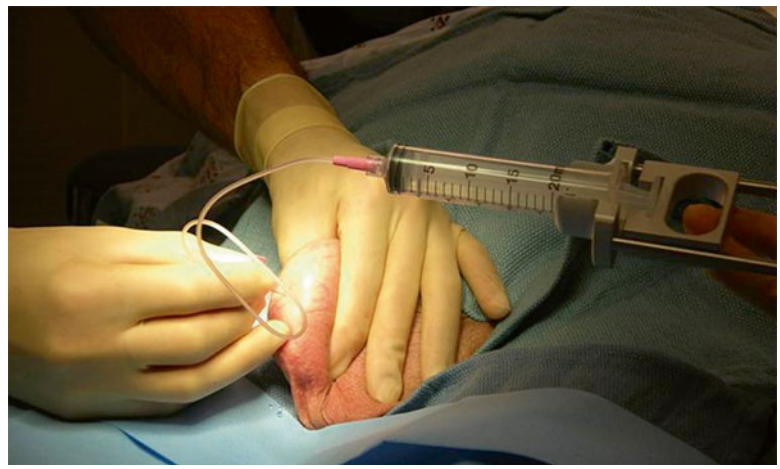

Fig. 13.1 Intraoperative photograph of a testicular sperm aspiration (TESA) procedure using a Cameco syringe holder, a 20 cc syringe, and 19 gauge butterfly needle attachment. (Used with permission of Springer Science + Business Media. Microsurgery for fertility specialists. A practical text. Sperm retrieval techniques in obstructive azoospermia. 2013. P 90. Shridharani A and Sandlow JI. © 2013)

Procedure: A spermatic cord block is placed by injecting 5–10 mL of 1 % lidocaine (without epinephrine) into the ipsilateral spermatic cord at the level of the pubic tubercle. Also, a small skin wheal is created subcutaneously with local anesthesia at each skin puncture site. A small nick is then made in the scrotal skin with a #11 scalpel. Next, a 23 gauge needle attached to a 20 mL syringe is passed through this nick into the testicle. At this point, a back and forth movement is begun with concurrent aspiration of the yellow appearing testicular fluid and tissue. The aspirated fluid and seminiferous tubules are removed for analysis following withdrawal of the needle. This procedure can be repeated several times, with a new needle being used for each new puncture site. A pressure dressing (consisting of gauze, ice packs, and an athletic supporter) should be applied after the procedure to minimize swelling.

Percutaneous Testicular Biopsy (Fig. 13.2)

Positioning: The patient is placed in the supine position, and the scrotum is shaved and sterilely prepared. PTB can be performed under local, regional, or general anesthesia.

Procedure: A spermatic cord block is placed by injecting 5–10 mL of 1 % lidocaine (without epinephrine) into the ipsilateral spermatic cord at the level of the pubic tubercle. Next, a small skin wheal is created subcutaneously with local anesthesia at the percutaneous biopsy site, and a small nick is made in the scrotal skin with a #11 scalpel. A 14 or 16 gauge automatic biopsy gun is advanced through the skin nick, through the tunica albuginea of the testicle, just into the testicular parenchyma. The biopsy gun is then fired to perform the PTB. Typically, percutaneous biopsies are taken in the longitudinal plane of the testicle to minimize the risk of vascular injury. The biopsy gun is withdrawn, the core biopsy tissue is flushed into a sterile container containing sperm wash media, and the tissue is then inspected beneath a microscope for the presence of sperm. This procedure can be repeated several times through the same skin opening, and the same biopsy device can usually be employed for the entire procedure. A pressure dressing (consisting of gauze, ice packs, and an athletic supporter) should be applied after the procedure to minimize swelling.

Open Multiple Testicular Biopsies or Testicular Sperm Extraction (Fig. 13.3)

The first successful case of a pregnancy for a patient with nonobstructive azoospermia via TESE with IVF/ICSI was reported in 1995 [50]. The open, multiple biopsy TESE technique has sperm recovery rates ranging from 56 to 87 %, and this approach provides direct access to and visualization of the testicle and seminiferous

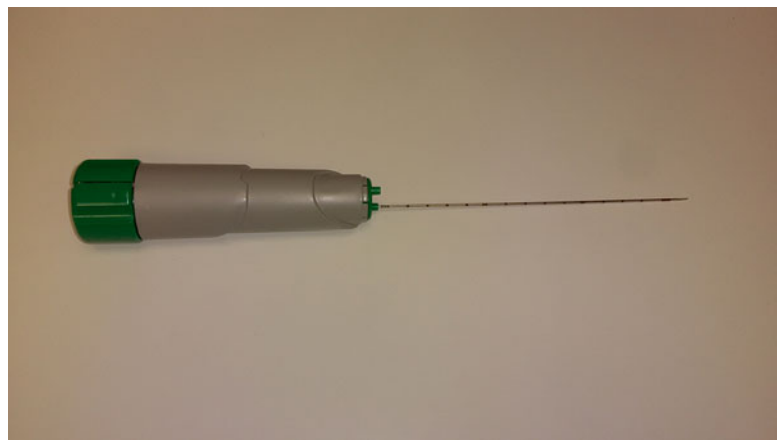

Fig. 13.2 Photograph of a spring-action biopsy gun used to perform testicular percutaneous needle biopsy (PNB) procedures

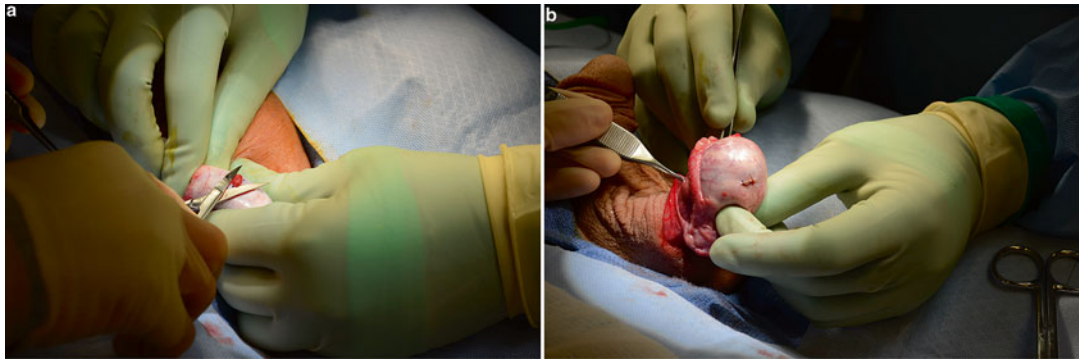

Fig. 13.3 (**a**) Intraoperative photograph of a surgeon using a pair of curved iris scissors to excise seminiferous tubules during a TESE procedure. (**b**) This photograph was taken during the same procedure after subsequent closure of the TESE site in the tunica albuginea using a 4-0 running suture

tubules (albeit in the absence of microscopic magnification) [30, 48, 51, 52]. Seminiferous tubules are typically excised from different geographical locations within the testicle.

Hemostasis is achieved using bipolar diathermy to reduce the risk of postoperative hematomas. While one study showed that 82 % of patients had evidence of hematoma and/or inflammation on scrotal ultrasound up to 3 months postoperatively, the vast majority of these findings represent normal postoperative changes, are clinically insignificant, and resolve spontaneously on ultrasound imaging within 6 months after the procedure [9]. Some patients in this study did have regions of permanent intratesticular devascularization, in particular when multiple biopsies were undertaken. The microscopic magnification of the micro-TESE procedure, which is discussed later in this chapter, assists in potentially reducing vascular injuries and optimizing hemostasis.

Positioning: The patient is placed in the supine position, and the scrotum is shaved and sterilely prepared. TESE can be performed under local, regional, or general anesthesia.

Incision: A transverse or a midline raphe scrotal skin incision is made and carried down through the dartos layer to the tunica vaginalis. The testis is delivered through the wound, and the tunica vaginalis is opened to expose the testicle. Next, an assessment of the testicle is performed, and horizontal incisions are made in the tunica albuginea in a fashion that minimizes injury to visible tunical vessels. If multiple ipsilateral TESE sites are necessary, some authors will make upper, middle, and lower pole transverse tunica albuginea incisions. Seminiferous tubules are extruded through the tunica albuginea incision site and sharply excised with the curved iris scissors. A wet prep specimen is made, and the microscope slide is inspected for sperm beneath a phase contrast microscope. Hemostasis is achieved with the bipolar microforceps, and the tunica albuginea is closed with a locked, running 5-0 permanent or absorbable suture. The tunica vaginalis is next closed using a 4-0 absorbable suture, and the dartos layer is closed with a 3-0 absorbable suture. Finally, the skin is closed with a 4-0 absorbable suture using a running horizontal mattress stitch, and gauze dressing, ice packs, and an athletic supporter are applied.

Microdissection Testicular Sperm Extraction (Fig. 13.4)

Micro-TESE is superior to TESE in regards to SRR, complications, and effects on testicular testosterone production [45, 53].

Positioning: The patient is placed in the supine position, and the scrotum is shaved and sterilely prepared. A general anesthetic is typically required for patient comfort.

Procedure: Either a midline median raphe incision or ipsilateral transverse scrotal skin incisions

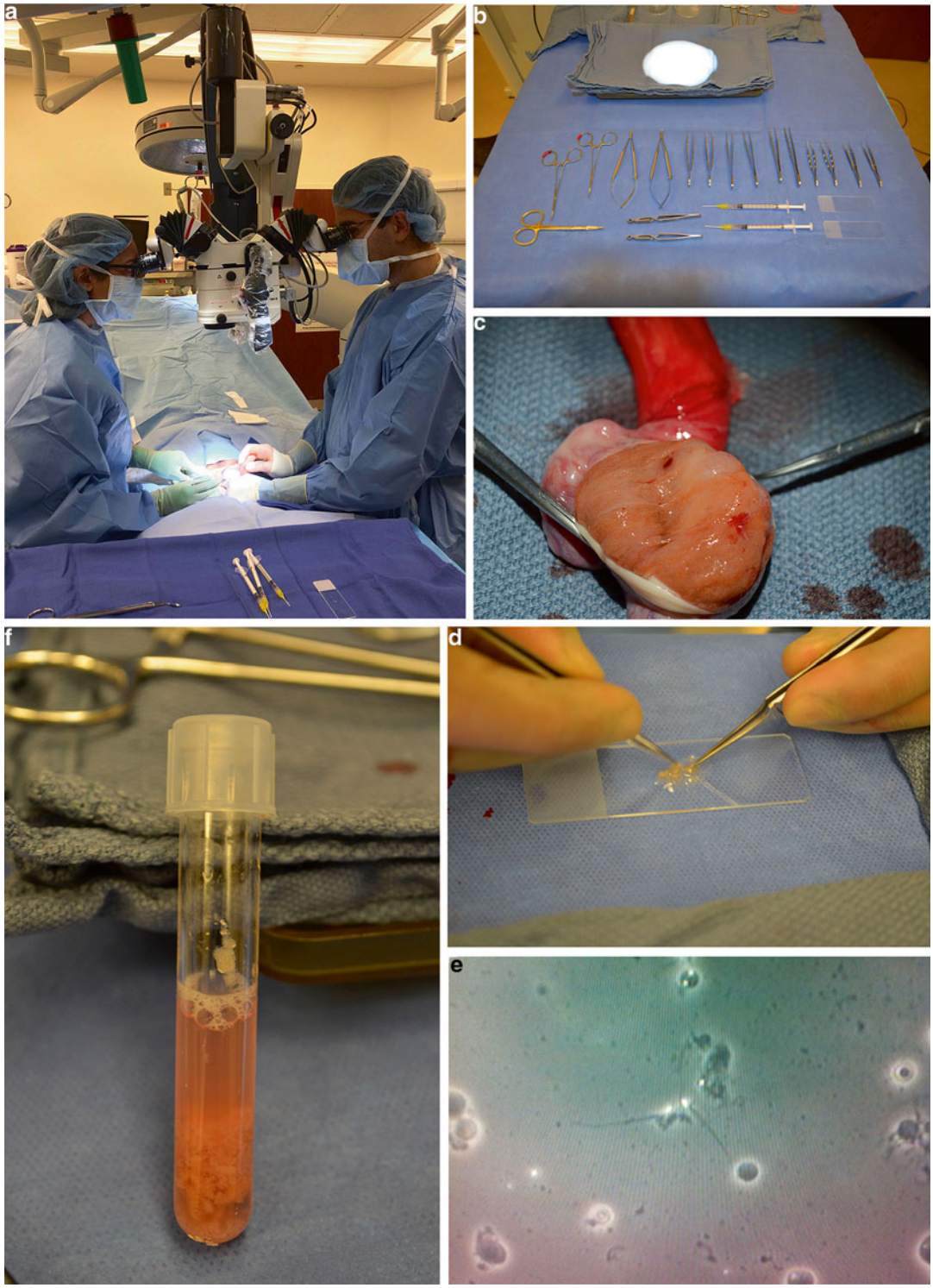

Fig. 13.4 (**a**) Photograph showing microsurgeons using an operating microscope to perform a micro-TESE procedure in a patient with post-chemotherapy, nonobstructive azoospermia. (**b**) Photograph of the microsurgical instrument tray. (**c**) Image of a bivalved testicle revealing the lobular anatomical organization of the seminiferous tubules. (**d**) Photograph showing the processing of excised testicular tissue using the jeweler forceps to create a wet prep slide. (**e**) Photographic image revealing spermatozoa seen on the testicular tissue wet prep slide when inspected under the phase contrast microscope. (**f**) Image of a vial of excised testicular tissue containing spermatozoa

