The Complete Guide to Male Fertility Preservation

Ahmad Majzoub
Ashok Agarwal
Editors

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We dedicate our book to Young Patients with Cancer seeking fertility preservation services before treatment of their disease. It is an honor to serve these brave men.

Foreword

With the dramatic increase in cancer rates in reproductive-age men, the rapidly emerging understanding of the genetics of infertility, and important advances in assisted reproductive technologies, our field has been waiting for a unifying treatise on the preservation of male fertility. Drs. Majzoub and Agarwal are to be congratulated for providing a state-of-the-art guide that focuses on critical techniques for fertility preservation, from the management of ejaculatory dysfunction to the use of microsurgical techniques for refractory nonobstructive azoospermia. They address complex developing topics including the role of postmortem sperm retrieval as well as cryopreservation issues in special populations such as adolescents and transgender patients. Careful focus is placed on the technical and compliance issues of maintaining a high quality sperm bank. The book closes with an important nod to the future, reviewing potential for developments in fertility preservation including germ and stem cell transplantation as well as induction of neospermatogenesis.

This text represents a *tour de force* in the field of fertility preservation. It will be an invaluable resource for urologists, medical oncologists, andrologists, reproductive endocrinologists, pediatricians, and a myriad of other health professionals who participate in the care of men in their reproductive years. These authors deserve great praise for providing an understandable, evidence-based review of fertility preservation options. The issue of preserving the ability to conceive is of paramount importance to our patients and deserves equal attention by health care professionals.

Cleveland, OH, USA Edmund Sabanegh Jr.

Preface

Fertility preservation has witnessed major advancements in the last decade along with other aspects of male reproduction. It is considered as an essential service in medical field due to societal changes leading to delays in parenthood coupled with rapid pace of scientific discoveries propelled by technological breakthroughs in cancer treatment and cryobiology. Cancer is well accepted as a significant circumstance where fertility preservation is an essential procedure. Global demographic and epidemiologic data signal a continuous increase in cancer prevalence, with over 20 million new cancer cases expected annually by 2025. Though finding a cure remains our utmost and paramount goal, nevertheless we are faced with a challenging outcome to treatment as a growing number of patients face quality of life issues resulting from the presence of malignancy as well as the subsequent treatment. Fertility remains the leading quality of life concern in young cancer survivors. As such, the need for fertility preservation strategies is crucial now as there is an increased long-term cancer survival rate. Recent evidence indicates that the 5-year relative survival rate for all cancers combined is now approaching 70% among adults and >80% among children. The focus on fertility preservation is currently limited to cancer patients during their reproductive age, yet its clinical relevance may well be expanded to noncancer patients. Consequently, much broader applications are expected in the near future.

Our book is unique in being a complete guide to male fertility preservation. Experts from various parts of the world have contributed to make this book an important reference and an extensive guide to the multidisciplinary approach which is favored in fertility preservation. The book is divided into 4 parts. Part I provides the reader with a thoughtful and comprehensive overview of the pathophysiologic processes interrelating cancer and its treatment with infertility. It also discusses different methods of sperm preservation and fertility outcomes in cancer patients. Part II explores male fertility preservation in various noncancerous conditions such as immunosuppressed, hypogonadal, and transgender patients. The manuscript reviews in its third part the fundamental principles of cryobiology and sperm optimization and more importantly offers essential building blocks for scientists to develop a sperm banking service and implement high standards of practice. Finally, the last

part offers an understanding of the current practices of male fertility preservation along with its psychological impact on patients and extends beyond to present a glimpse of future innovative methods (tissue preservation, xenografting and artificial gametes) being implemented in this field.

We are confident that our book will be a useful guide for scientists, embryologists, and other healthcare workers practicing reproductive medicine and oncology. In addition, it will be a valuable resource for students and researchers wishing to learn more about this subject. We are highly thankful to a large number of experts who worked hard to contribute the latest, well-written and well-researched articles; without their active support, this book would not be possible. We wish to express our deep gratitude to the superb organizational and management skills of Michael D. Sova, Development Editor, and the overall support and supervision of this project by Kristopher Spring, Editor at Springer. This book is dedicated to our parents, families, mentors, and patients.

Doha, Qatar Ahmad Majzoub, MD
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Dr. Majzoub has done extensive work in the field of Medical Research and has been very active with over 70 research publications in peer-reviewed journals and several book chapters mainly focusing on Andrology and men's health. He is a reviewer at several high impact medical journals and is an active member of the American Urological Association, the American Association for Reproductive Medicine, the European Association of Urology, the European and International Societies of Sexual Medicine, and the Arab Association of Urology. He holds editorial positions at a number of scientific journals and has had several speaker participations at national and international conferences.

