Chapter 3
Fertility Preservation in Males

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Introduction

Childhood malignancy is estimated to affect 1 in 168 Americans between the ages of 15 and 30 years [1]. Among the 1.75 million people diagnosed with cancer from 2004 to 2008, 9% were younger than 45 years of age, with 1% under 20 years of age [2]. In addition to the relatively large incidence of cancer diagnoses, young cancer patients are living longer due to ongoing improvements in cancer detection and treatment. The majority of childhood cancer patients experience long-term survival, with 5-year survival rates of 75–80% in patients under 50 years of age [2–6]. This results in an estimated prevalence of 1 in 1,300 young US males who have survived childhood cancer [7]. As approximately 30% of these patients received therapies with secondary effects on fertility, the long-term impact of cancer treatment on reproductive function has become increasingly relevant in recent years [8].

To address the role of fertility preservation in cancer treatment, both the American Society for Reproductive Medicine (ASRM) and the American Society of Clinical Oncology (ASCO) have established recommendations for clinicians managing malignancy, including childhood cancers [9, 10]. In these documents, physicians are encouraged to address the topic of fertility preservation at the earliest opportunity, discuss available options, and consider referring patients to a qualified reproductive specialist [10]. Despite the recognition that fertility preservation is an important survivorship issue, numerous factors continue to hamper its routine integration into clinical practice. These barriers include a lack of knowledge, time constraints, financial concerns, discomfort discussing the topic, and lack of available

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fertility preservation services ([11–16]; and see Chaps. 8, 9, and 11 in this volume for further discussion). These limitations are due, in part, to the developing nature of the field of oncofertility [17].

Although the term "oncofertility" was only recently popularized, the impact of cancer treatments on male fertility has long been recognized as a clinically significant aspect of patient care [18]. As a new discipline, oncofertility is centered on improving short- and long-term reproductive function in cancer patients while maintaining the current efficacy of available cancer therapies. As a newly emerging field, oncofertility has resulted in an increasingly available network of resources to both clinicians and patients with the aim of preserving both the quantity and quality of life of young cancer survivors.

Given the broad scope of oncofertility, this chapter will focus predominantly on fertility preservation in males with an emphasis on reviewing known etiologies for cancer-induced infertility as well as available treatment options. Additional brief overviews of the embryology and physiology of fertility, psychological impact of infertility, ethical considerations, provider implementation of fertility preservation into an oncologic practice, as well as future directions in male fertility preservation techniques will also be reviewed.

Embryology and Physiology of Testicular Development and Spermatogenesis

Testicular Development

Beginning at 7 weeks of gestation, the sex-determining region of the Y chromosome (SRY), located on the short arm of the Y chromosome, induces the gonadal ridge to become a testis. At weeks 7–10, Sertoli and Leydig cells form and produce testosterone with resultant differentiation of the Wolffian duct into structures including the epididymis, vas deferens, seminal vesicles, and ejaculatory ducts [19]. Testosterone is converted to dihydrotestosterone by 5-alpha reductase, which results in further differentiation of the urogenital sinus and external genitalia into male structures. Among other roles, Sertoli cells secrete Müllerian inhibiting factor (MIF or anti-Müllerian hormone [AMH]), which causes regression of female structures of the Müllerian duct. At 10–15 weeks of gestation, the fetal testicle begins its descent from its position medial to the kidney toward the scrotum via several hypothesized mechanisms, including intra-abdominal pressure, testosterone effects, insulin-like factor-3 effects, and gubernacular traction.

Spermatogenesis

Spermatogenesis in the seminiferous tubules begins at puberty and results in the transformation of immature germ cells into mature spermatozoa. Prior to pubertal

development, the testis of a 10-year-old male is estimated to contain 83,000,000 diploid germ cells [20]. The process of transformation from spermatogonia (2n) to spermatozoa (1n) includes mitosis and two rounds of meiotic divisions over a period of approximately 74 days. Spermatogonia are further subdivided into A (dark), A (pale), and B spermatogonia [21–23]. A (dark) stem cells function as a diploid reserve pool and are, for the most part, mitotically silent except during pubertal development and following stem cell depletion during toxic exposure [24–32]. A (pale) cells act as progenitor cells and remain mitotically active throughout the lifelong process of spermatogenesis, with self-renewal occurring during each spermatogenic cycle [33, 34]. Due to the increased mitotic activity, A (pale) cells are more susceptible than A (dark) cells to gonadotoxic insults, including radiation and chemotherapy. These germline stem cells are also unique in that they are theoretically immortal [35, 36]. Additionally, they are unipotent and are the only cell type that gives rise to cells that undergo meiosis [37]. Final spermatic maturation occurs in the epididymis, where sperm become motile and gain the ability to fertilize an egg.

Reproductive Physiology and Endocrinology

Successful completion of differentiation to mature spermatozoa is highly dependent on the specialized intratesticular microenvironment and hormonal milieu. At the initiation of puberty, the hypothalamus releases gonadotropin-releasing hormone (GnRH) in a pulsatile fashion, which subsequently induces the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH predominantly acts on Sertoli cells to promote spermatogenesis, whereas LH targets intratesticular Leydig cells to produce testosterone. Sertoli cells produce multiple substances including AMH (active during fetal life), inhibin (which negatively feeds back to regulate FSH secretion), and androgen-binding protein (to increase testosterone levels in the seminiferous tubules), among others [38, 39]. Testosterone similarly provides negative feedback to the pituitary to regulate the release of LH.