are used. Exposure of the testicle is performed in an identical fashion to that used in the TESE procedure. After the tunic albuginea has been exposed, the operating microscope is brought into the field. With the microscope at 15×–25× magnification, a transverse incision is made in the mid pole of the testicle with a 15° ophthalmic blade, a microknife, or a #11 blade. An effort is made to avoid vascular structures. While a longitudinal tunica albuginea incision can be utilized, there is an increased risk of injury to the subtunical arteries given their transverse plane of orientation. With the microscope at 20×–25× magnification, the seminiferous tubules can be seen within septa. Microdissection of the seminiferous tubules facilitates access to deeper tubules and enhances the ability to preserve the intratesticular vasculature. This is important to reduce the risk of postoperative bleeding and scarring. The magnification also markedly assists in visualizing the larger and more opaque seminiferous tubules that are more likely to contain active spermatogenesis. The microdissection approach optimizes sperm yield, whilst reducing the tissue volume requiring biopsy 70-fold [54, 55]. Once identified, a candidate tubule is incised with iris scissors and a 24-gauge angiocatheter is used to process the tissue in a flush-through technique to increase sperm yield on the wet prep. If sperm are identified with adequate specimens, the procedure can be terminated following bipolar hemostasis and closure of the tunica with a 5-0 absorbable or nonabsorbable suture. The tunica vaginalis and dartos layers are closed with 4-0 and 3-0 absorbable sutures, respectively, and the skin is closed with a 4-0 absorbable suture using a running horizontal mattress stitch. If no sperm can be seen intraoperatively, some authors have advocated an additional, thorough laboratory search using enzymatic treatment. This approach has been shown to increase sperm detection by 25–30 % when sperm are not initially identified [56].

Conclusions

Approximately 50 % of males will develop cancer in the course of their lifetime. The vast majority of these patients will enjoy 5-year survival due to advances in cancer treatments. Unfortunately, though, some men will be rendered permanently azoospermic as a result of their chemotherapy and/or radiation therapy. In men in whom this azoospermia persists, TESA, PNB, TESE, and micro-TESE are all options to consider for TESE. Sperm that are successfully harvested can be used in the setting of IVF/ICSI, without apparent increase of risk of miscarriage or congenital anomalies in resultant offspring.

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Stem Cells and Fertility

14

James F. Smith and Kirk C. Lo

Introduction

Each year, over 700,000 malignancies are diagnosed in men in the United States [1]. For many of these men, the risk of infertility after cancer treatment is of primary concern [2]. Unfortunately, one of the side effects of many cancer treatments is temporary or permanent sterility [3]. In men undergoing stem cell or bone marrow transplants (BMT), at least 85 % are at risk of severe fertility impairment [4]. Worldwide, an estimated 50–60,000 of these transplants are performed annually with 60 % occurring in adults of reproductive age [5]; at UCSF, over the past 5 years, 513 adult men received BMT with a median age of 50. In children, more than 200 received BMT at UCSF in the past 5 years for both benign (e.g., Wiscott–Aldrich syndrome, severe thalassemia, sickle cell anemia) and malignant (e.g., leukemia, non-Hodgkin's lymphoma) conditions. Many more boys receive chemotherapies putting them at high risk for sterility (e.g., neuroblastoma, sarcoma).

Sperm cryopreservation is a well-established, standard clinical technique for adult men and postpubertal boys; however, *no established technique in humans exists to speed or correct this reproductive damage*. American Society Clinical Oncology (ASCO) [6], American Society Reproductive Medicine (ASRM) [7, 8], and American Academy of Pediatrics (AAP) [9] guidelines recommend fertility preservation counseling for all patients; however, sperm cryopreservation, the primary approach to preserving a man's fertility, does not allow future spontaneous conception [6]. A testicular biopsy performed prior to initiating chemotherapy can preserve spermatogonial stem cells (SSCs) and give prepubertal patients a chance at future fertility [10]. For men or postpubertal boys using cryopreserved sperm, advanced reproductive technologies (ART) such as intrauterine insemination (IUI) or in vitro fertilization (IVF), successful but expensive and invasive procedures, are required to conceive a child. The costs of these treatments can be prohibitive and impose significant barriers to their use [11, 12]. Development of SSC transplantation techniques to restore spermatogenesis after cancer treatment may allow couples to conceive naturally without costly and invasive ART. Concomitantly, SSC differentiation to mature spermatozoa from pediatric testicular tissue would allow boys the option of utilizing IVF. Many questions remain to be

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answered about which boys should be candidates for testicular biopsy. Exciting research advances on the horizon offer many potential solutions to help these boys achieve fatherhood after cancer therapy.

Methods for Fertility Preservation in Children and Adolescents Facing Sterilizing Therapy

Technical advances in semen cryopreservation in conjunction with IVF and intracytoplasmic sperm injection (IVF/ICSI) have revolutionized the options for fertility preservation in postpubertal male children [13] and cryopreservation of semen is a well-established method for sexually and reproductively mature boys [14]. The quality and number of healthy sperm cryopreserved determine the reproductive options available to these boys after completing therapy. Banking as many samples as possible and referring these patients for sperm banking early in the cancer therapy process is very important as the total number of moving sperm in a sample determines the future options for using this sample. Sperm cryopreservation options were discussed in more detail in Chap. 9 in the text.

Spermatogenesis starts before puberty and spermarche, the beginning of mature sperm production, often precedes the ability to ejaculate. Sperm may be detected in urine samples from these boys [15, 16], generally occurring around age 12 or 13 [17]. Therefore, it may be possible to obtain sperm from peripubertal boys using sperm retrieval techniques such as epididymal and testicular sperm aspiration [18]. Sperm obtained and cryopreserved from these techniques can be used later with IVF and ICSI, allowing for successful fertilization even in cases of severe oligospermia.

For boys who have not yet reached spermarche, investigational options provide significant hope for the future. These techniques rely upon the isolation and cryopreservation of SSCs from the prepubertal testicle prior to chemotherapy. This technique requires a testicular biopsy and cryopreservation of either whole tissue or isolated cells. After the patient is disease free, this tissue or cells may be thawed and used for induction of in vitro spermatogenesis or autologous transplantation into the patient's own testes.

Who Should Undergo Testicular Tissue Cryopreservation?

Two key questions underlie the decision to consider fertility preservation in children: will these children survive to adulthood and what is the risk of infertility as a result of their treatment? We know that fertility preservation is very important to survivors of childhood cancer survivors [2] and that these boys are much less likely than their peers to have children. While this latter observation is often directly related to impaired fertility, other factors, including the inability to maintain a long-term relationship, fear of cancer recurrence, and fear of death from cancer, can strongly impact the decision to seek fatherhood [19, 20]. Assessment of fertility damage remains problematic in childhood. For postpubertal males, semen analysis represents a good indicator of spermatogenesis and testicular function, and allows for sperm cryopreservation. This is not the case for prepubertal children or those who are unable to produce an ejaculate. Assessment primarily relies on the development of secondary sexual characteristics, including testicular and penile size, as well as the presence of pubic and axillary hair. Clinical examination may show soft testes of diminished size [21]. However, many patients can have severely impaired spermatogenesis but retain normal Leydig cell function and testosterone levels. Inhibin B, secreted by Sertoli cells, might be an indicator of diminished sperm production as a result of cytotoxic chemotherapy [22].

While the absolute assessment of fertility risk is critically important for patients and clinicians, no unified risk calculator yet exists. Development of comprehensive risk estimates based upon prospective cohorts of boys undergoing therapy would be of enormous value. For now, a number of manuscripts have documented fertility risks of many common cancer treatments (Table 14.1).

Table 14.1 Probability of fertility impairment in population of men cryopreserving sperm before fertility threatening treatment[a] [87]

Condition (treatment)	Probability of normality[b]	Probability of oligospermia[b]	Probability of azoospermia[b]
NHL (CHOP or variant)	42	12	46
HL (ABVD)	N/A	N/A	11.5
HL (MOPP, ChIVPP)	23	14	63
Leukemia	30	10	55
Testicular (BEP)	53	33	14
Testicular (radiotherapy)	65	17	18
Sarcoma	100	0	0
Carcinoma	68	11	21
Brain	N/A	N/A	67
Non-malignancy	72	11	17

[a]Sample size very small in nearly all groups. Follow-up ranged from 1 to 194 months. Sample size ranged from 1 to 154 men in each group. Percentage of men following up was also low and ranged from 9 to 45 %
[b]Normal defined as sperm concentration >15 million/mL; Oligospermia: <15 million/mL; Azoospermia: no sperm identified

Choosing the cut points at which one should recommend testicular biopsy is difficult and should be done in discussion with parents and referring oncologists. The following agents and treatment regimens put boys at the lowest risk for impaired fertility (ideally, this would be defined as a fertility risk less than a 25 % probability of future oligospermia or some other cutoff of induced subfertility; however these data do not exist). Methotrexate, vincristine, vinblastine, and mitoxantrone can induce temporary or permanent azoospermia but the majority of patients recover spermatogenesis [3]. Drugs and treatments in the intermediate risk of future fertility impairment (A useful clinical estimate would be 25–50 % probability of future oligospermia but this estimate does not exist) include bleomycin, etoposide, cisplatin, doxorubicin, fludarabine, and agent combinations like ABVD (adriamycin, bleomycin, vinblastine, dacarbazine). Patients receiving alkylating agent therapies are in the highest risk for future infertility (Ideally defined as >50 % probability of oligospermia or worse as an adult). Representative individual and multimodal therapies in the high risk category include cyclophosphamide, busulfan, cytarabine, agent combinations like CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone), gonadal irradiation, and total body irradiation [23]. While these agents are highly toxic to spermatogenesis, these effects are dose dependent. Some patients retain significant spermatogenesis and azoospermia does not occur in all patients. Scientists from St. Jude's Children's Research Hospital developed the cyclophosphamide equivalent dose (CED) as a means of comparing treatment regimens [24, 25]. Azoospermia was rare when CED was less than 4000 mg/m^2; however, many of these children will still develop significant degrees of oligospermia.

These data are critical to determining who should receive a testicular biopsy and becomes an ethical conundrum: at what level of infertility risk from chemotherapy is the risk of doing a testicular biopsy worthwhile? To put it simply, clinicians and researchers should utilize treatments that offer clinical benefit (beneficence) and minimize the risks and harms that arise from these treatments (non-maleficence) [8]. Furthermore, what benefits can patients and their parents realistically expect from allowing their sons to undergo a testicular biopsy? How big of a biopsy should be taken? In work from Belgium, the balance of risks and benefits suggested that taking 5 % of a single testis was an ethically appropriate amount [10]. However, the size of the biopsy should arguably be based upon the size needed to successfully allow fertility preservation, particularly given the high future risk of impaired testicular function in boys selected to undergo this biopsy.

This work from Belgium has demonstrated that spermatogonia were found in these testicular biopsies; yet, it is not known how many of these cells are necessary as starting material for in vitro differentiation of sperm nor the SSC concentration needed for testicular cell transplantation (TCT). It could be argued that a much larger biopsy is important to allow a boy a significant chance at fertility preservation or restoration; however, this larger biopsy might result in reduced testicular function and hypogonadism [26]. Although this observation may be true for adult patients not undergoing chemotherapy, the real question is how much additional risk does this biopsy entail above that the boy will experience from the chemo- or radiation therapy?

Ethical Challenges of Testicular Biopsy for Fertility Preservation

Postpubertal Boys

Depending on their age, postpubertal boys are often able to ejaculate and provide sperm for cryopreservation. Nevertheless, it is important to discuss this option with them in a comfortable setting, including discussions outside of the presence of their parents. For most families, semen obtained by ejaculation usually poses few ethical problems though religious and moral objections sometimes limit this approach [27–29]. The religious and cultural implications of the necessary techniques for male prepubertal fertility preservation have yet to be explored.

Prepubertal Boys

Methods of fertility preservation involving testicular biopsies and cryopreservation for prepubertal children are still experimental and should be conducted under Institutional Review Board (IRB) guidance [8]. Since these biopsies may ultimately prove beneficial for these children, they should be done with the boy's formal assent (for boys aged 7–17) in addition to the parental consent. While the potential enormous benefit of future fertility outweighs the small risks of testicular biopsy prior to cancer treatment [8], enthusiasm for this approach should be tempered by the understanding that successful development of fertility restoration in humans may take decades or never be achieved [18]. However, if the testicular biopsy does not occur prior to cancer treatment, the child may lose his best opportunity to protect his fertility.

Another important issue is the risk of mutations induced by the cytotoxic treatment. It is well established that some cancers have a genetic predisposition (e.g., Li-Fraumeni, retinoblastomas) [30]. However, sporadic cancers, including most pediatric malignancies, are thought to arise from low penetrance gene–environment interactions [31]. Although offspring of cancer survivors have not had an increased risk of developing cancer or congenital anomalies than the general population, these observations have been made in offspring of natural conception rather than men using ARTs [32, 33]. Selection of sperm with IVF/ICSI bypasses natural barriers to conception with an unknown risk of congenital anomalies for cancer patients.