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Abbreviations

AAS	Anabolic-androgenic steroids
AI	Aromatase inhibitor
ART	Assisted reproductive technology
ASRM	American Society for Reproductive Medicine
BMP4	Bone morphogenic protein 4
BTB	Blood-testis barrier
CXCL12	C-X-C chemokine ligand 12
CXCR4	C-X-C chemokine receptor type 4
EEJ	Electroejaculation
ESHRE	European Society of Human Reproduction and Embryology
FDA	Food and Drug Administration
FGF2	Fibroblast growth factor 2
FSH	Follicle-stimulating hormone
GCT	Germ cell transplantation
GDNF	Glial cell-derived neurotrophic factor
GFR alpha-1	GDNF family receptor alpha-1
GS cell	Germ-line stem cell
hCG	Human chorionic gonadotropin
HH	Hypogonadotropic hypogonadism
HIV	Human immunodeficiency virus
HPG	Hypothalamic-pituitary-gonadal
ICSI	Intracytoplasmic sperm injection
IHH	Idiopathic hypogonadotropic hypogonadism
IVF	In vitro fertilization
LH	Luteinizing hormone
NHL	Non-Hodgkin lymphoma
NOA	Non-obstructive azoospermia
PGCLC	Primordial germ cell-like cell
PMSR	Postmortem sperm retrieval
RCT	Randomized controlled trial
rhFSH	Recombinant human FSH

ROS	Reactive oxygen species
RPLND	Retroperitoneal lymph node dissection
SERM	Selective estrogen receptor modulator
SSC	Spermatogonial stem cells
T	Testosterone
TST	Testosterone supplementation therapy
WPATH	World Professional Association for Transgender Health

Part I
Fertility Preservation in Cancer Patients

Chapter 1
Etiology of Cancer-Induced Male Infertility

Julie Won-Ching Cheng and Edmund Y. Ko

Introduction

Cancer can significantly impact male fertility in multiple ways. A patient's endocrine system, testicular parenchyma, and sexual function can be individually or collectively impaired by malignancy, surgical intervention, and chemoradiation. The purpose of this chapter is to discuss how the disease process and multidisciplinary treatment of cancer can induce or exacerbate male infertility. This discussion is organized in a similar format as the workup of male infertility in examining the pretesticular, testicular, and post-testicular components of the male reproductive system. Although cancer affecting various systems in the body can influence male fertility, less common diseases and treatments are mentioned with the primary focus on cancers affecting males of reproductive age.

The Hypothalamic-Pituitary-Gonadal Axis

Sexual development and reproduction begin with a functional endocrine system comprised of the hypothalamus, pituitary gland, and gonads. This is known as the hypothalamic-pituitary-gonadal (HPG) axis (Fig. 1.1). The hypothalamus is part of the limbic system and acts as a link between the nervous and endocrine systems. The hypothalamus releases gonadotropin-releasing hormone (GnRH) to activate gonadotrophs within the anterior pituitary to produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH acts on Leydig cells to stimulate testosterone production whereas FSH acts on Sertoli cells to promote spermatogenesis.

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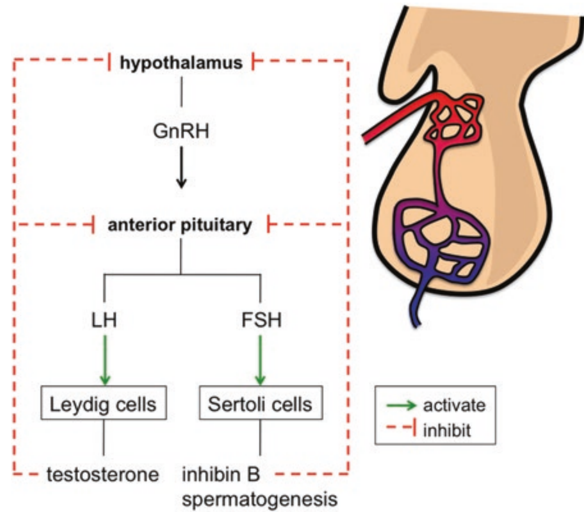

Fig. 1.1 The hypothalamic-pituitary-gonadal axis. The hypothalamus signals the anterior pituitary to release LH and FSH to, respectively, stimulate testosterone production and spermatogenesis in the testicles. This system is regulated through feedback inhibition

This system is regulated by a negative feedback loop that maintains a homeostatic balance when testosterone and sperm are produced appropriately. Hypothalamic and anterior pituitary activity can be suppressed by inhibin from Sertoli cells, testosterone from Leydig cells, or other circulating factors.