Etiology of Cancer-Induced Infertility

Infertility associated with malignancy may occur secondary to numerous factors, including the primary malignancy itself, surgery, radiation, chemotherapy, or a combination (Fig. 3.1; also see Chap. 2 in this volume). The most common malignancies in males 0–19 years of age are leukemias and central nervous system (CNS) tumors; for men 20–44 years of age, testicular cancer, melanoma, and non-Hodgkin lymphoma are most prevalent, and for men over the age of 45 years, prostate, lung, and colorectal cancers are seen most often [2, 40]. A wide array of therapeutic regimens are used to treat the various cancers affecting men of reproductive age. Each regimen is associated with its own risk of reproductive harm. A thorough understanding of the etiology of malignancy-related infertility enables the treating physician to effectively counsel patients and plan therapy to optimize prospects for successful fertility preservation.

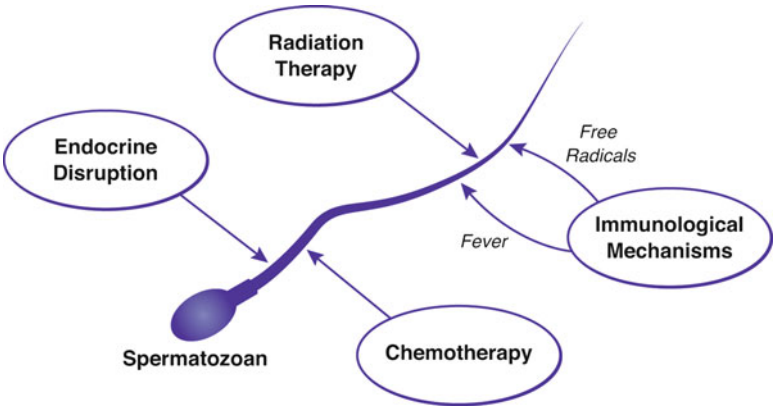

Fig. 3.1 Mechanisms of sperm damage

Primary Malignancy

Although the underlying mechanisms have not been fully described, certain malignancies, in and of themselves, may have a direct effect on fertility. Several studies have demonstrated preexisting impaired semen quality in patients with leukemia, lymphoma, and testicular cancer [41–44]. Rueffer and colleagues reported reduced semen quality in 70% of males with Hodgkin lymphoma [45]. Some patients with cancer present with baseline oligospermia, azoospermia, or sperm chromosomal aneuploidy. Following treatment of their malignancy, some patients experience improvement in semen parameters. These findings implicate the primary malignancy or the immune response to the cancer as a contributing factor to impaired fertility in these patients [46–48]. In a recent study, Schover et al. examined 764 men who presented with various malignancies and had been referred for sperm banking. The authors found abnormal semen parameters in 64% of men; 12% had azoospermia or oligospermia with nonmotile spermatozoa [49]. Further analysis found patients with testicular germ cell tumors to have the highest risk of abnormal semen analyses when compared to other malignancies.

Possible contributing factors for malignancy-induced infertility likely include a systemic inflammatory state with an enhanced immune response, tumor and host cytokine release, febrile status, and multiple system impairments resulting from the chronic disease state and malnutrition [50]. Each of these conditions can lead to suboptimal gonadal conditions, and some of the reproductive impairment may be reversible with successful treatment of the underlying malignancy.

In addition to the direct effect of the primary malignancy, fertility may be secondarily affected by variations in systemic hormones. Both testicular germ cell tumors and hematopoietic malignancies can impair fertility through hormonally mediated mechanisms. For example, certain testicular germ cell tumors release high levels of human chorionic gonadotropin (hCG), which can exert negative feedback on native

LH secretion and thus testosterone production. Hematopoietic malignancies may involve the CNS with direct invasion and subsequent decline in function of the hypothalamus, pituitary, and testis [51]. Thus, reproductive hormones may be decreased in patients with cancer due to a direct, malignancy-related systemic stress response or indirectly from resultant metabolic conditions.

Surgery

Surgical treatment for malignancy is another potential cause of fertility impairment. Orchiectomy is currently the standard of care for diagnosis and initial management of primary testicular germ cell tumors, and it may occasionally be performed for treatment of other malignancies such as prostate cancer. Although orchiectomy directly leads to decreased germ cell mass, unilateral testicular loss does not necessarily result in infertility. Herr and colleagues confirmed this finding with a reported 65% paternity rate in men on active surveillance following unilateral orchiectomy for primary testicular cancer [52].

Surgery may alter the ability to conceive through natural ejaculation by damaging the neurologic or functional mechanism of sperm delivery. Retroperitoneal lymph node dissection (RPLND), prostatectomy, cystectomy, pelvic exenteration, low anterior colonic resection, or any similar deep pelvic surgery may result in damage to the vas deferens, ejaculatory duct, or seminal vesicles, which collectively comprise the excurrent ductal system of the testis. These procedures may also result in cavernous nerve injury with erectile dysfunction, autonomic nerve damage with ejaculatory dysfunction, and physical interruption or obstruction of the sperm delivery pathway, as well as contribute toward erectile and/or autonomic nerve dysfunction.