Future Fertility Restoration Options for Prepubertal Boys

Two approaches may potentially help infertile boys become fathers after cancer treatment. Testicular tissue taken prior to chemotherapy or radiation therapy could be differentiated into mature sperm either in vitro or as a transplant to another species (xenotransplantation). This approach combined with IVF and ICSI has been successful in a neonatal mouse model [34]. Alternatively, autologous SSC transplant has been shown to restore spermatogenesis leading to healthy offspring in many non-primate models for more than 20 years and, 3 years ago, Hermann et al. demonstrated its success in primates [35]. However, neither approach has been attempted in humans.

In Vitro Growth of Spermatozoa

Despite advances in fertility treatment that have led to the routine use of IVF/ICSI for men with ejaculated or surgically obtained sperm concentrations approaching zero, these techniques are not possible for prepubertal boys. However, their testicles do contain SSCs [36] with the potential to expand and differentiate into mature sperm. Developing a technique to expand SSCs for autologous transplantation or to differentiate SSCs into mature sperm would be of tremendous value. Currently, much work has been done in animal models, yet the identification, isolation, and expansion of highly purified SSCs in humans have been limited by gaps in our understanding of human extracellular surface antigen expression and the cellular environment ("niche") necessary for SSC growth and differentiation [37–40]. Demonstrating the feasibility of this approach, in a neonatal mouse model, testicular tissue was harvested, cryopreserved, thawed, and cultured with resulting complete spermatogenesis [34]. IVF with ICSI was performed and two generations of healthy offspring were observed. The success of this technique in mice suggests that this organ culture method may be modified for application in other mammalian species, providing a solid technical base for use in fertility preservation for prepubertal boys facing sterilizing chemotherapy [34, 41]. Preliminary results using human SSC suggest that it may be possible to induce meiotic differentiation; however, arrest of maturation occurred and mature spermatozoa have not been observed [42, 43].

Several labs have successfully purified and expanded mouse SSCs using surface antigens (THY1, GFRα1, GPR125, and CD49f) [44–47]. Transplantation of SSCs expanded in vitro into germ cell depleted mouse testes has demonstrated a restoration of fertility for these animals [48]. While a number of recent studies presented data that human SSCs may be identified by expression of THY1, CD49f, EpCAM, and SSEA-4 [49–52], only SSEA-4 is highly expressed in embryonic stem cells and in both human fetal and prepubertal SSCs [36, 53]. Recently these hypotheses were supported by strong evidence suggesting cell populations expressing SSEA-4 are highly enriched for human SSC [37]. Previous work attempting to confirm spermatogenesis by transplanting putative SSC into germ cell depleted mouse testes resulted in limited colonization of human cells or cells expressing germ cell markers. The failure to initiate spermatogenesis was presumably due to interspecies differences [50–55]. Interestingly, mouse and human SSCs have been reported to have the capability to turn into testis-derived pluripotent stem cells during in vitro culture [50, 51, 56–60]. However, in contrast to studies in mice, recent human studies suggest that the human testis-derived pluripotent stem cells derived from in vitro culture of putative human SSCs are actually cells of mesenchymal rather than germ cell origin [37]. Future studies need to fill these gaps in our understanding of the human SSC niche [38–40, 61].

Due to the limited availability of human tissues, the lack of in vitro or in vivo xenograft models capable of supporting human spermatogenesis, and the significant ethical and logistical challenges associated with human germ cell research, current data on the identification, isolation, and expansion of human SSCs are mixed and highly controversial. To shed light on this controversy and lay the groundwork for a new therapy for young male patients facing sterilizing treatments, a detailed characterization of SSCs and the required somatic niche capable of supporting SSC expansion are required [62].

Testicular Cell Transplantation

Autologous SSC transplant is an exciting technique that has demonstrated success in restoring spermatogenesis in many non-primate models for more than 20 years [63–65] and, most recently, in primates [35]. These transplants have led to viable embryos and offspring in nonhuman models [35, 48, 66]. So far, these studies have not shown an increased risk of developmental or epigenetic defects in these offspring [67]. For cancer patients who may have malignant cells within their testes, TCT suggests the dangerous potential for malignant cell reintroduction.

Two potential transplantation approaches are possible: either using a mixture of testicular cells containing all cell types found in the testicle, or using specific populations of sorted, highly specific testicular cell populations. Whole cell testicular cell transplants have led to viable embryos and offspring in a number of non-primate and rhesus macaque models [35, 63, 66]. However, transplant of even a very small number of malignant cells back to a mouse can be lethal [68, 69]. While it is not yet possible to transplant a pure population of SSC nor is it known precisely which aspects of the cellular niche are most important for restoring sperm production in humans, recent data suggests that it is possible to purify and amplify human SSC and critically important supporting cells in human testicular tissue [37]. A significant advantage of transplanting isolated, purified SSC and other cell populations critical for spermatogenesis would be a much lower risk of malignant cell transfer. This risk is particularly important for boys with hematological malignancies such as leukemia and solid tumors with possible spread to the testicle. Using non-cancer xenografts, studies have demonstrated the safety of transplanting nonmalignant human testicular cells to nonhuman species without development of malignancy [70]; however, to achieve complete spermatogenesis, the testicular microenvironment is critical [71].

Xenotransplantation

Xenotransplantation offers another potential means to allow future fertility for boys cryopreserving testicular tissue. Cryopreserved tissue would be transplanted to a nonhuman species to complete spermatogenesis. Sperm differentiated in this way could be used for IVF/ICSI. Successful testicular tissue xenografting has been reported resulting in the completion of spermatogenesis in a variety of species [72–80]. While this approach has demonstrated the presence of spermatogonia and early cells of spermatogenesis, no studies have demonstrated complete human spermatogenesis after xenotransplant [81], rather success has been defined by growth of interconnected cells presumed to be spermatogonia or products of differentiation (Fig. 14.1).

A xenograft can survive for more than 135 days when fetal human testicular tissue is transplanted subcutaneously onto nude mice [82]. Sato et al. reported accelerated germ cell differentiation from spermatogonia to pachytene spermatocytes by grafting 3-month-old human testicular tissue onto nude mice [83]. Van Saen and colleagues reported that 9 months after xenografting, germ cells differentiated up to the level of secondary spermatocytes in the grafted testis from postpubertal boys, while no differentiation was seen in the prepubertal testis grafts [84]. The same group examined the effect of exogenous administration of recombinant human follicle stimulating hormone (FSH), which did not result in further differentiation in prepubertal human xenografts [85]. Wyns and colleagues successfully harvested and cryopreserved testicular tissue from five prepubertal cancer patients, aged 7–14 years, prior to chemotherapy [70]. This tissue was later xenotransplanted into the scrotums of nude mice and survived for more than 6 months. Further analysis showed the presence of premeiotic spermatocytes, a few spermatocytes at the pachytene stage, and spermatid-like cells. Furthermore, a group from the United Kingdom examined the short-term (6 week) xenotransplantation of early to mid gestation human fetal testes, and reported >75 % graft survival with normal morphology and germ cell differentiation from gonocytes ($OCT4^+$) to pre-spermatogonia ($VASA^+$) [86]. Although these advances are exciting, a great deal of additional research is necessary before xenotransplantation could become a practical and safe fertility restoration technology.

Conclusion

A growing number of boys survive cancer and desire the chance to father children. For postpubertal boys, standard fertility preservation techniques like sperm cryopreservation and testicular or epididymal cryopreservation are possible. For prepubertal boys, only experimental techniques

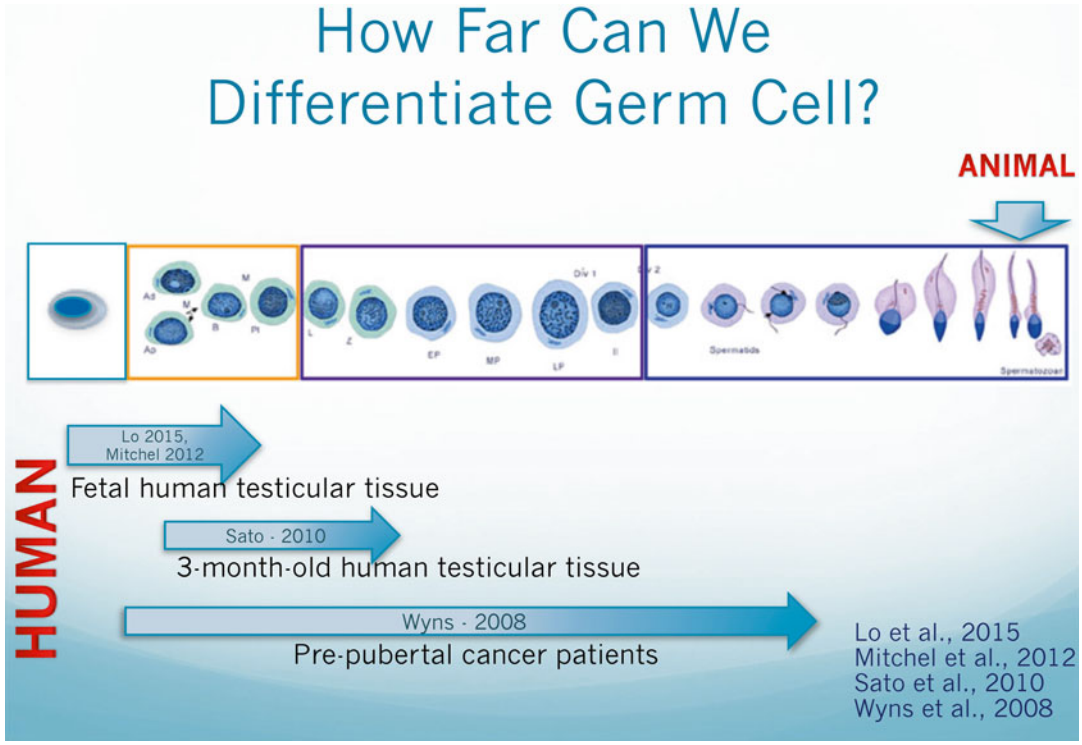

Fig. 14.1 Degree of experimental differentiation of testicular tissue in animals and humans

utilizing cryopreserved testicular tissue are currently possible. Advances in SSC technology have led to great optimism in the field and may offer these boys a chance at fatherhood. In vitro differentiation of SSC in conjunction with IVF/ICSI, testicular cell or spermatogonial cell transplant to restore spermatogenesis, and xenotransplantation with IVF/ICSI are leading techniques to make these dreams a reality.

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The Role of Assisted Reproduction in the Cancer Patient

15

Pasquale Patrizio and Paolo Emanuele Levi-Setti

Introduction

Advances in the diagnosis and treatment strategies for patients with cancers have led to remarkable improvements in the survival rates. However, the gonadotoxic risk of the various treatments (chemo and or radiotherapy) may impair the future reproductive chances unless fertility preservation options are utilized. Older women (>35 years old) are more likely to lose their ovarian function and become infertile post-chemotherapy than younger women [1]. However, it should be stressed that even for women who resume regular menstruation post-chemotherapy, the incidence of premature ovarian failure and resultant infertility is extremely high [2].

In general, there are various methods available to help "at risk" or future infertile patients to achieve a pregnancy. Some are classified under the general concept of "assisting" conception and include the use of medications to promote ovulation with or without the concomitant use of intrauterine inseminations (IUIs). The term assisted reproduction technology (ART) instead applies to techniques which involve the handling of gametes (sperm and oocytes) and embryos in vitro. Thus the term ART includes in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), embryo transfer, use of oocyte or embryo freezing, and the use of sperm surgically extracted (testicular or epididymal sperm). Many additional technologies exist such as preimplantation genetic screening (PGS) or diagnosis (PGD), in vitro maturation of oocytes (IVM), oocyte and sperm donation, and gestational surrogacy, but these are all additional treatments relying on the basic ART services of IVF/ICSI.

This chapter addresses both methods of assisted conception and assisted reproduction for infertile patients in general and for cancer survivors in particular.

General Overview of Ovulation Induction and the Medications

Ovulation induction is a pharmacological treatment for women who either do not have a spontaneous ovulation or to allow, by overriding the natural mechanism of mono-ovulation in humans, the development of more than one mature follicle.

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Table 15.1 Common medications used for controlled ovarian stimulation (COS)

Gonadotropins
1. Recombinant follicle stimulating hormone (rFSH)
2. Recombinant luteinizing hormone (rLH) produced by recombinant technology or extracted from urine of postmenopausal women
3. Human menopausal gonadotropin (hMG) extracted from urine of postmenopausal women (1 vial contains 75 IU of FSH and 75 IU of LH)
4. Urinary FSH (uFSH)
5. Recombinant human chorionic gonadotropin (rhCG) or extracted from urine of pregnant women
Gonadotropin releasing hormone (*GnRH*) *agonists/antagonists*
1. GnRH agonists: Leuprolide acetate; Nafarelin acetate
Buserelin acetate; Triptorelin acetate
2. GnRH antagonist: Cetrorelix acetate; Ganirelix acetate
Oral agents
– Clomiphene citrate (CC)
– Letrozole

It is also known as controlled ovarian stimulation or hyperstimulation (COS or COH) and is used in conjunction with either timed intercourse or for IUI or for IVF/ICSI.