Infertility caused by failure of the HPG axis is termed *hypogonadotropic hypogonadism* or secondary testicular failure. This implies that a lack of gonadotropic activity is the source of decreased testicular function. The etiology of endocrine dysfunction may be congenital or acquired with the latter occurring in cancer.

The function of the HPG axis can be inhibited by factors produced by tumors. Human chorionic gonadotropin (hCG) is a tumor marker that is produced by testicular germ cell tumors. As this hormone shares a similar chemical structure with the hormones produced by the anterior pituitary, hCG may affect fertility indirectly through the negative feedback loop. Testicular cancer patients with elevated hCG exhibit lower LH, FSH, and motile sperm count compared to those with normal hCG [1]. Alpha-fetoprotein has also been associated with decreased total sperm count in patients with nonseminomatous testicular cancer [2]. The impact of these tumor markers on fertility is not fully understood as other studies demonstrate that there may not be an association between tumor markers and spermatogenesis [3].

An excess of physiologic factors can also inhibit the HPG axis. Prolactin, produced by the anterior pituitary gland, acts as an inhibitory factor to the HPG axis by limiting hypothalamic GnRH secretion. Hyperprolactinemia has been associated with sexual dysfunction and premature ejaculation [4]. Hyperprolactinemia can occur in cancer, such as from a functional prolactinoma, or as a side effect of treatment. Nasopharyngeal and intracerebral non-pituitary tumors are treated by radiation with potential impact upon the pituitary gland and hypothalamus. Hyperprolactinemia has been identified in patients treated with radiotherapy directed at head and neck cancers through suspected neurovascular damage and

demyelination of cranial structures [5, 6]. Radiation injury to these structures has also been reported with decreased function at gonadotrophs as well as somatotrophs and corticotrophs [5–7]. These patients can exhibit signs of gonadotroph dysfunction with reduced levels of LH and FSH [5–7] and the symptoms can worsen with concurrent chemotherapy [6].

Testosterone Production and Function

Testosterone significantly contributes to the development and function of the male reproductive system. LH stimulates Leydig cells in the testicle. Testosterone is then released into systemic circulation, where it is bound by sex-hormone-binding globulin and albumin until it is released to act on target tissues. Testosterone is converted to dihydrotestosterone by 5α-reductase. These two androgens play a significant role in sexual development and maturation throughout embryonic development, puberty, and adulthood.

As Leydig cells produce testosterone, androgen-binding protein sequesters this hormone to maintain intratesticular testosterone levels that are 40–100 times higher than serum levels [8]. Intratesticular testosterone then binds androgen receptors in Sertoli cells to promote spermatogenesis [9]. The HPG axis further contributes to this process as FSH determines the expression of androgen receptors in Sertoli cells [10]. Changes to the hormonal environment within the testicle can impact spermatogenesis. Suppression of intratesticular testosterone levels can decrease sperm count by up to 98% [8]. Knockout mice without Sertoli cell androgen receptors are infertile and demonstrate arrested spermatogenesis, decreased spermatocyte and spermatid production, and increased germ cell apoptosis [11–13].

Gonadotoxic chemotherapy can damage Leydig cells. Patients treated with chemotherapy for testicular cancer [14, 15] and lymphoma [16] can have decreased testosterone levels and corresponding increases in LH. Long-term follow-up in men treated for childhood Hodgkin's lymphoma demonstrates normalization of testosterone levels but increased LH levels, which suggests permanent Leydig cell damage and higher LH requirements [17].

The Germinal Epithelium

The long, convoluted seminiferous tubules coiled within the testicle serve as the site of spermatogenesis. The lumen of these tubules is surrounded by the germinal epithelium (Fig. 1.2), which is comprised of columnar Sertoli cells anchored to the basement membrane and germ cells. These germ cells are located between Sertoli cells at varying stages in development as spermatogonia, spermatocytes, and spermatids. The population of Sertoli cells directly correlates with sperm production [18] as they provide structural and nutritional support to developing spermatozoa.