Improvements in surgical technique, including nerve-sparing modifications and template retroperitoneal resections, have helped to decrease the risk of erectile dysfunction and ejaculatory dysfunction, respectively. Foster and colleagues demonstrated a 95–98% rate of preservation of antegrade ejaculation and 76% paternity rates in males undergoing a nerve-sparing RPLND for testicular cancer [53]. Despite ongoing improvements, select surgical procedures can result in the loss of ability to conceive naturally, thus necessitating the use of assisted reproductive techniques (ARTs).

Radiation and Chemotherapy Mechanism of Infertility

Both radiation and chemotherapy can result in damage to the seminiferous tubules, including spermatogonial cells and Sertoli cells with resultant detrimental effects on fertility (see Chap. 2 in this volume for more information). Low levels of gonadal toxicity can result in damage to A (pale) cells with preservation of A (dark) stem cells, which later repopulate the testis. High doses of gonadotoxic therapy may cause apoptosis of both A (pale) and A (dark) cells, resulting in depletion of

stem cells and permanent infertility [54, 55]. In the case of high-dose treatments, seminiferous tubules may become hyalinized with a resultant Sertoli-only histological pattern remaining [55, 56]. This susceptibility to radiation and chemotherapy occurs not only after initiation of spermatogenesis but throughout all ages, including before puberty [57, 58].

Following sustained damage, stem cells initially recolonize the seminiferous tubules and subsequently divide to form differentiating germ cells. The degree of recovery achieved depends on several factors, including the type of therapy administered, dose, and fractionation/delivery schedule [31]. In contrast to the seminiferous epithelium, Leydig cells are relatively resistant to both radiation and chemotherapy, and cancer treatments rarely result in clinical hypogonadism [59].

Radiation

The extent of testicular injury sustained by radiation therapy is directly related both to the dose of radiation delivered as well as the underlying cell type. Seminiferous tubules are particularly sensitive to radiation with energies as low as 0.1 gray (Gy), resulting in temporary arrest of spermatogenesis [60]. Increasing doses have been shown to cause azoospermia at 0.65 Gy with doses of <1 Gy, 2–3 Gy, and 4–6 Gy, resulting in azoospermia lasting 9–18 months, 30 months, and 5 years to permanent, respectively [60–63]. Other studies have demonstrated permanent testicular damage with 1.2 Gy, far below commonly utilized protocols for testicular cancer of 16–18 Gy [64].

In addition to damage to seminiferous tubules, radiation has been shown to directly result in injury to other testicular cell types including DNA fragmentation in sperm. This is particularly relevant when considering ART in patients who previously received testicular radiation. Leydig cells are more radioresistant, and exposure to 20 and 30 Gy in pre- and postpubertal males, respectively, is required before Leydig cell dysfunction and hypogonadism are typically encountered [65]. Prepubertal and postpubertal males have been shown to have permanent azoospermia with hypogonadism requiring androgen replacement with doses of 24 Gy for testicular leukemia [66, 67]. Although radiation therapy doses less than 20 Gy are associated with normal levels of testosterone, plasma LH levels are notably elevated, indicating likely subclinical Leydig cell injury [59, 63, 68, 69].

The impact of radiation on germ cells is directly related to increasing fractionation and inversely related to patient age. Delivering radiation over an extended period of time with increasing fractionation leads to increased damage to spermatogonia, possibly due to repeated sustained injuries and the inability to repair and regenerate the reserve stem cell population [63, 69].

The timeline for sperm nadir and recovery following radiation is dose dependent, with the lowest sperm counts frequently reported at 6 months following completion of therapy [70]. With doses of 0.2, 1, and 10 Gy, spermatogonia may be detected as early as 6 months, 9–18 months, and 4 years, respectively [60, 62, 71].

Chemotherapy

Chemotherapeutic agents have a wide and variable range of impact on male fertility. The effect of chemotherapy administration is age independent with chromosomal abnormalities detected in spermatocytes up to 24 months after the final treatment [46, 72]. It is often difficult to identify the specific impact of individual drugs, given the frequency of combination therapies. Alkylating agents such as cyclophosphamide, chlorambucil, procarbazine, and busulfan cause the greatest impairment in male fertility, whereas antimetabolites, platinum-based agents, vinca alkaloids, and topoisomerase inhibitors are also noted to be gonadotoxic [73–75].

Alkylating agents result in impaired spermatogenesis in a dose-dependent manner [76–78]. Cyclophosphamide administered at doses of 7.5–9 g/m^2 has been shown to impair fertility, dosages over 10 g/m^2 are associated with a high risk of gonadal damage, and dosages greater than 19–20 g/m^2 typically result in permanent sterility [58, 76, 79]. As is the case with radiation therapy, repeated smaller doses have been shown to have a greater impact on overall fertility compared to fewer, larger doses [80].

Similar to alkylating agents, platinum drugs such as cisplatin and carboplatin have a dose-dependent negative impact on fertility [81]. Ohl and Sonksen reported on patients treated with cis-platinum-based chemotherapy for testicular cancer; the majority of patients developed azoospermia with subsequent recovery within 4 years of last therapy [82, 83].