Fertility medications commonly used for COH are: clomiphene citrate, aromatase inhibitors, gonadotropin hormones (follicle stimulation hormone—FSH, Luteinizing hormone—LH), agonists or antagonists of gonadotropin releasing hormone (GnRH), and human chorionic gonadotropin (hCG) (Table 15.1). Depending upon the type of ovulatory disorder, various protocols for COS can be planned. For example, at one end of the spectrum there are women with hypothalamic-pituitary failure and amenorrhea secondary to excision of pituitary tumors and typically have low follicular stimulating hormone (FSH), low luteinizing hormone (LH) and estradiol levels. In these instance, COS should be carried out with a combination of FSH and LH so to replace the deficiency in endogenous gonadotropins. Male patients may also have defective spermatogenesis due to pituitary tumors causing deficiency of gonadotropins (FSH and LH).

On the other side of the spectrum there are patients with elevated FSH, LH and low estradiol/testosterone levels and whose gonads have been damaged by chemotherapy or radiotherapy. These patients will suffer from premature ovarian insufficiency or testicular failure and are the most difficult to treat. For patients whose gonadotropin levels are normal, COS can be obtained with a variety of protocols.

Medications and Protocols for COS

Clomiphene Citrate

Clomiphene citrate is the most commonly used oral agent for induction of ovulation and stimulates endogenous FSH secretion by decreasing central estrogen negative feedback via estrogen receptor antagonism. It is typically begun on the third or fifth day after the onset of a spontaneous or progestin-induced menses. The starting dose is 50 mg daily for a 5 day interval and with increases, by 50 mg increments, for subsequent cycles until ovulation is achieved. Most women who respond to clomiphene do so at either the 50 mg (52 %) or 100 mg (22 %) dosage [3]. Spontaneous pregnancy rates are the highest during the first 3 cycles of clomiphene citrate treatment. Usually the LH surge is observed from 5 to 12 days after the last clomiphene citrate tablet. Studies in humans have not found an association between clomiphene citrate and congenital defects. Some of the common side effects include transient hot flushes and mood swings (10 %). Other mild and less common side effects include breast tenderness, pelvic pressure or pain, and nausea. However, if neurological side effects like headaches or visual changes (i.e., blurred or double vision, scotomata, or light sensitivity) arise, the clomiphene citrate treatment must be stopped. The risk for multiple pregnancies, mostly twins, is about 10 % [4]. Although some retrospective studies have initially reported an increased risk of ovarian cancer, recent studies have completely dismissed this claim [5].

Letrozole

The aromatase inhibitor letrozole is another option for COS, for either breast cancer patients/survivors or for patients diagnosed as clomiphene-resistant anovulatory women. At doses of 2.5–5 mg, letrozole decreases the estradiol levels by 97–99 % and is completely absorbed after oral administration, with a mean half-life of approximately 45 h (range: 30–60 h). Clearance from the systemic circulation is mostly by the liver. Contrary to clomiphene citrate, aromatase inhibitors block the peripheral estrogen production, causing release of pituitary FSH and LH. Letrozole does not have direct anti-estrogenic effects on the endometrium (less risk for thin endometrium) as it is seen with clomiphene citrate. In previous studies, endometrial proliferation was uniformly normal even though peak estrogen levels were 60–75 % lower than was observed during previous clomiphene treatment. Letrozole treatment is used at the dosage of 2.5 mg cycle days 3–7 and ovulation is triggered with exogenous hCG [6]. Letrozole has been shown to be more effective than clomiphene in hyperandrogenic anovulatory patients with polycystic ovarian syndrome [7]. Teratogenic effects of letrozole have been suggested but not proven in humans [8] and follow-up of babies delivered so far is reassuring. The common adverse effects of aromatase inhibitors are gastrointestinal disturbances, asthma, hot flashes, headache, and back pain [9].

Gonadotropins (FSH, LH, hCG)

Gonadotropins are either extracted from the urine of postmenopausal women or synthesized in vitro by using recombinant DNA technology [i.e., recombinant human FSH (rFSH), recombinant human LH (rLH), and recombinant human chorionic gonadotropin (rHCG)] [4]. The gonadotropins obtained from the urine of postmenopausal women may contain equal amounts of FSH and LH activity, while others contain primarily FSH with very low levels of LH or LH activity. Recombinant human FSH (rFSH), LH, and hCG are available as prefilled syringes or pen devices. Recombinant medications are widely used due to greater batch-to-batch consistency than the urine-derived products and decreased risk of urinary contaminants. Disadvantages of gonadotropins treatment include the need for frequent ultrasound to monitor follicular growth and the risk of multiple pregnancies which occurs with rates of up to 20 %. When planning COS for IUI, the starting dose of gonadotropin is low, about 50–75 IU/day, with close monitoring to avoid the risk of exaggerated ovarian response and ovarian hyperstimulation syndrome (OHSS).

Human Chorionic Gonadotropin

To achieve ovulation during COS and to promote the final stages of oocyte maturation with the progression from Prophase I to Metaphase II, the "LH surge" is mimicked by the administration of hCG at dosages between 5000 and 10,000 IU. When using recombinant hCG, the dose is 250 μg which is equivalent to approximately 6500 IU of hCG.

Intrauterine Insemination

Patients younger than 35 years old who have either not lost or have resumed their ovarian function after chemotherapy are good candidates for attempting some cycles of IUIs with COS prior to resorting to ART [10]. A recent Cochrane review showed that the addition of COS to IUI treatment improves live birth rates [11]. Many authors recommend switching to ART after three to six unsuccessful COS and IUI. Patients older than 35 years who have resumed their ovarian function and have unsuccessfully attempted pregnancy for 6 months or more, should be offered ART since reduced ovarian reserve may or may not be apparent, but oocyte quality is certainly a factor for their reduced chances of pregnancy.

The conventional approach starts with use of clomiphene citrate (50–100 mg daily for 5 days starting on cycle day 3) or letrozole (2.5 mg cycle day 3–7) CC alone with IUI before resorting to

the use of injectable gonadotropins (FSH and LH) and IUI.

The clinical pregnancy rate with IUI is 11–33 % for all causes of infertility after 3–4 cycles (Levi Setti). Semen is "processed" in the laboratory by either swim-up or filtration gradients methods and the final sample containing normal motile sperm in a small volume (about 0.4–0.5 mL) is inserted into the uterine cavity using a small catheter.

For the correct timing of the insemination, cycle's monitoring is generally carried out by ultrasound assessment of follicle growth. When one or two follicles are ≥18 mm, ovulation is triggered by hCG. It is still debated whether the IUI should be carried out the day after hCG administration or 36 h later. Likewise, it is still debated whether one IUI per cycle is sufficient as opposed to two consecutive ones. A recent study examining single versus double IUI for unexplained infertility has shown no clear benefit of double over single inseminations. The incidence of multiple pregnancies after treatment with COS and IUI varies between 10 % (with clomiphene or letrozole cycles) and 30 % (when using gonadotropins) and the overall contribution of IUIs to multiple births is estimated to be around 30 % [10].

Ovarian Stimulation for ART: IVF/ ICSI

The choice of a protocol of stimulation varies according to the cause of infertility, age of the patient, body mass index (BMI), ovarian reserve (AMH), and response to previous cycles of assisted reproduction or ovulation induction [4]. During COH, the ovarian response is monitored with both hormonal blood levels and ultrasound imaging. In general, the ovarian stimulation protocols can be generally divided into two groups: (1) long or luteal phase protocols and (2) short or follicular phase protocols with GnRH antagonists. There is still an ongoing debate on whether one treatment is superior to the other [12].

GnRH Agonists or Antagonists

The rationale for using GnRH agonists and antagonists during ART cycles is to prevent premature LH surges and thus avoiding cycle cancellation [4]. The chemical structure of the native GnRH is a decapeptide with three sites responsible for the physiological actions: (a) activation of the GnRH receptor on the pituitary cells (amino acids [aa] in positions 1–3); (b) regulation of GnRH receptor affinity (aa 5–6); and (c) regulation of biologic activity (aa 9–10). Modifications in any of these areas change the properties of the native GnRH molecule (GnRH analogs). The GnRH analogs are classified as either agonist or antagonist. Contrary to the GnRH antagonist, the GnRH agonists first stimulate an acute release of pituitary FSH and LH known as the "flare" effect which last for about 5–7 days, causing ovarian stimulation and a rise in estradiol levels. The continuous administration of GnRH agonist no longer causes activity of the pituitary gonadotropin receptors in a process known as desensitization or down regulation.

Among the many formulations of GnRH agonists, the most commonly used are leuprolide acetate (administered subcutaneously) and nafarelin acetate (administered by intranasal spray). For leuprolide acetate, the glycine in position 6 has been replaced by D-leucine and the glycine in position 10 has been removed. The substitution at position 6 helps protect against enzymatic degradation.

The GnRH antagonists are structurally very similar to the GnRH receptor; they exert a competitive binding with the native pituitary receptor causing an immediate (within hours) decline of FSH and LH levels without the flare effect as seen with the GnRH agonist [13]. The two GnRH antagonists available for clinical use are ganirelix and cetrorelix, both equally potent and effective. Amino acid substitutions at positions 1, 2, and 3 are important for the antagonistic effects and substitution at position 6 helps protect against enzymatic degradation and enhances aqueous solubility. Substitutions at positions 8

and 10 help to reduce the histamine release effects that plagued earlier generations of GnRH antagonists.

GnRH Agonist Followed by Stimulation with Gonadotropins: "The Long Protocol" for IVF

In a typical long suppression cycle, GnRH agonist treatment begins during the midluteal phase about 1 week after ovulation, at a time when endogenous gonadotropin levels are at or near their nadir. When using leuprolide acetate, the dosage begins with 0.5 mg daily for approximately 10 days and is then reduced to 0.25 mg when the gonadotropins are added with the onset of menses (Fig. 15.1). For nafarelin, the initial dose is typically 400 μg twice daily and is decreased to 200 μg when stimulation starts. Before the initiation of gonadotropin stimulation, suppression of serum estradiol levels (less than 75 pg/mL) and the absence of ovarian cysts should be documented. The dose of the gonadotropins is usually kept constant for the first 4–5 days of COH. For patients with normal ovarian function and younger than 38 years old, the starting dosage of gonadotropins is between 150 and 225 IU. The patient's initial response is assessed by transvaginal ultrasound (to visualize the number and size of developing follicles) and serum estradiol level on cycle day 5. If the follicular growth proceeds slowly (follicular diameters less than 11 mm and estradiol levels <150 pg/mL), the gonadotropin dosage can be increased. On the other hand, if the follicular response is rapid (estradiol levels higher than 500 pg/mL), the dose of gonadotropin should be decreased. hCG is administered to trigger release when an appropriate number of mature follicles have developed ranging in size between 17 and 20 mm in diameter.

Antagonist and Gonadotropin Protocol

Gonadotropin releasing hormone (GnRH) antagonists were introduced in recent years in ovarian stimulation for assisted reproductive technologies (ART) to exert an immediate inhibition of a premature rise in luteinizing hormone (LH). Because of the immediate effect of the GnRH antagonists can be administered when criteria suggesting a risk for an LH surge are met (i.e., follicle size of 13 mm or larger, or estradiol level >400 pg/mL).

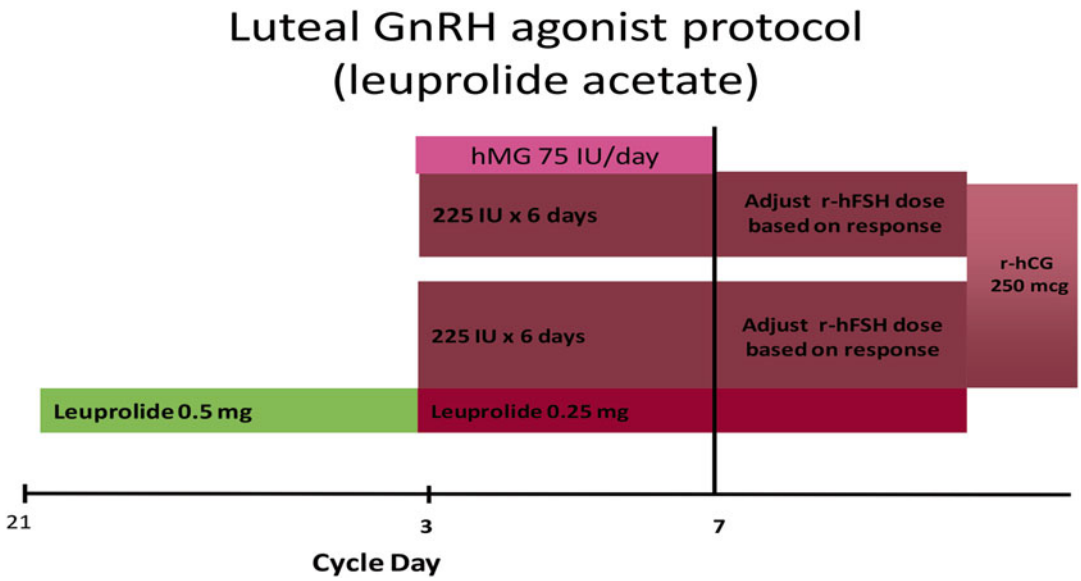

Fig. 15.1 The luteal GnRH agonist protocols for ART

The two GnRH antagonists commonly used for clinical use as mentioned before are ganirelix acetate and cetrorelix acetate. For both, the minimum effective dose to prevent a premature LH surge is 0.25 mg administered subcutaneously every day until the day of hCG [13]. An alternative option is a single dose where a 3 mg dose of GnRH antagonist is given on cycle day 7 during ovarian stimulation. Four randomized controlled trials have so far been performed comparing a fixed (on day 6) versus a flexible (by a follicle diameter of 14 mm) protocol of GnRH antagonist administration [13] showing no difference in pregnancy rates.