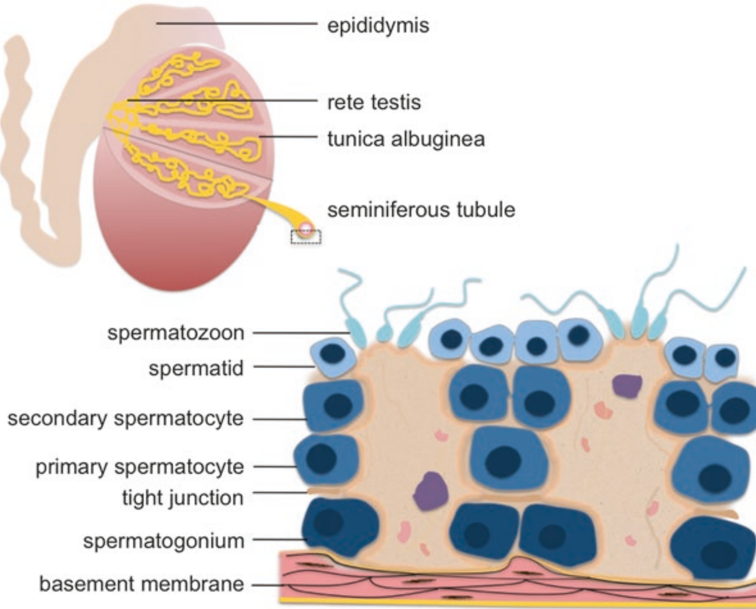

Fig. 1.2 The germinal epithelium. The germinal epithelium within the seminiferous tubules acts as the site of spermatogenesis. Sperm precursors (spermatogonia, spermatocytes, spermatids) are sandwiched between Sertoli cells and gradually move toward the lumen of the seminiferous tubules as they progress through development

Sertoli cells are interlocked by tight junctions forming the blood-testis barrier. This barrier divides the germinal epithelium into basal and adluminal compartments. The adluminal compartment contains developing spermatozoa in an environment that is sealed by the blood-testis barrier. This limits the entry of ions, metabolites, and potentially toxic substances from systemic circulation and also protects spermatozoa from the immune system. After spermatocytes undergo genetic recombination during meiosis, they display antigens that are no longer in concordance with those of host tissue. This makes developing spermatozoa appear foreign to the immune system and therefore vulnerable to being attacked. The blood-testis barrier serves an important function by creating an immune-privileged site to protect spermatozoa from the immune response.

Hormonal signaling from FSH promotes seminiferous tubule growth, protein production, and spermatogenesis. The production of spermatozoa starts with a diploid spermatogonium germ cell that undergoes a series of mitotic and meiotic divisions to ultimately produce four haploid spermatozoa. These cells migrate from the basement membrane toward the lumen of the seminiferous tubules as division and development progress from spermatogonium to spermatocytes to spermatids. Spermatids then undergo spermiogenesis to differentiate into a reproductive cell specialized for motility and fertilization. These newly transformed spermatozoa are finally shed into the lumen of the seminiferous tubules and deposited into the

epididymis. Spermatozoa then undergo structural and functional changes to complete maturation within the epididymis [19]. Spermatozoa dysfunction can occur in patients with cancer as these cells are sensitive to genetic disruption, oxidative damage, and immunologic response. A more extensive description of the structural and functional integrity of spermatozoa can be found in Chap. 2.

Primary testicular failure occurs when parenchymal tissue within the testicle is no longer able to produce sperm or testosterone. This is also known as *hypergonadotropic hypogonadism* as the HPG axis produces FSH and LH at elevated levels due to a lack of feedback inhibition from a nonfunctional testicle. Infertility from primary testicular failure can result from baseline testicular dysfunction, pathologic disruption from cancer, or direct damage to or removal of reproductive tissue from cancer treatment.

Baseline Testicular Dysfunction

Sperm production depends on both the quality and quantity of testicular tissue. The quality of testicular tissue can be altered in men with poor testicular development. Cryptorchidism represents a failure of testicular descent that can affect testicular development. This congenital anomaly has been identified as a risk factor for both impaired fertility [20, 21] and testicular cancer [22, 23] regardless of the location of the testis and whether cryptorchidism was unilateral or bilateral.