Combination Therapies

As many malignancies are commonly treated with combination therapies of chemotherapeutic agents and in conjunction with radiation therapy, it is difficult to determine the effects of individual treatments on overall fertility. When compared with their siblings, patients receiving both alkylating agents and radiation were one-half as likely to achieve pregnancy 5 years or more following their diagnosis [22]. Similarly, patients with acute lymphoblastic leukemia undergoing chemotherapy and radiation therapy were found to have spermatogonia present in fewer than 40% of seminiferous tubules [84].

Treatment of hematopoietic malignancies in general requires multiagent therapy with or without radiation and is frequently associated with infertility. Therapies for Hodgkin lymphoma have undergone an evolution, resulting in improved fertility outcomes over time. Previously, regimens were composed of more than three courses of mechlorethamine, oncovorin, procarbazine, and prednisone (MOPP) and resulted in azoospermia in 85–90% of patients at 1-year follow-up, with an associated increase in LH, decrease in testosterone, and development of gynecomastia [10, 76, 85, 86]. Further studies revealed permanent sterility in 80–100% of patients treated with MOPP or COPP (with cyclophosphamide rather than mechlorethamine) [87, 88]. With alterations in Hodgkin lymphoma to regimens of adriamycin, bleomycin,

vinblastine, and dacarbazine (ABVD), 90% of patients experience a return to normal sperm counts 12 months after therapy [89]. When combined with total body irradiation of 10–13 Gy, 85% of patients developed azoospermia with oligospermia noted in the remainder [71]. Similarly, cyclophosphamide or melphalan combined with total body irradiation (TBI) resulted in permanent sterility in 83% of patients [71, 90].

Recovery of spermatogenesis with combination therapies is often diminished and sometimes absent. After undergoing TBI at doses of 10–13 Gy, recovery of spermatogenesis was seen in 15% of patients within 10 years, with no recovery present prior to 4 years.

Similar to hematopoietic malignancies, testicular cancer frequently requires multimodal therapy for successful treatment. Common chemotherapeutic regimens for testicular cancer include bleomycin, etoposide, and cisplatin (BEP), and these regimens generally present a low risk of infertility. In those receiving cisplatin-based chemotherapy, sperm recovery occurred in 55–80% of patients, and normospermia was achieved in 63% patients at 1 year and 80% at 5 years [91]. Compared with cisplatin, carboplatin results in even lower rates of azoospermia with less spermatogonial damage [92]. Retroperitoneal radiation performed for seminoma resulted in a return to baseline sperm counts in an average of 2 years [82], with other reports identifying permanent infertility in up to 25% of patients [93]. Para-aortic radiation alone for stage I seminomas resulted in no reports of azoospermia.

In contrast to the mechanisms of infertility seen with treatment of hematopoietic and testicular cancers, cranial malignancies may result in secondary gonadal failure following cranial irradiation. Cranial doses of 35–45 Gy have been shown to reduce plasma gonadotropins, which may secondarily delay puberty in prepubertal males [94]. While these effects may be overcome with gonadotropin administration, such patients are less frequently encountered clinically in an infertility practice due to the relatively poorer prognosis of primary CNS malignancies [95].

Clinical Evaluation

Patient Perceptions

In addition to the challenges associated with a new diagnosis of malignancy, young patients in particular are faced with the long-term distress of possible future infertility [96]. The loss of fertility may have either a perceived or real impact on a patient's physical, economic, social, and sexual life [49, 97–99]. Concerns regarding infertility are second only to questions regarding mortality in this group. A majority of 13–21-year-old cancer survivors surveyed indicated their willingness to participate in fertility preservation had it been offered, further highlighting the importance of the issue [100, 101].

The desire for future fertility is prevalent in young cancer survivors, with one study indicating that 73% of male and female patients 12–28 years of age expressed the desire for a child in the future [98, 102]. Schover and colleagues further reported

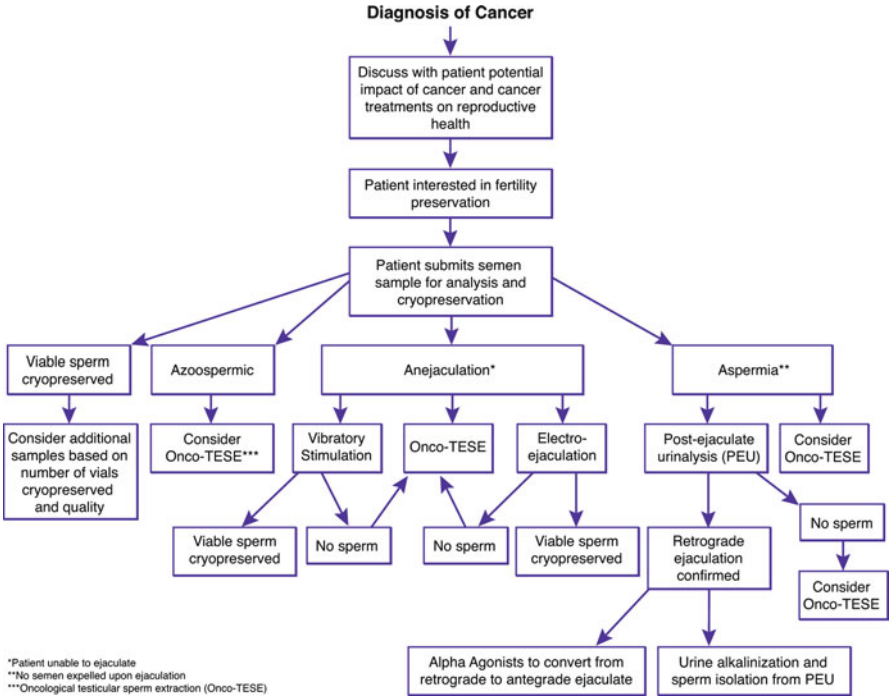

Fig. 3.2 Male fertility preservation decision tree

that 76% of male cancer survivors wished to have children following their cancer treatment, with a particular desire for parenting their own biologic offspring rather than through adoption or other methods [49]. Of interest, this group felt that the cancer experience heightened their appreciation of family life and enhanced their parenting ability through the development of mature coping skills, among other factors.