Protocols for Breast Cancer

Protocols for ovarian stimulation in breast cancer patients need to minimize the rise in estradiol concentrations since experimental data have suggested that estrogen can have an indirect mitogenic and growth-promoting effect on breast cancer cells, especially in tumors positive to estrogen receptors [4]. Safer stimulation protocols include tamoxifen alone or combined with gonadotropins, or, most commonly, the use of aromatase inhibitors (for example, letrozole) to keep estradiol at very low levels.

Combined letrozole, gonadotropin and antagonist protocols have shown significantly lower peak estradiol levels than standard IVF [14]. Letrozole is started orally on the second day of the menstrual cycle at a dose of 5 mg/day until the day of hCG. 150–300 IU/day of recombinant follicle stimulating hormone (depending on patient's age and ovarian reserve) is initiated on the third day of the cycle and GnRH antagonist is administered when the leading follicle has reached 13 mm in size or with estradiol levels ≥300 pg/mL. GnRH analog can be administered to trigger ovulation instead of hCG because the latter has a longer half-life and thus may prolong high estradiol levels. Letrozole is reinitiated after oocyte retrieval and continued until the estradiol level falls below 50 pg/mL [15]. Initial follow-up data of breast cancer patients who have used this stimulation protocol did not demonstrate an increased risk of recurrence.

Poor Ovarian Responders

Women with a family history of premature ovarian failure, a personal history of chemo-radiotherapy or removal of ovarian tissue generally are poor responders to ovarian stimulation. Stimulating follicle production in poor responder patients is a challenge to many providers. When the patient has no prior stimulatory cycles, a poor responder is generally defined as someone with low anti-mullerian hormones (AMH), low antral-follicle count (AFC < 4), small ovarian volume, elevated FSH (>15 IU/mL), or older than 42 years of age [16]. For patients with prior ovulation induction cycles, a poor responder is someone who had 3 or less oocytes, or estradiol less than 500 pg/mL in her previous cycles. This incidence of poor responder varies wide from 9 to 26 % because the definition of poor responder varies in different studies [16]. The alternative management options include using GnRH antagonist instead of a long acting agonist, or using a mini-dose GnRH agonist protocol, or beginning with higher doses of gonadotropin stimulation. However, doses greater than 450 IU daily generally provide little if any benefit. There are no drugs that can boost fertility in cases of ovarian failure. In these instances, oocyte donation can be considered or moving to other options such as adoption or accepting childlessness.

Random-Start Protocol

Many cancer patients have to go through a cycle of ovarian stimulation, for either oocyte or embryo freezing, quickly. To minimize delays, random-start protocols for ovarian stimulation have been devised, exploiting recent evidence indicating waves of recruitable follicles during various stages of the entire menstrual cycle [17–19]. Briefly, patients in need for emergency fertility preservation, irrespective of where they are in their menstrual cycle, can begin ovarian stimulation almost immediately. Even patients that are on cycle day 15 (immediately post-ovulatory) of a 28 days menstrual cycle can start assuming FSH (and letrozole if diagnosed with hormonally

sensitive cancers such as breast, endometrial, and ovarian) while GnRH antagonist is added few days later. The random-start ovarian stimulation protocol is plausible especially when the endometrial development is irrelevant as is the case in fertility preservation. The total duration of stimulation can last between 9 and 12 days. The three patients in the case series started letrozole and FSH while on cycle days 11 (late follicular phase), 14 and 17 (early luteal phase) of the menstrual cycle, respectively. GnRH antagonist was provided after 5 days of stimulation for the two patients who were in luteal phase and from the outset of stimulation for the patient in the late follicular phase. In all three cases, there were follicles that reached preovulatory stage after 9–12 days of stimulation. Oocytes were collected from each case (range 9–17) and each patient had frozen embryos (range 7–10) [17]. It must be mentioned, however, that as of now, it is not known whether oocytes or embryos frozen with random-start ovarian stimulation will provide pregnancy rates comparable with those originating from conventional stimulation cycles. Bedoschi et al. described emergency ovarian stimulation in two cases, one with breast cancer and the second with Hodgkin lymphoma where 12 mature oocytes were retrieved in both cases and all mature oocytes were subjected to ICSI with fertilization and cleavage rates of 83.3 % and 70 %, respectively [20].

It cannot be left unmentioned that another strategy to maximize outcomes for emergency fertility preservation is in vitro maturation of oocytes immature at the time of retrieval [19, 20].

IVF/ICSI

When the development of the ovarian follicles is optimal (leading follicles of 18–20 mm in diameter), the patient is instructed to administer hCG, an equivalent of luteinizing hormone (LH), so to assure the completion of oocyte maturity. About 36 h after hCG, the oocytes are harvested through the vagina with a needle under ultrasound guidance (Fig. 15.2) [21, 22]. The oocyte retrieval is completed in about 15 min and can be performed either under general anesthesia or with sedation and local anesthesia. Once the retrieval is completed, the oocytes are prepared for insemination. The process of insemination with husband or partner's sperm or with donor sperm can be done either with conventional insemination, i.e., IVF, or with the technique of ICSI. With ICSI, a single spermatozoon is loaded into a microneedle and then injected into a mature egg. Since the chemotherapy or radiotherapy treatment may render the female infertile, ICSI may be utilized in order to maximize the fertilizing potential of any available oocytes for cases of fertility preservation. In addition, ICSI minimizes the risk of possible, unanticipated failed fertilization due to an undiagnosed male factor infertility.

For convention, the day of the egg retrieval is identified as day 0, while the day for fertilization check is day 1 (this is the day when the fertilized oocyte shows two distinct pronuclei produced by the fusion of the male and the female nucleus). The next day (day 2) is when the first cell division, also known as cleavage, occurs. On day 3, the embryo may have between 4 and 10 cells, while on day 4 is at a stage known as morula (about 60–80 cells) and on day 5 is at a stage of development known as blastocyst (>80 cells) and begins the formation of a fluid-filled cavity known as blastocele which contains the inner cell mass (the future fetus) and, on the outer layer, the trophoblast cells (the future placenta),

Today, the majority of embryo transfers take place either on cycle day 3 or on cycle day 5. The embryo transfer (ET) is carried out with a soft plastic catheter passed through the cervix and the aid of an abdominal ultrasound allows visualization of the catheter for proper positioning and release of the embryo(s) in the uterine cavity. From the time of oocyte retrieval to the time of the pregnancy test, the patient is instructed to take progesterone supplements as supportive of the luteal phase. In established IVF clinics, the across all age odds of pregnancy and birth after embryo transfer are about 30 %. Younger patients (<35 years old) have successful birth rates of about 45 % per transfer, while women older than 40 have about 20 % and women between 43 and 44 years about 8 % and

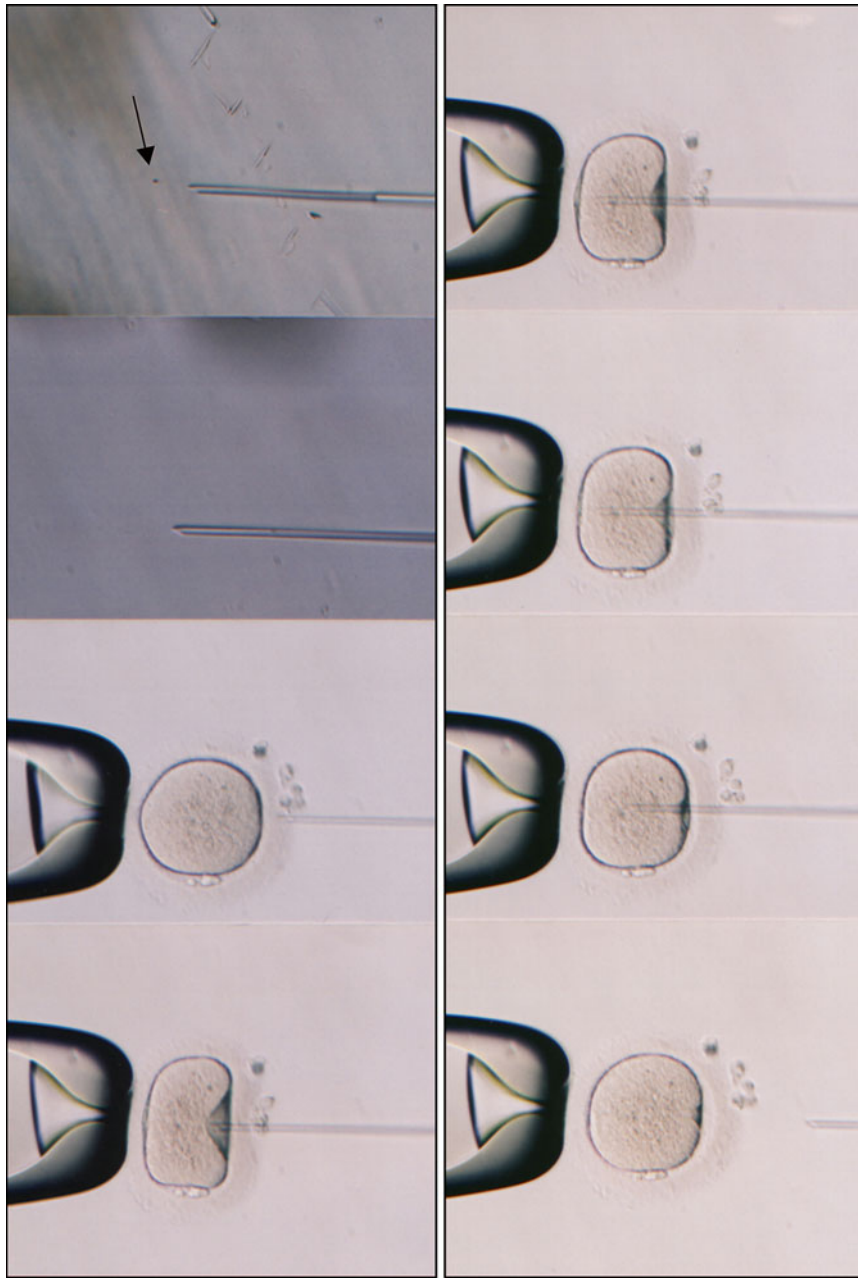

Fig. 15.2 Sequence of events to perform intracytoplasmic sperm injection (ICSI). The *arrows* indicate a spermatozoon ready to be picked up by the microinjection needle

4 %, respectively. At age 45, the odds of delivering a child after IVF using their own eggs is about 1 %. The actual woman's age together with the markers of ovarian reserve (AMH, AFC) remains the most significant predictor for live birth even in cancer survivors who have retained or regained their menstrual function.

Cancer patients who undergo ART for fertility preservation have to choose either cryopreservation of oocytes (mostly if they have no partner) or embryos. Oocyte cryopreservation with the relatively new technique of vitrification as opposed to slow freezing has been shown to be an efficient method to preserve fertility with high

oocyte survival rates. Briefly, mature oocytes are denuded of their cumulus cells and exposed to solutions containing various concentrations of cryoprotectants before being plunged directly in liquid nitrogen.

Embryo freezing is generally carried out either at the pronuclear stage of development (day 1 after oocyte retrieval) or at the cleavage stages (mostly at the blastocyst stage which is 5–6 days after oocyte retrieval). Embryo freezing has been available and offered for many more years than oocyte freezing and thus many more centers have the required experience to anticipate high embryo survival rates after thawing.

PGS/PGD

Another procedure for IVF is the examination of oocytes and embryos for chromosomal and genetic abnormalities. Preimplantation testing can be for chromosomal aneuploidy or aberrations screening (called PGS for preimplantation genetic screening) or for genetic diagnosis (called PGD for preimplantation genetic diagnosis). Embryo testing was originally developed for couples at high risk for passing to their children a genetic disorder such as cystic fibrosis or Tay-Sachs disease. PGS/PGD can be carried out on polar-bodies (analyzing the DNA of the first and second polar body), on blastomeres (analyzing a single cell from a day 3 embryo) or, more recently, on trophectoderm cells (by examining extra-embryonic cells from a day 5 embryo). Today, trophectoderm biopsy is the most favored because is safer, more accurate, and least invasive for the embryo. In addition to PGD, PGS is carried out for screening all the 23 pairs of chromosomes with technologies known as array comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP). Next generation sequencing (NGS) is the newest methodology and it could test for about 3000 genes on just one chip.