There is a small window of opportunity for surgical intervention to lower the risk of cancer and maintain fertility. Prepubertal orchiopexy has been found to significantly decrease the risk of developing testicular cancer [23–25] but may be too late for fertility preservation. Biopsies of cryptorchid testes from patients ranging from 3 months to 5 years of age demonstrate a decline in fertility potential with age [20]. Fertility potential is determined by a measured spermatic index, which is calculated as number of germ cells within a given number of tubular cross sections. Regardless of whether the testis in question is located within the abdomen or the inguinal canal, there is a risk of decreased fertility by 9 months of age followed by a sharp decline in spermatic index by 15–18 months of age [20]. A separate study also demonstrated a significant difference in testicular volume at long-term follow-up in patients that underwent orchiopexy by age 2 compared to those that underwent surgery at an older age [21].

As males undergo puberty, some may develop baseline spermatogenic dysfunction with **testicular dysgenesis syndrome**. Testicular dysgenesis can manifest with a variation of alterations to the germinal epithelium including testicular microlithiasis, poor Sertoli cell differentiation, and a significant reduction of germ cells. In *hypospermatogenesis*, there is a decrease in the number of germ cells. Normal spermatogenesis can still occur but at a lower rate with a subsequent reduction in sperm count. *Germinal aplasia,* also known as Sertoli cell-only syndrome, exhibits a germinal epithelium with Sertoli cells but a total absence of germ cells. At the end of

the spectrum, patients with end-stage testes have scarred germinal epithelium with a thickened basement membrane and no viable Sertoli or germ cells.

Although the neoplastic process and multidisciplinary treatment of testicular cancer may impair fertility, a proportion of men with testicular cancer may already have underlying testicular dysgenesis and impaired fertility. Testicular biopsies of men with testicular dysgenesis syndrome have demonstrated both carcinoma in situ and reduced sperm counts [26, 27]. Spermatogenesis can also be disrupted in the contralateral testis. Biopsies of the contralateral testis at the time of orchiectomy demonstrate impaired spermatogenesis and dysgenic features, such as immature tubules, microcalcifications, and Sertoli cell-only patterns [3, 28, 29].

Spermatogenesis can also be influenced by the quantity of viable reproductive tissue. Decreased testicular volume is associated with lower sperm concentration and proportion of sperm with normal morphology or forward motility [30]. Comparison of semen analyses before and after radical orchiectomy demonstrates a statistically significant decrease in sperm count in patients with nonseminomatous and seminomatous testicular germ cell tumors [31–33]. After orchiectomy, the degree of sperm count decline may be correlated with higher tumor stage and history of cryptorchidism [34]. Orchiectomy does not appear to permanently affect testosterone production although there is a corresponding increase in LH and FSH as well as a decrease in inhibin. Compensatory testicular hypertrophy in the remaining testicle and normal spermatogenesis have both been demonstrated within 2–3 years following orchiectomy in patients with early-stage seminoma despite the initial negative impact on fertility [32].

Systemic and Local Disruption from Cancer

The temperature of the scrotum is physiologically cooler than core body temperature. Heat exchange through vasculature, evaporative heat loss through scrotal skin, and positional changes via the cremasteric reflex maintain this temperature gradient. High-grade fevers of 39–40 °C can cause a temporary change in sperm concentration, total count, and motility [35, 36]. Systemic inflammation in cancer can clinically manifest as constitutional symptoms or the B-symptoms of fevers, night sweats, and weight loss associated with lymphoma. Patients with Hodgkin's lymphoma experiencing B-symptoms have elevated erythrocyte sedimentation rates suggestive of inflammation. These patients have poor sperm quality on semen analysis prior to treatment [37, 38] and there is a dose-dependent effect by which fever affects sperm count and motility [38].

Scrotal temperature can also rise as a result of altered blood flow in addition to changes in core body temperature. Doppler ultrasound has demonstrated hypervascularity at cancerous lesions in men with lymphoma [39]. Venous drainage may also be impaired by tumors. Varicoceles may arise when pelvic masses, retroperitoneal masses, or an intravascular thrombus cause compression or obstruction of the testicular vein, renal vein, or inferior vena cava. As decreased venous drainage limits blood flow and countercurrent heat exchange, patients with varicoceles may experience

increased scrotal temperature [40]. Varicoceles have been linked with infertility as sperm morphology, motility, and concentration levels are affected in men with varicoceles and elevated scrotal temperature [40, 41]. Sperm protein expression may also be affected in high-grade varicoceles and improvements in sperm motility and concentration have been demonstrated following varicocelectomy [42].