Despite the heightened concern for fertility preservation, young patients with cancer are frequently not familiar with cancer-related infertility. More specifically, 30–60% of survivors report that they did not receive information on the risks of infertility from their health-care team [49, 102–104]. Additionally, young cancer survivors frequently have an inaccurate understanding of their own fertility, including misconceptions about an increased risk of malignancy in their progeny [49].

Pretreatment Evaluation

The optimal time for discussion of fertility preservation is at the initial cancer diagnosis. Both the ASRM and ASCO recommendations address the importance of informing cancer patients about potential risks of their malignancy and treatment as well as available preservation options [9, 10]. Figure 3.2 details a clinical decision

tree for fertility preservation in males. The ability to preserve fertility in males is dependent upon the age at presentation. Twenty percent of males at Tanner stage II or above with testicular volumes greater than 10–12 ml have achieved spermiation, with the ability to provide sperm for cryopreservation. Currently, there are no widely accepted options for fertility preservation in prepubertal males, although experimental procedures such as testicular tissue cryopreservation are being performed at some institutions under IRB-approved protocols. It is imperative that the patient and his family understand of the experimental nature of these procedures [9] and that there is currently no technology in place to transform prepubertal testicular tissue into functional, mature sperm.

Previous investigations have examined the utility of GnRH (or LH releasing hormone, LHRH) agonists to create an iatrogenic arrest of spermiogenesis in the hope of limiting damage to germ stem cells. Despite initial successes in rodent models, similar results have not been reproducible in human studies, possibly due to continued germ cell proliferation via a gonadotropin-independent pathway [105–109].

Sperm Cryopreservation

Sperm cryopreservation is the most common technique utilized for fertility preservation in pubertal and postpubertal males. The potential benefits of cryopreservation have significantly increased following advancements in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) techniques, as only one appropriately preserved sperm is required for oocyte fertilization and future paternity [110, 111]. In addition to preserving future fertility, many patients have reported that sperm banking is a method of coping with their malignancy, even if the samples remain unused [112, 113]. Sperm banking may provide both a sense of security and reassurance of the future, as both young cancer patients and their parents wish to be offered fertility preservation options [103].

While sperm cryopreservation should be considered for all patients prior to cancer therapy, ultimately, some patients are fortunate and recover sufficient levels of spermatogenesis posttreatment to sustain normal fertility [114]. One consideration for providers is that the starting cancer treatment regimen may be less toxic than the ending regimen, so stratifying patients by chemotherapeutic regimen at the beginning of treatment may not capture the actual full gonadotoxic exposure. Along these lines, utilization rates of banked sperm are reported to be between 10% and 15%, but it is important to keep in mind that such studies have a limited period of follow-up [115] and some patients may use their sperm after the study end date, resulting in an underreporting of sperm utilization rates.

The technique utilized for sperm retrieval will depend on the underlying integrity of the reproductive system. In the absence of any anatomic or neurologic impairment, sperm are retrieved via ejaculation following a period of abstinence of 48 h [116]. Two to three samples are typically collected due to frequently reduced semen quality in cancer patients, and samples are obtained prior to cancer treatment to ensure optimal DNA integrity and sperm quality [10].

Successful sperm collection can be challenging in certain populations, including adolescents and those with alterations in normal ejaculatory physiologic mechanisms [117]. Patients with retrograde ejaculation can undergo catheter placement in the bladder prior to ejaculation, followed by bladder drainage and washing with sperm wash media. An aliquot of sperm wash media is then instilled into the bladder, the catheter is removed, and the patient is asked to ejaculate. The catheter is then reinserted, the bladder is drained, and the ejaculated sperm are isolated from the solution by centrifugation of the fluid. The sperm can then be resuspended in fresh media with cryoprotectants prior to cryopreservation.

Patients with neurologic compromise resulting in aspermia can undergo vibrostimulatory ejaculation if the sacral reflex arc is intact. In the absence of an intact spinal pathway, electroejaculation can be performed, although this requires anesthesia if the patient does not have a complete spinal cord injury. Although electroejaculation is not frequently used in adolescents, Hovav and colleagues reported its use in six adolescents aged 15–18 years with successful sperm isolation in all cases [118].

In the case of anatomic obstruction (absence of the vas, prostatectomy, prior vasectomy, epididymal obstruction, etc.), sperm can be obtained via multiple techniques, including microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA), testicular sperm extraction (TESE), or testicular sperm aspiration (TESA). Testicular microdissection (micro-TESE) may be utilized in the setting of poor sperm retrieval using the above techniques. Sperm obtained via these methods can be frozen for future use or can be utilized immediately (Fig. 3.3).