Hundreds of genetic traits are associated with an increased risk of neoplasia and about 5–10 % of common adult cancers are hereditary. Many of the genes responsible for hereditary cancer predisposition are involved in the control of cell growth and differentiation or in DNA repair and maintenance of genomic integrity. In the context of ART, patients with a known strong familiar predisposition to cancer can benefit from PGD/PGS to screen embryos free of BRCA1/2 mutations, Lynch syndrome, HLA-matching, and many more other genetic conditions.

Table 15.2 Outcome of oocyte cryopreservation in cancer patients

N. patients/N. cycles	30/37
N. reaching embryo transfer	25
N. eggs cryopreserved	208
Survival rate %	85 %
Clinical pregnancies	12
Deliveries	10

Clinical Outcome After Oocyte Freezing in Cancer

Few studies have been published specifically addressing the clinical outcome of oocyte freezing in cancer patients, while the literature is abundant for outcome data on oocyte cryopreserved and then used for other indications such as oocyte donation and for infertile patients. Overall, the great majority of patients who have frozen oocytes are affected by breast cancer followed by Hodgkin and non-Hodgkin lymphoma; the oocyte survival rate is about 85 % and the delivery rate about 30 %. Of note, there have been no reports of increased incidence of congenital anomalies [23–27] (Table 15.2).

Conclusions

IVF and other forms of assisted reproduction have now become a routine medical intervention for infertility resulting in the birth of millions of children.

Particularly in the context of fertility preservation for cancer patients, the various forms of ART play a pivotal role in safeguarding future chances for parenthood as long as patients are referred early to reproductive specialists.

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Third Party Reproduction

16

Gretchen Collins and James M. Goldfarb

Abbreviations

ART	Assisted reproductive technology
DI	Donor insemination
DSR	Donor sibling registry
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
UPA	Uniform Parentage Act

Third Party Reproduction in Cancer Patients

Patients who will be undergoing cancer therapy that will likely result in sterility should be counseled on fertility preservation treatments if the cancer treatment timeframe allows. However, sometimes fertility preservation treatments are not done because cancer treatments need to be started shortly after diagnosis, patients are not offered fertility preservation treatments, or patients do not choose to undergo fertility preservation treatments. In these cases, third party reproduction is an option once their cancer treatment is completed. Third party reproduction is any reproduction in which gametes or gestation is provided by a third party or donor other than the two parents who will raise the resulting child. This chapter will discuss the medical, legal, and psychological implications of third party reproduction treatments for both men and women cancer patients.

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Women

Women who had either chemotherapy or radiation to the ovaries, or surgical resection of the ovaries, have the option of either oocyte donation or embryo donation. Alternatively, women whose cancer treatment has left them without a uterus may still use their own ovaries for embryo formation, but must use a gestational carrier for the pregnancy.

Oocyte Donation

History of Oocyte Donation

In the mid-1980s, two scientific teams on two separate continents (North America and Australia) were working simultaneously to produce the first

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donor oocyte pregnancy. Both teams reported successful pregnancies in 1984. These pregnancies were conceived using two different methods. The Los Angeles group, led by M. Bustillo and J. Buster, first inseminated donors with the recipient's husband's sperm followed by uterine lavage with a special catheter. Embryo recovery took place 5 days after insemination, and the synchronization of the recipient's endometrium was done using oral contraceptives [1, 2].

The first successful donor oocyte cycle is generally attributed to P. Lutjen (Australia), who reported in *Nature* on a 25-year old with primary ovarian insufficiency. Donor oocytes from a patient with tubal factor infertility were inseminated with the recipient partner's sperm. Synchronization of the endometrium was achieved with a combination of oral estradiol valerate and an intravaginal progesterone pessary. The resulting single two-cell embryo was transferred to the recipient's uterus, and the recipient was maintained on continuous estrogen and progesterone throughout the pregnancy, with delivery at 38 weeks via scheduled cesarean section [3]. This was the first time a recipient with no ovarian function of her own was found to reliably produce receptive endometrium with exogenous estrogen and progesterone.

Medical Aspects of Oocyte Donation

Guidance and Standardization in Donor Programs

Oocyte donation has important socio-medical-economic implications for all the participants. Egg donor programs involve donation of part of the genetic pool, or all of the pool if used in combination with sperm donation. The recipient, her partner, offspring, supporting social structures, and the medical team all play important roles. It is imperative for a successful reproductive donor oocyte program that donors and recipients undergo adequate screening and counseling provided by a team of specialists familiar with third party reproduction (i.e., reproductive endocrinologist, mental health specialist, nursing staff, and embryologist) [4]. This creates a system with multiple medical checkpoints that will increase program safety by correctly and carefully identifying inclusion and exclusion criteria.

Cost is a major factor for most people desiring third party reproduction, as there is not insurance coverage for fertility treatments in most states in the USA. The most common scenario for couples undergoing egg donation is for the couple to obtain an anonymous egg donor through an egg donation agency. The total costs for this tend to run in the $25,000 range—approximately $5000 payment to the egg donor, $5000 agency fee, and $15,000 for the medical procedures. Costs per cycle can be decreased by using frozen eggs obtained from an egg bank. However, there has been some questions as to whether this is truly more cost effective in the long run since the success rates tend to be somewhat lower using frozen rather than fresh eggs. Another way of decreasing cost is for the egg donor to be used for 2 recipients or for a person going through in vitro fertilization (IVF) for themselves to share eggs with a recipient couple [5]. Fortunately for cancer patients, there is financial help available through Livestrong, Fertile Hope, pharmaceutical companies, and programs through some fertility centers.

Oocyte Donor Selection and Screening

Donated oocytes are available in two forms: fresh oocytes donated from an IVF cycle specifically performed for the recipient, or frozen oocytes purchased from an egg bank. Oocyte donation, as any other medical procedure, requires patients/donors to be fully informed of all the proceedings, selection requirements, pre- and post-selection medical workups, medications, side effects, short- and long-term associated risks, and realistic outcomes. Preconception testing and counseling are recommended for all parties. There are strict FDA screening requirements, which are listed in Table 16.1.

There are three sources of eggs for donation:

1. Anonymous donors
2. Known donors
3. IVF patients who will share their eggs

In all cases, eggs can be used fresh or they can be frozen. In addition, particularly with anonymous donors, the eggs can be donated exclusively to one recipient or can be shared between more than one (usually 2) recipients.

Table 16.1 FDA required infectious disease testing and other recommendations for third party reproduction

FDA requirement	Sperm donor	Oocyte donor	Embryo donor	Biologic parent (for gestational carrier)	Gestational carrier
Infectious disease testing	–	–	–	–	–
HIV-1 and HIV-2 antibody	X	X	X	X	–
Hepatitis B surface antigen	X	X	X	X	–
Hepatitis B core antibody IgG and IgM	X	X	X	X	–
Syphilis (RPR or VDRL)	X	X	X	X	–
Chlamydia trachomatis	X	X	X	X	–
Neisseria gonorrhea	X	X	X	X	–
Human T-lymphotropic virus (HTLV), types I and II	X	–	–	–	–
Cytomegalovirus	X	–	–	–	–
1. Offered psychology consultation		X	X	X	–
Medical Questionnaire to assess risks for communicable diseases	X	X	X	X	–
Specific physical exam to assess for signs of communicable diseases	X	X	X	X	–

Known donors are often relatives, sisters, nieces, etc. Clearly when a known donor is used, counseling to rule out coercion is essential [6–8]. Shared IVF oocyte donor programs have been shown to be cost-effective, increasing the number of donor cycles, decreasing the waiting period, and resulting in equivalent fertility outcomes [6, 9].

The ASRM recommends that donor age be between 21 and 34 years, although there is considerable variation among centers [10]. If the donor is older, recipients should be properly informed about the increased cytogenic risk and the effect of donor age on pregnancy rates. Personal health and sexual history should be obtained with the intent of excluding those women at high risk for HIV and other sexually transmitted diseases. Family, medical, and psychiatric history should be reviewed. The donor and their partner should also have a psychological evaluation by a qualified mental health professional assessing the psychological adequacy of a potential donor. This will lead to informed consent that is tailored to each donor and potentially reduce the possibility of financial or emotional coercion [11].

Oocyte Donor Process

The process of oocyte harvesting is similar for donor and non-donor women undergoing controlled ovarian hyperstimulation (COH), oocyte retrieval, and IVF/ET, although some minor differences exist. A particular challenge is imposed by the use of fresh donor oocytes, which requires a timely and precise synchronization of the donor stimulation protocol with endometrial preparation of the recipient. This requires an experienced reproductive team that will monitor the donor and recipient closely. Even though the majority of donor cycles will be synchronized with embryo transfer (ET) after fresh retrieval, the use of newer cryopreservation techniques (i.e., vitrification) with excellent outcomes is becoming more popular and does not require synchronization

between the donor and recipient [12–15]. Given the increased success of egg freezing with the use of vitrification, patients are now able to freeze eggs for future fertilization, or purchase frozen eggs from a commercial egg bank [16].

Most donor stimulation protocols will involve daily injections of gonadotropins with an FSH-like action commencing in the first 2–5 days of the menstrual cycle and continuing for 7–12 days, depending on the ovarian response. Substantial variations of protocols are common, and the one chosen will depend on the profile of the particular donor and the achievement of suitable endometrial development in the recipient. Stimulation of the donor is now commonly started with gonadotropins, and as the follicles develop, a GnRH antagonist is added to prevent premature ovulation. With this protocol, leuprolide can be used as the trigger shot. The use of this protocol essentially eliminates the risk of ovarian hyperstimulation for the egg donor [17]. Multiple studies comparing GnRH antagonist to other protocols have found no significant difference in the number of oocytes retrieved or ongoing pregnancies when donor eggs are being used [18, 19]. Monitoring the donor is done through frequent ultrasounds targeted at observing the growth of the developing follicles and serum estradiol levels. Once the follicles are of the size that indicates the eggs inside them are ready to be matured, a trigger shot (generally HCG or leuprolide with or without a small dose of HCG) is given to finalize the maturation of the eggs. The eggs are then retrieved from the ovary approximately 36 h later. The eggs are retrieved by a vaginal route under ultrasound guidance. The patient is generally given mild sedation.

Recipient Endometrium Preparation

If fresh embryos are to be used, the cycles of the donor and recipient must be synchronized so the endometrium will be receptive to the embryos. There are many ways to synchronize the cycles including the use of oral contraceptives and GnRH agonists. Once the onset of menses is synchronized, the recipient is generally given estradiol orally or vaginally to stimulate proliferation of her endometrium. Early initiation of estradiol therapy suppresses follicular development and ovulation [20]. Because the donor stimulation interval may be shorter than the follicular phase of a normal menstrual cycle, it is common to start the recipient's estradiol a few days before initiation of the donor's gonadotropin therapy [21–23]. Most protocols utilize a constant oral (4–8 mg/day) or transdermal (0.2–0.4 mg/day) estradiol dose. An ultrasound is done about 10 days after initiation of estradiol to ensure adequate endometrial response to the estradiol. If the recipient is menopausal due to previous chemotherapy, there is obviously no need for her to use oral contraceptives or GnRH agonists. Estradiol in this case can merely be started several days before the donor begins her stimulation.

In recent years, the efficacy of using frozen embryos has increased greatly. Therefore, it is becoming more common for embryos from donor eggs to be frozen rather than transferred fresh. This eliminates the need to synchronize donor and recipient cycles. Most commonly, the preparation of the endometrium is done the same as when fresh embryos are used. However, when frozen embryos are used, it is also possible to transfer the embryos without use of estradiol priming if the recipient's cycle is very regular.

When estradiol is used to prepare the endometrium, the use of exogenous progesterone is vital to replicate the physiological postovulatory endogenous production of progesterone by luteinized granulosa cells, transitioning the endometrium to a secretory pattern that is receptive to embryo implantation. Escriba et al. randomized recipients of fresh donor oocytes to start progesterone the day before, the day of, and the day after oocyte retrieval (OR) and reported higher pregnancy rates (OR 0.87, 95 % CI 1.13–3.08) in those who received replacement therapy on the day of, or the day following egg retrieval [24]. Progesterone supplementation may be given via IM injection, vaginal tablet, or vaginal gel [25]. For fresh embryo transfers, IM and vaginal progesterone seem to have comparable pregnancy rates. There is a greater debate regarding the efficacy of IM versus vaginal progesterone in frozen embryo cycles. Small randomized trials and larger retrospective studies have conflicting

results showing either equal or higher pregnancy rates for IM progesterone.

The transfer is a minor procedure that entails atraumatic transfer of the embryos into the endometrial cavity. Generally anesthesia is not required except for the very rare patient for whom the transfer is very difficult. US guidance is generally performed at the time of transfer to visualize the embryo transfer catheter and place the embryos ideally between 10 and 20 mm from the uterine fundus [26, 27]. There are no restrictions after transfer [28].

Legal Aspects of Oocyte Donation

An egg donor is a woman who contributes her genetic material, for reproductive purposes, to another. A donor has no intention to be a parent of any resultant child and waives any rights she may have to the eggs upon the donation. Currently, fewer than 15 states in the USA have laws addressing egg donation. In jurisdictions without statutory or precedential case law that establishes parental rights, there is no absolute assurance that the intended mother would be considered the legal parent. Fortunately, litigation generated by controversy among the parties in these arrangements is extremely rare. A major reason why there are not more litigation cases is attributable to the carefully drawn safeguards that are regularly practiced by infertility centers. This includes recommendations for psychological evaluations and careful and thorough informed consents, as well as offering legal consultation for both donors and intended parents. Thus far all litigation cases have eventually determined the intended parent, and not the donor, to be the parent.

Psychological Aspects of Oocyte Donation

If the effects of cancer treatments and the connection to future fertility are discussed at the time of cancer diagnosis, the need for use of donor eggs in the future will be decreased because many women will freeze their own eggs prior to treatment with chemotherapy. Those who cannot or decide not to freeze eggs should be made aware of donor eggs as a future option. The transition to deciding to use an egg donor can be difficult on many levels, and can lead initially to a sense of shock or revulsion, along with anger, resentment, depression, fear, and loss. For many, even as the comfort level with oocyte donation increases, fears and fantasies may linger. Commonly, donor oocyte-recipient women express fear that they will not "love" or feel attached to the child, and vice versa. She may also feel that her partner may not see her as the legitimate mother of the child or that the child may not be accepted as a legitimate member of the family. The loss of the genetic tie to the offspring is often profound and prolonged. Male partners may also resist using oocyte donation because the idea of not having a child that is related to his partner is deeply emotionally painful.

Because family building via oocyte donation can be an emotionally difficult decision for so many individuals and couples, the need for psychosocial consultation and education is critical and can be immensely helpful by providing emotional support and information. This notion is supported by the American Society for Reproductive Medicine [29]. This consultation includes acquisition of information about infertility history, marital and relationship history, alcohol/drug use, past or present abuse/neglect, availability of social support systems, and thoughts and feelings about disclosure/openness versus nondisclosure/privacy, as well as addressing the recipients' thoughts and feelings about donor selection. The goals of the psychosocial assessment and psychoeducational counseling are not only to establish a positive, supportive relationship with the recipient but also to evaluate emotional readiness to move forward with the oocyte donation procedure [30]. The oocyte donor should also be encouraged to undergo a psychological consultation. This may help assess for any real and/or expected relationship that the donor has or will have with the recipients as well as the resulting offspring. The intent of this conversation is to protect all parties involved and to decrease the possibility of later regret or strained relationships, particularly if it is a known donor.

Embryo Donation

History of Embryo Donation

The first embryo donation dates back to 1983 when a fresh donor embryo was specifically created for a biologically unrelated recipient, and the remainder of the embryos were created for the donor couple's own use. Once the menstrual cycles of both women were synchronized, they both underwent embryo transfer on the same day [22].

Medical Aspects of Embryo Donation

Women who have undergone cancer treatments that resulted in premature ovarian insufficiency and have a male partner who has minimal or no sperm, are ideal candidates for embryo donation. Couples where the sperm count is not an issue may also choose embryo donation instead of oocyte donation as the cost of embryo donation is significantly less. As previously described, oocyte donation cost includes donation agency fees, donor compensation, ovarian stimulation medications, and oocyte retrieval of the donors. The costs associated with the transfer of cryopreserved embryos are significantly less than undergoing a fresh IVF cycle and are generally similar to those of a frozen embryo transfer [31].

Unlike gamete donation, guidelines for embryo donation dictate that there should be no compensation for donors [11]. Couples donating their embryos are usually doing so because they had the number of children they desire, and have extra embryos remaining. Because of this, the embryos tend to be of "good quality" and therefore the pregnancy rates with use of donor embryos are quite good.

FDA screening and testing requirements for tissue donation should be met by both parties involved in providing the embryos (Table 16.1). Embryos may be used even if screening of donors is not possible at the time of donation, provided there is adequate documentation specifying the recipient couple realizes that complete FDA screening was not done. The ASRM, however, deems it safer to withhold embryo transfer in these situations. Both donors must sign informed consent forms relinquishing all rights to donated embryos or persons resulting from them. A minimum 3-month waiting period is recommended, though not required, between the time the donors sign consent forms and the actual transfer for the embryos in the recipient.

The ASRM also has requirements for embryo recipients. The recipient couple must take full responsibility for the embryos and all children resulting from transfer of these embryos. Specifically, donors must be released from all liability with regard to potential complications from procedures or pregnancies, congenital abnormalities, heritable diseases, etc. The assisted reproductive technology (ART) program should also be free of liability from any of these potential issues. Recipients must be willing to undergo all the screening tests that the donors have undergone, and they must be willing to adhere to established guidelines for embryo donation established by the ART program performing the procedures. Recipients and donors should be offered a psychological consultation with a qualified mental health professional. Issues that arise from raising nongenetically related offspring and those of appropriate disclosure to these children may be addressed during these visits.

The process for embryo transfer is very similar to that which was described under the "Recipient Endometrium Preparation" section under "Oocyte Donation." The same process applies in which estrogen is given to prepare the endometrium, a mid-cycle ultrasound is performed to ensure the lining of the uterus is at least 8 mm, and vaginal or intramuscular progesterone is provided for 3–7 days prior to transfer depending on the stage of the cryopreserved embryo and the route of progesterone administration. A natural cycle frozen embryo transfer may also be done if the patient prefers this method and has regular ovulatory cycles. The cycles in this case are carefully monitored by ultrasound and ovulation predictor kits to determine the precise timing of ovulation and subsequent embryo transfer. Ovulation can also be induced by administration of a human chorionic gonadotropin (hCG) injection once the dominant follicle exceeds 18–22 mm [32].

Legal Aspects of Embryo Donation

The main difference between the legal implications of oocyte donation and embryo donation results from the definition of the term embryo. Various legal definitions related to embryos appear in federal and state statutes and regulations. More recent ART or parentage statutes have been passed in about a dozen states that permit embryo donation in the same manner as egg or sperm donation and further state that embryo donors are not legal parents of the resulting child.

At the federal level, embryo donation for procreation was encouraged by the George W. Bush administration by earmarking funds for an "awareness campaign" for "embryo adoption," and waiving FDA screening and testing requirements for cryopreserved embryos intended for donation to "enhance the availability of embryos for donation" [33]. While the Obama administration has retained the FDA regulations, in its FY2013 budget it declined to fund the awareness campaign, which had received a total of over $16 million in funding the previous 5 fiscal years [34].

As mentioned, in 12 states embryo donation statutes clarify the legal status of the parties and any resulting child. These laws range from a straightforward mirror image of sperm donation laws to more comprehensive statutory documents that encompass egg and embryo donation, as well as surrogacy arrangements. The Uniform Parentage Act (2002), a model law, proposes that many of the parentage issues for children born from donor gametes, embryos, or surrogacy should turn solely on the intent of the parties, not genetics. Following this trend, all of the state embryo donation statutes, except Louisiana's, explicitly relieve an embryo donor from all parental rights or responsibilities and transfer such rights to the intended parents. The laws typically require prior written consents of both the donors and the recipients, and these should be completed prior to undergoing any treatments. These consents and contracts should clearly define each party's respective roles, obligations, intentions, and expectations regarding the donation and any resulting child. Independent legal counsel should separately advise donors and recipients as to their interests, including whether in their specific state a pre- or post-birth order of parentage, and/or a post-birth adoption may be recommended as legally protective for the offspring and all involved [35].

Psychological Aspects of Embryo Donation

Embryo donation shares characteristics of, and is also distinct from, other forms of family building that involve the use of ART. Rather than one parent having a genetic link, as found in donor insemination or egg donation, both parents share a similar lack of genetic connection to the resulting offspring. The fact that the parents raise a child with whom they do not share a genetic connection makes embryo donation more akin to adoption. Yet parents have an important prenatal gestational connection with the child not found in adoption. The chance to experience pregnancy and childbirth may be a significant benefit over adoption for many couples. In addition to control over the prenatal environment, the pregnancy allows the couple to experience typical social and communal rites of passage into parenthood via pregnancy and childbirth, such as baby showers, prenatal birthing classes, and shared discussion of pregnancy symptoms and childbirth experiences. The experience of pregnancy also contributes to the mother's perception of self as mother [36].

An important question for practitioners, donor and recipient parents, and society at large is whether children conceived via donor embryo have psychological difficulties later in life. In spite of the societal ambivalence over embryo donation, research to date does not suggest adverse psychological outcomes for embryo-donation children. This is generally consistent with findings regarding children conceived using assisted reproduction [37, 38].

Similar to couples who use oocyte donation, many families have to decide if they will proceed with secrecy and nondisclosure of the embryo-donation status. There is a growing trend toward greater openness in most forms of gamete donation and mental health professionals advocating for disclosure to the offspring [39]. Currently, the limited research on the issue suggests that embryo

donation parents are less likely to disclose information concerning conception and genetic origins to their child than parents of adoptive or IVF children [40]. In a sample of British families used in several related research studies, only one-third of the embryo-donation couples had told, or were planning to tell, the child about his or her origins, in contrast to 100 % of the adoption couples and 93 % of the IVF couples. Almost 43 % of the embryo-donation parents were not planning on disclosing to the child [41]. The amount of information a couple has regarding the donor couple appears to impact their willingness to disclose to the offspring [42]. If parents are unable to give more information about the donor, they may not disclose the use at all [40]. The most popular reasons for nondisclosure involved a desire to protect the child and to avoid damaging the family relationship, particularly the parent–child relationship. Non-disclosing mothers feared that the lack of genetic connectedness as well as the lack of available genetic information would upset the child [40]. However, now with the modern genetic and social information sharing, it may be unrealistic to assume that secrecy can be maintained. No research to date specifically compares the impact of disclosure, nondisclosure, or inadvertent disclosure on embryo-donation children.

As with oocyte donors, embryo donors should also be offered psychological consultation. There is less research on the psychological impact of embryo donation on the donor couple, but relinquishment of the embryos has been described as sad, bittersweet, and imparting a sense of finality.

Gestational Carrier

History of Gestational Carrier

A gestational carrier is a woman who is carrying a baby that is not genetically related to her. This is different than "traditional surrogacy" in which a woman is inseminated with sperm and carries a baby who is genetically hers.

The first birth utilizing a gestational carrier occurred in April 1986. The couple involved had a long history of infertility, and the wife had lost both of her fallopian tubes due to ectopic pregnancies. She was able to conceive with IVF, but at about 22 weeks' gestation her uterus ruptured and a hysterectomy was performed. After hearing of this story, physicians at Mount Sinai Medical Center in Cleveland, Ohio took on the challenge of having embryos conceived from her oocytes and his sperm and implanting them into another woman's uterus. The greatest difficulty at that time was coordinating the recipient's uterus with the embryos, and it required three attempts and two gestational carriers before the cycle was successful. The baby was born in April 1986 and made history not only as the first baby born by a gestational carrier, but also as the first baby to be legally handed over to a non-birth mother without having to be adopted [43].

Medical Aspects of a Gestational Carrier

A gestational carrier is an option for women who had any gynecologic malignancy that resulted in loss of the uterus. This is currently the only option for women without a uterus; however, promising research is currently being done in Sweden regarding uterine transplantation. Thus far there have been nine uterine transplants performed at one institution with one birth of a child post-transplantation [44]. This treatment option is still considered experimental.

If the woman still has her ovaries, it is possible for her to undergo ovarian stimulation with IVF and then use a gestational carrier. The method for supplying eggs necessary for use in a gestational carrier is a process very similar to that described earlier in the chapter, entitled "Oocyte Donor Process." Similar to the recipient of the oocyte or embryo donation, the endometrium needs to be prepared so that the carrier's uterus is receptive to the embryo implantation, with either fresh or frozen embryos.

Prior to embarking on ovarian stimulation, it is important to assess the biological mother's ovarian reserve in order to establish a protocol for stimulation of her ovaries that will allow her to produce an appropriate amount of eggs. This can be done through the use of blood tests including cycle day 3 follicle stimulating hormone (FSH), estradiol, and anti-Mullerian hormone levels. Additionally, an ultrasound can be performed to determine the number of antral follicles. If fresh embryos are to

be used, the gestational carrier is started on a gonadotropin-releasing hormone agonist (GnRHa), commonly leuprolide, which stops the pituitary from stimulating the ovaries, so they become inactive. Because the ovaries are inactive, there are no ovarian hormones to stimulate the endometrium. Therefore, exogenous hormones can be given to stimulate the endometrium. There are many treatment regimens available for this preparation that are similar to the frozen embryo transfer protocol previously described. One protocol uses 2 mg oral estradiol three times per day, which is started about 2 days before the biological mother is started on her gonadotropins. A transvaginal ultrasound is performed after 10–11 days of oral estradiol, and if the endometrium is less than 8 mm, 2 mg of vaginal estradiol is added twice daily. On the day of the egg retrieval, intramuscular progesterone 50 mg/day is added unless the gestational carrier is over 39 years old, in which case 100 mg of intramuscular progesterone is added. Vaginal progesterone may also be used. The implantation rates (i.e., the chance of an embryo successfully implanting in the gestational carrier's uterus) are greatly influenced by the age of the biological mother. These rates may also be influenced by the quality of the eggs and embryos as the patient may have poorer quality embryos if chemotherapy or radiation cancer treatments were performed.