Rates of paternity utilizing ART with cryopreserved sperm from former cancer patients are reported to be between 33% and 56% [115]. Although cryopreservation is associated with decreases in semen parameters noted upon thawing, with advances such as IVF/ICSI, only low numbers of viable sperm are necessary to achieve fertilization, pregnancy, and live birth [5, 56, 103, 119]. Although the exact length of time that cryopreserved sperm remains viable is unknown, successful paternity has been demonstrated with sperm stored up to 28 years [120–122].

Despite improvements in ART, one study found that the current rate of sperm cryopreservation in men with newly diagnosed malignancy and at risk for infertility is 27% [49]; the most common reason given by males for not choosing cryopreservation was the lack of information on the topic. This study is in contrast to other reports of successful cryopreservation in 67–83% of pubertal boys, highlighting the discrepancy between the capability to preserve sperm and routine clinical implementation in actual practice [58, 123].

Testicular Tissue Cryopreservation

For prepubertal patients who have not initiated spermatogenesis, investigators are examining cryopreservation of testicular tissue through either cell suspension or whole tissue as a possible option for fertility preservation. Tissue can be obtained through the techniques previously described, including TESE, MESA, and testicular

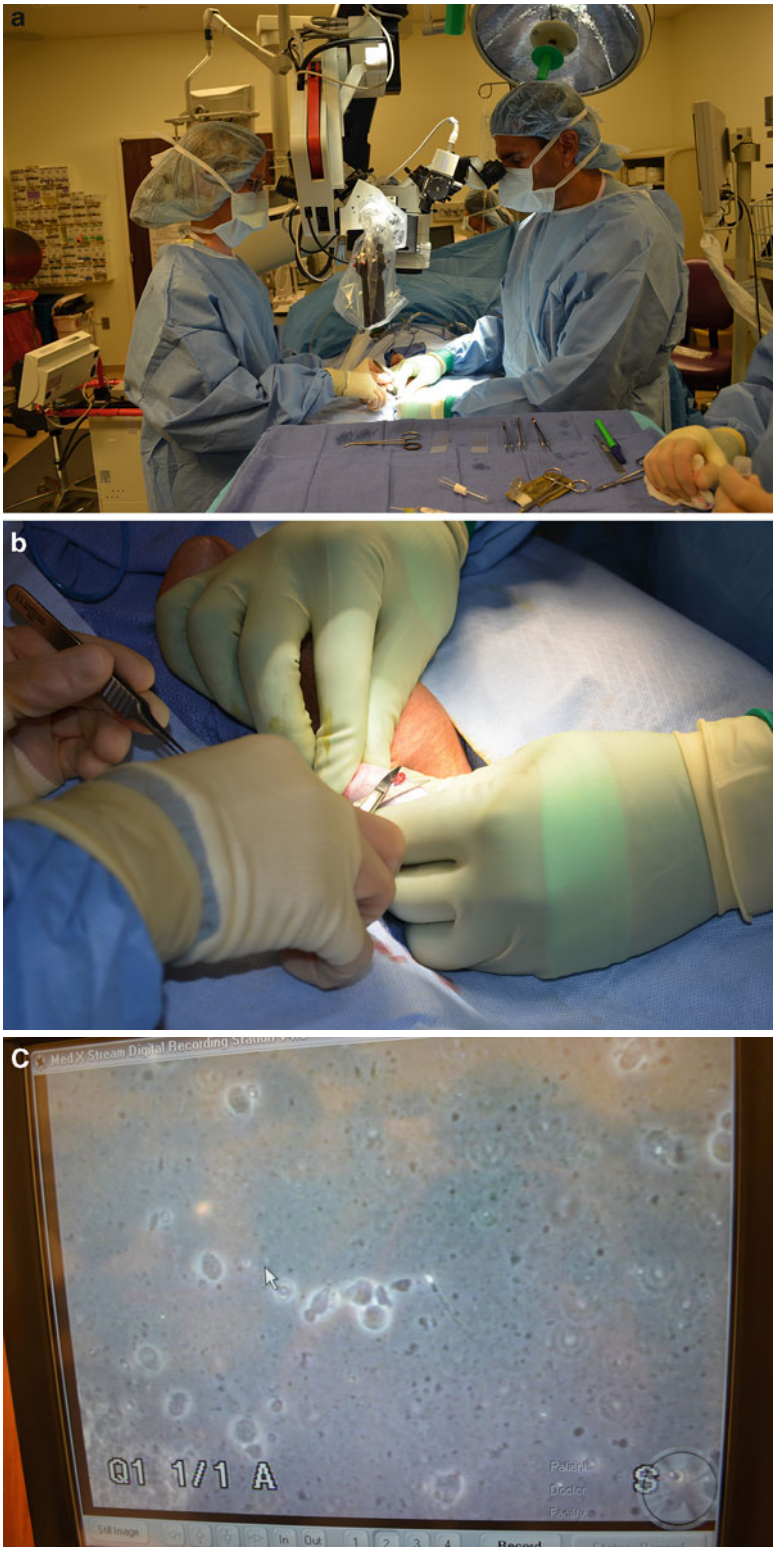

Fig. 3.3 Demonstration of micro-TESE with intraoperative sperm retrieval

biopsy. Although prepubertal germ cells do not contain mature spermatozoa, they do demonstrate the presence of spermatogonial diploid stem cells, which maintain the capacity to differentiate into mature cells given the appropriate microenvironment. The hope is that one day technology will evolve to permit successful use of this immature, cryopreserved, testicular tissue in ART.