The gestational carrier should have a complete medical history taken and physical exam to make sure pregnancy will not be of increased risk to her. From an obstetrical standpoint, women are at increased risk of complications if they had a large number of deliveries. Therefore, it is recommended that the gestational carrier should not have more than a total of five previous deliveries and no more than three deliveries by cesarean section [45]. The couple and the gestational carrier must address several questions before proceeding. There are no "right or wrong answers" to these questions, but the answers must be the same for the couple and the gestational carrier. These questions include:

How many embryos to transfer?
Would selective reduction be done if more than the desired number of embryos implant?
Will genetic testing be done?
If genetic testing is done and is found to be positive, what will be done?

The FDA has established eligibility standards for third party reproduction. Since May 2005, all infertility centers have to comply with these standards. The FDA requirements for the biological parents are listed in Table 16.1. Of note, the infectious disease tests must be drawn for the biological mother within 28 days of the egg retrieval. The biological father must have these laboratory tests drawn within 7 days of when his sperm is collected, which is why frozen sperm is generally used.

The FDA does not require any screening procedures for the gestational carrier; however, most programs still do extensive screening on the carrier as she could transmit disease to the baby she would be carrying. If there are any positive findings in the screening process of the biological parents, they would be considered "ineligible donors." This does not preclude them from using a gestational carrier as long as the carrier signs a document stating that she knows the couple is considered "ineligible donors," and understands why they are considered ineligible donors.

Legal Aspects of a Gestational Carrier

Child custody matters are usually decided by state, rather than federal courts, and are generally handled at the trial court level. There are much more significant legal issues with traditional surrogacy than with gestational carriers, but in many states the enforcement of even gestational carrier contracts are prohibited by statute [46]. The most visible traditional surrogacy case is the *In re Baby M* matter. In this case, a woman agreed, by contract, to be inseminated with sperm from the intended, and genetic, father and to carry the child, give birth, and place the child with the father and his wife. The surrogate had a change of heart during the pregnancy and refused to relinquish the baby. The father sued for custody, and the New Jersey Supreme Court, in declaring both the surrogate and the father parents, ruled that the surrogacy contract was unenforceable and void as against public policy.

The genetic father and his wife were granted custody, and the surrogate was granted visitation rights [47].

The laws dictating gestational surrogacy are largely determined by case law. In the early 1990s, the Ohio Court of Common Pleas determined that the genetic mother of a child gestated by the mother's sister was the legal mother [48]. This notion of parentage being determined by genetics was challenged by a case in California in 1998. This case involved a married couple who created an embryo with donor gametes and arranged to have the embryo implanted in a gestational surrogate. The couple then separated and the wife claimed that the husband was required to pay child support even though he did not have a genetic linkage to the child. The courts determined that a man can be a legal parent with no other tie to the child except intent. The court established that consent to engage in a gestational carrier arrangement, even without a genetic or gestational link, is sufficient to allow a determination of parentage [49]. Yet another Ohio case involved a married couple using a donor egg and gestational carrier. At the time, the married couple lived in Ohio, the gestational carrier lived in Pennsylvania, and the egg donor lived in Texas. The gestational carrier delivered premature triplets, and after birth the carrier asked that the triplets be discharged from the hospital to her, because she thought the original couple were inappropriate as parents. The Pennsylvania court granted custody to the gestational carrier and her husband, but the Supreme Court of Ohio found that the carrier did not have standing to seek custody of the triplets and the original father ultimately received custody [50].

Fewer than two dozen states have statues governing gestational surrogacy. Five states' laws prohibit enforcement of gestational surrogacy contracts: Arizona, Indiana, Michigan, New York, and Nebraska. Seven states' laws regulate or restrict the practice: Florida, New Hampshire, Nevada, Texas, Utah, Virginia, and Washington. One state, Illinois, permits surrogacy and provides regulatory structure so no court action is necessary. At a minimum, medical practitioners should be aware that all parties involved in gestational carrier arrangements require independent legal counsel. The experienced counsel should know and access the appropriate protocols for obtaining a pre-birth order, or other determination of parentage, with the concomitant declaration that the gestational carrier, and her husband/partner, is not the parent of any child. Even if the carrier is a friend or relative of the intended parent(s), and perhaps especially in those circumstances, the contract and independent legal counsel are critical, and the legal referral should be made.

Psychological Aspects of Use of a Gestational Carrier

In 2012, the ASRM Practice Committee published recommendations for fertility practices using gestational carriers to "address the complex medical and psychological issues that confront the gestational carrier and the intended parents, as well as the children" [51]. For the intended parents, the guidelines point out the "complexity" of the decisions that go into using a gestational carrier and strongly recommend psychosocial education and counseling by a qualified mental health professional. The clinical interview and psychological assessment include a discussion of the medical and psychological demands of using a gestational carrier, couples' history of infertility and methods of coping, the risks of unsuccessful cycles, pregnancy loss, multiple pregnancy, multifetal pregnancy reduction, and elective termination. It also includes counseling couples about the importance of establishing a respectful relationship with the gestational carrier as well as the importance of reaching an agreement with her on medical decisions regarding her body. Criteria for rejection include, besides abnormal psychological evaluation, unresolved or untreated addiction, unresolved or untreated psychiatric disorders, current marital or relationship instability, and intended parents' inability to maintain a respectful and caring relationship with the gestational carrier.

For the gestational carrier, a clinical assessment and psychological testing to determine her ability to cope with the psychological demands of being a gestational carrier are recommended.

These include her ability to understand and cope with potential medical issues such as treatment failure, pregnancy loss, pregnancy complications, multiple pregnancy, multifetal pregnancy reduction, and elective termination. The assessment should also review her current life stressors; history of pregnancy and childbirth, whether she has experienced postpartum depression or other reproductive problems; as well as her social, sexual, and psychiatric history. Reasons for rejection include inability to give informed consent, addiction, uncontrolled depression and other current psychiatric disorders, chaotic lifestyle, and evidence of emotional inability to relinquish the baby at birth.

Men

Fortunately, since 1992, men who have severe oligospermia following cancer treatment are candidates for IVF with intracytoplasmic sperm injection (ICSI). For men who have undergone cancer treatments that have rendered them azoospermic, and did not undergo fertility preservation, the only option for third party reproduction is the use of donor sperm for either donor insemination (DI) with intrauterine insemination or occasionally for IVF.

Sperm Donation

History of Sperm Donation

In the late 1700s, the first successful insemination of a woman was reported. In this case, the wife of a man with male infertility related to hypospadias proceeded to have a normal pregnancy after vaginal insemination with his sperm. In 1884, Dr. William Pancoast was approached by a Quaker couple where the male partner had azoospermia. One of the residents on his service donated sperm, and the wife was anesthetized, inseminated, and gave birth 9 months later. The first report of this insemination appeared in a medical journal in 1909 after the death of Dr. Pancoast.

In 1953, the first human pregnancy with frozen sperm occurred, and this allowed for the development of commercial sperm cryobanks. With the discovery of HIV in the 1980s, requirements for donor screening were developed. This new standard of care included testing men for sexually transmitted infections at the time of donation, cryopreserving and holding the sperm for 6 months, and then retesting to confirm the lack of infection. In 1992, the first births after ICSI were reported. ICSI is a procedure in which one sperm is directly injected into the cytoplasm of the egg. This allowed for men with very low sperm counts to still achieve a pregnancy. For cancer patients who are able to produce even a small number of sperm prior or after treatment, this remains an option for fertility. It does, however, require that the female partner undergo IVF.

Medical Aspects of Sperm Donation

As mentioned, when men have undergone either chemotherapy, radiation, or surgery that has left them with azoospermia, the only treatment option is the use of donor sperm. To find a sperm donor, most couples access a commercial sperm cryobank. Varying information about the sperm donor is provided, and each sperm cryobank provides different background information. The cost of donor sperm varies significantly depending on the sperm bank.

The ASRM recommends the following requirement for sperm donors [11]: Sperm donors should be 18–40 years old as increasing male age may be associated with an increase in the prevalence of chromosomally abnormal sperm [52]; a history of fertility is desired but not required; employees or clinicians of the office practice cannot be a donor to a patient at the practice. All sperm donors are required, by the Food and Drug Administration to undergo communicable disease testing as listed in Table 16.1. The anonymous donor samples are quarantined for 180 days after the date of donation, at which time the donors are then retested for communicable disease. If the testing is negative, the samples are released for use. The FDA does not require quarantine for directed donation. However, Third party reproduction:men:sperm donationthe ASRM recommends that directed donors (donors known to the couple) have the same protocols as

anonymous donors, that recipients be made aware of any increased risks or presence of disease in the donor, and follow the same quarantine regulations [11].

The process for using donor sperm for insemination is much less complicated than the process required for egg or embryo donation. The female partner of the male requesting DI could undergo natural cycle insemination or could use fertility medications. Prior to insemination, the female should be evaluated for fallopian tube patency with a hysterosalpingogram (HSG). However, if she is low risk for tubal disease and is concerned about the cost or pain associated with an HSG, she may choose to decline this test after appropriate counseling. To facilitate conception, the sperm should be placed in the upper genital tract at the time of ovulation. The time of ovulation can be known by checking ovulation prediction kits or ultrasound and/or blood monitoring.

Assuming the male is the only source of infertility, the pregnancy rate with donor insemination is mostly determined by the female partner's age. Shenfield et al. evaluated the effects of age on pregnancy rates using cryopreserved donor sperm. The cumulative conception rates after 3, 6, and 12 cycles of treatment were 21, 40, and 62 % for patients <30 years of age compared with 17, 26, and 44 % for those aged ≥30 years ($p = 0.008$) [53].

Donor sperm may be used for IVF as well, if the female partner has infertility factors necessitating IVF.

Legal Aspects of Donor Sperm

The legal, moral, and ethical issues surrounding DI have taken place in courts and religious circles. A 1954 article on DI in the British Medical Journal prompted the Archbishop of Canterbury's Commission to label DI a criminal offense. The Pope declared DI a sin. And in 1963, the Supreme Court of Cook County in the USA ruled that DI was "…contrary to public policy and good morals…adultery on the mother's part." Furthermore, the court determined that children so conceived were born out of wedlock and therefore illegitimate [54].

The tide of opinion began to turn in the mid-1960s. A year after the Cook County case, Georgia passed the first statute legitimizing children conceived with DI on the condition that both the husband and wife consented in writing. In 1968, a California case (People v. Sorenson) determined that a DI child was legitimate. Finally, in 1973 and 1974, the Uniform Parentage Act (UPA) was approved by the Commissioners on Uniform State Laws and the American Bar Association. This act stated that if DI is done with physician supervision and husband consent, the child is considered if he/she were the natural child of the recipient [54]. The husband, or legal spouse, cannot challenge parentage of the child under either act if he consented to sperm donation before or after the birth of the child [55, 56].

To date, few states have laws or regulations governing sperm donation [57]. When state law does not address aspects of sperm donation, courts apply existing law on a case-by-case basis [57]. In states that address the parentage of sperm donors, the sperm donor typically is not considered the legal father of a child born by using artificial insemination [58].

Psychological Aspect of Sperm Donation

The psychological components of DI have an impact on three distinct sets of parties: donors, recipients, and the resultant children. The motivations for donating are generally twofold: the small fee they collect for each specimen and some desire to be helpful to Third party reproduction:men:sperm donationsomeone less fortunate [59–61]. The most controversial area surrounding DI is regarding donor anonymity. Donor anonymity has been deemed a barrier to a prospective child having access to half of his/her genetic history. At the same time, the threat of personal and financial claims against donors is seen as a primary reason for maintaining anonymity [62]. While anonymity tends to be the rule at US sperm banks, some sperm banks offer donor contact information, and a Donor Sibling Registry (DSR) was established in 2000 that allows a mechanism for contact between siblings of the same donor.

The majority of research about recipients has been limited to men's emotional reactions to their infertility. Men first have to come to terms with their inability to procreate before they can consider the option. Ideally, for cancer patients, sperm will be frozen before therapy so they will not need donor sperm in the future. If donor sperm is needed, many recommend a slow approach to the topic because it raises issues of loss, defectiveness, shame, and humiliation [63]. Recent research indicates that both men and women believe that the use of donor sperm would lead to more marital difficulties than the use of donor eggs [64]. Therefore, since the use of donor sperm can cause social anxiety and the fear of disturbed marital relationships, mental health consultation should be considered a routine part of treatment for DI users as it is with donor ova.

A recent comprehensive review of outcome studies with children conceived with DI indicates that their development is comparable to children with genetic links to both of the parents who raise them [64]. Again, the question of disclosure arises. Certainly the decision to not disclose can have no ramifications if offspring never discover they are donor conceived. However, family therapy literature suggests that family secrets can have a detrimental impact. Some parents believe the children have a right to the information. These parents generally want to do away with the burden of secrecy and prevent disclosure by someone else or accidental discovery by the child [63]. Disclosure has been associated Third party reproduction:men:sperm donationwith better communication between parents and children and parental satisfaction [65]. Consultation with a mental health specialist prior to DI will help couples determine the right course of action for their family.

Conclusion

Patients who receive a cancer diagnosis are often confronted with multiple physicians and numerous treatment options. If fertility preservation is not feasible, third party reproduction options should be discussed with these patients. Using third party reproduction can be a positive and hopeful experience. Even though there are medical, legal, and psychological implications of third party reproduction, as long as patients are educated on these aspects, third party reproduction remains a viable treatment option for sterile cancer patients who desire a family.

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