Testicular tissue cell suspensions are achieved through mechanical and/or enzymatic digestion of testicular tissue followed by preservation in media including agents such as ethylene glycerol, DMSO, and propanediol [124–128]. Again, tissue processing and cryopreservation result in decreased cell survival, with a postthaw viability of 60% [129, 130]. Direct preservation of testicular tissue is achieved in a similar manner to cell suspensions, with anticipated potential future uses in tissue autografting.

Investigators from The Children's Hospital of Philadelphia recently published reports of 24 prepubertal boys who underwent testicular biopsy with tissue cryopreservation for fertility preservation at the time of surgical central line placement [5]. Despite this and other similar experimental protocols reporting preservation of prepubertal testicular tissue, no study to date has demonstrated a technique to transform this immature, cryopreserved testicular tissue into functional gametes either in vivo or in vitro. Thus, testicular tissue cryopreservation is performed strictly on an experimental, IRB-approved protocol, as no clinically proven means to use such tissue for reproductive purposes exists at this time.

Beyond the current technical limitations in being able to transform cryopreserved spermatogonia into mature, functional sperm, hypothetical risks associated with tissue preservation exist. Given the underlying malignancy in patients undergoing testicular tissue extraction, there is concern regarding the potential for reseeding the cancer when the cryopreserved tissue is reintroduced into the native host [131]. Rat studies have demonstrated leukemic relapse in previously treated animals when as few as 20 leukemic cells were reintroduced [132]. This is further supported by the finding of increased risk of CNS relapse in leukemic patients undergoing traumatic lumbar punctures [133]. This concern, combined with technological factors, limits the current utility of testicular tissue preservation.

Posttreatment Evaluation

Following treatment for malignancy, patients may face either transient or permanent reproductive impairment. Clinical evaluation of patients after treatment includes history, physical exam, semen analysis, and laboratory testing to assess a patient's current level of fertility. Examination of the infertile male may demonstrate reduced testicular size secondary to diminished seminiferous tubule volume [134]. Laboratory testing should include semen analysis, which offers information regarding the patient's current fertility potential, as well as FSH and testosterone measurements. Elevated FSH is indicative of testicular germ cell damage or absence, and FSH levels may be followed over time to assess the return of germ cell function [83, 135]. Similarly, inhibin B, a glycoprotein secreted from Sertoli cells that functions in

feedback regulation of FSH and spermatogenesis, is utilized as a potential marker for gonadotoxicity by some investigators. Wallace and colleagues have measured reductions in inhibin B following chemotherapy, noting its potential role as an indicator of decreased sperm production [136].

In addition to laboratory evaluation, counseling as to optimal reproductive behavior and feedback regarding reproductive health are important in optimizing patient satisfaction and education. Although chemotherapy has been shown to result in chromosomal aneuploidy and therefore has a hypothetical mutagenic effect on host germ cells, multiple reports have shown no increase in birth defects in the offspring of patients who were treated for malignancy [137–142]. Offspring born to fathers treated for prior malignancy do not demonstrate an increased risk of primary malignancy or congenital malformations, with the exception of familial hereditary cancers [143–145]. Similarly, sperm retrieved from men treated for a childhood malignancy demonstrates an equal rate of DNA damage compared with controls, with no reduction in the total time span of fertility [146, 147]. Despite these reassuring findings, studies examining offspring born via ART utilizing sperm from patients treated for malignancy are lacking. There is at least some theoretical concern that the use of banked sperm from patients with preexisting impaired fertility for IVF/ICSI may result in the introduction of damaged sperm, thereby bypassing natural selection mechanisms.

For males who have completed a course of chemotherapy or radiation therapy, the latency period of recovery of spermatogenesis can be quite variable. While no formal guidelines exist regarding timelines for posttreatment contraception, most clinicians do recommend some period of posttreatment contraception to allow time for recovery of treatment-induced sperm DNA damage and improvement in sperm parameters [148, 149]. Contraception recommendations typically span 6–48 months following completion of chemotherapy or radiation therapy. For those men with no fresh ejaculated sperm or previously cryopreserved sperm after treatment for malignancy, evaluation and treatment are conducted in a similar manner to those without a preexisting malignancy. A full discussion of infertility evaluation and treatment is beyond the scope of this chapter but is more thoroughly reviewed in the AUA best practice statement on the optimal evaluation of the infertile male [40].

Special Considerations

Psychological Impact

Male-factor infertility following treatment for malignancy may result in long-term distress and disappointment [150, 151]. Cultural conceptions linking male virility to masculinity and strength may result in a perception of decreased masculinity in males with posttreatment infertility [152]. Some authors suggest that this is supported by the routine practice among couples of “matching” donor sperm to the phenotypic traits of the father, possibly in an attempt to avoid the appearance of

infertility [153, 154]. Additionally, there is suggestion as to a possible disparity in how the medical field approaches infertility in males versus females. These gender norms and perceptions appear to have potential influence on both the patient and the provider, as one study examining referral patterns demonstrated a higher rate of referrals for infertility evaluation from female oncologists compared with male oncologists [155]. In contrast to cultural and gender perceptions, 80% of interviewed cancer survivors reported a positive outlook as potential parents, with no gender differences noted in the desire to have children or distress with inability to conceive. Similarly, fertility was found to impact identity, well-being, and life planning at equal rates in males and females, with both men and women desiring the ability to have children [99, 156].

Ethical Considerations

Clinicians frequently encounter ethical issues in the management of malignancy-related fertility preservation [discussed in more detail in Chap. 9 of this volume]. Care should be taken to distinguish between routine fertility preservation procedures and experimental interventions, with any experimental techniques being conducted on a protocol with IRB approval [10, 157]. Young patients present further ethical issues related to age of assent and consent to procedures and rights of ownership of preserved specimens. Additional challenges include concerns over potential delay of cancer treatment for cryopreservation, postmortem disposition of sperm, and rights attributed to banked testicular material. Each of these issues should be considered and addressed when appropriate, preferably at the time of initial fertility preservation consultation.

Barriers to Fertility Preservation

Oncologists managing patients at risk for infertility report several common barriers to the routine implementation of fertility preservation practices. Desire to avoid delays in cancer treatment, concerns about costs associated with fertility preservation, and lack of insurance coverage contribute to a low number of referrals of cancer patients for fertility preservation [158–160]. Other obstacles include a lack of adequate facilities, lack of awareness of referral sites, lack of oncologist knowledge of preservation techniques, incorrect perception of inability of adolescents to sperm bank, poor underlying prognosis, and lack of time to discuss the topic [158, 161–163].

Despite formal recommendations for a discussion of fertility preservation options with patients at the time of initial malignancy diagnosis [9, 10], actual provider referrals demonstrate a varied pattern of fertility preservation practices. A survey of 249 oncologists who treat female cancer patients revealed that a discussion of the impact

of treatment on fertility occurs 95% of the time [164]; however, patient surveys report a 57–60% recollection of health-care professional discussion of fertility preservation prior to treatment with 51–55% of men reporting being given the option to sperm bank prior to therapy [4, 49]. These data underscore the challenges inherent in initiating discussions about potential infertility and fertility preservation options in the time just after a cancer diagnosis (this topic is reviewed in Chap. 8 of this volume). Nevertheless, the most common reason given by male cancer survivors for not sperm banking was a lack of information about cryopreservation options [49]. Failing to inform patients about fertility preservation in a timely manner can result in patient regret and has the potential to result in legal liability [112].

Future Directions

Currently accepted options for fertility preservation for males are limited to sperm or testicular tissue cryopreservation. For prepubertal males, no mature sperm is available for cryopreservation, and testicular tissue harvesting and cryopreservation are performed only in the context of experimental protocols. At the present time, no proven methods of transforming the immature germ cells into mature, functional sperm exist, although this is an area under active investigation.

Germ cell transplantation has previously been reported in mice, and germ cell extraction, cryopreservation, and reinjection have been performed with subsequent recovery of fertility [165–167]. In a 1999 study, testicular biopsies were obtained from men with solid organ tumors, and, following successful cancer treatment, the germ cells were transferred back into seven of the twelve patients; the results in terms of fertility outcomes are pending [168, 169]. Utilization of this technique is currently limited by the inability to perform 100% accurate cell sorting, thus leaving open the possibility of reintroducing malignant cells into the native host [132]. For this reason, the technique will likely be limited to patients with solid organ tumors for the near future.

An alternative technique for fertility preservation is testicular tissue harvesting for later reimplantation. Animal studies have demonstrated successful recolonization of seminiferous tubules in recipient nude (immunosuppressed) mice following transfer of testicular material from donor animals, thus providing proof of concept [91, 165, 166, 170, 171]. Additional studies have demonstrated the capacity for sperm to undergo complete maturation several months following xenografting [172]. The capability to perform xenologous transfers from donor patients to recipient animal hosts may provide the ability to detect residual cancer cell infiltration while creating a suitable testicular microenvironment to allow maturation and differentiation of germ cell lines. Despite initial successes in animal models, transfer of human testicular tissue results in limited tissue survival, with adult testicular tissue especially prone to degeneration [173, 174]. To our knowledge, there are no current studies being conducted to examine results of human autologous grafting.

As an alternative to tissue reintroduction, in vitro spermatogenesis offers the possibility of generating mature spermatozoa without the risk of reintroducing malignant cells. Some success in the maturation of sperm at the later stages of spermatogenesis has been achieved, but there have been no reports of successful generation of spermatozoa from stem cell progenitors [175]. In vitro spermatogenesis is hampered by the challenging process of recreating the complicated testicular microenvironment required for sperm maturation and the difficulties in facilitating reductive meiosis.

Conclusions

Oncofertility is a field that has grown remarkably in recent years, and the education of patients and clinicians, the implementation of fertility preservation programs, and the development of fertility preservation techniques have all advanced the field. Clinicians treating reproductive-aged males with newly diagnosed malignancy should discuss possible fertility preservation options including sperm cryopreservation. Additional techniques to preserve testicular tissue, particularly in prepubertal males, should be treated as experimental in nature and conducted under the direction of IRB-approved protocols.

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