Local changes to testicular parenchyma and altered semen analysis can occur in patients with testicular cancer. Histological evaluation of surgical specimens after orchiectomy demonstrates that tumors can cause local structural damage prior to orchiectomy. The degree of parenchymal disruption has been associated with larger tumor size [3], close vicinity to the tumor [43, 44], and malignant tumors [44]. The subtype of testicular germ cell tumor may also influence spermatogenesis. Both seminomatous and nonseminomatous testicular cancers were found to have a significant impact on semen parameters. Some studies have identified lower sperm concentration [34] and total count [2] in patients with seminomatous cancers compared to those with nonseminomatous ones. In contrast, other studies have found that patients with nonseminomatous tumors have greater alterations in sperm concentration, morphology, and motility [33, 45].

Patients with lymphoma and leukemia also demonstrate changes in semen parameters prior to treatment. Altered sperm concentration, motility, and morphology have been reported in patients with Hodgkin's lymphoma [46–49], non-Hodgkin's lymphoma (NHL) [48], and leukemia [50]. Patients with NHL demonstrate better sperm quality with regard to motility and morphology on pretreatment semen analysis compared to those with Hodgkin's lymphoma [51]. Testicular size or hormone levels do not appear to affect these parameters [51]. Elevated erythrocyte sedimentation rate and more advanced disease are associated with poorer semen quality even before treatment in patients with Hodgkin's lymphoma [49].

Gonadotoxicity from Chemotherapy

Gonadotoxic agents can damage the germinal epithelium within the seminiferous tubules. The germinal epithelium can be particularly sensitive to treatments that target rapidly dividing cells as spermatogenesis is an ongoing process in postpubertal men. This includes both the chemotherapy regimens (Table 1.1) and radiation treatments used in treating cancer.

The mechanism of various chemotherapy agents is to target a particular stage in the cell cycle to prevent cells from replicating, regardless of whether these cells belong to malignant tumors or benign host tissue. The germinal epithelium can be especially sensitive to chemotherapy. The damage inflicted upon germ cells and Sertoli cells becomes evident in the sperm count, motility, and altered morphology at 3 months to 1 year after the initiation of chemotherapy [52–54]. This corresponds to the 2–3-month cycle of spermatogenesis. The gonadotoxic effects of chemotherapy in lymphoma patients may be even more pronounced in patients with testicular infiltration [55] and more widespread disease compared to those with localized disease [56].

Table 1.1 Chemotherapy regimens

Regimen	Agents	Indication
CHOP	Cyclophosphamide[a] Doxorubicin (Hydroxydaunorubicin) Vincristine (Oncovin) Prednisone	Non-Hodgkin's lymphoma
ABVD	Doxorubicin (Adriamycin) Bleomycin Vinblastine Dacarbazine	Hodgkin's lymphoma
MOPP/MVPP	Mechlorethamine[a] Vincristine (Oncovin) Procarbazine[a] Prednisone	Hodgkin's lymphoma
BEP	Bleomycin Etopisode Ciplatin	Testicular cancer

The dose-dependent gonadotoxicity of chemotherapy regimens can depend on the presence of alkylating agents. Chemotherapy regimens and the agents involved in the treatment of cancers that commonly affect men of reproductive age are listed

[a]Alkylating agents

Table 1.2 Chemotherapy agents with high risk of gonadoxicity

Alkylating agents
Cyclophosphamide
Chlorambucil
Procarbazine
Mechlorethamine
Busulfan
Platinum agents
Cisplatin
Carboplatin

Some chemotherapy agents can be more gonadotoxic than others (Table 1.2). Alkylating agents, in particular, have been found to cause more damage to the germinal epithelium. These agents damage cells by attaching an alkyl group to DNA and interfering with replication. Testicular biopsies of patients treated with cyclophosphamide demonstrate significant damage to the germinal epithelium [57]. A dose-dependent effect on sperm concentration and gonadal damage has been demonstrated in adult males treated with alkylating agents for lymphoproliferative disorders [58, 59] and testicular cancer [60]. The gonadotoxicity of cyclophosphamide may be related to gonadal activity as a greater degree of damage has been reported in sexually mature males than in prepubertal males [58].

Gonadal dysfunction may be permanent and has been demonstrated in patients previously treated with alkylating agents. Regardless of pubertal status during treatment, childhood cancer survivors treated with high doses of cyclophosphamide for

sarcoma [61], leukemia, Hodgkin's lymphoma, NHL, and testicular cancer [62, 63] are found to have persistent gonadal dysfunction with decreased sperm count at follow-up ranging from 4 to 42 years since treatment.

Chemotherapy regimens for lymphoma vary by the specific type of disease. Treatment of NHL involves **CHOP** (cyclophosphamide, doxorubicin (hydroxydaunorubicin), vincristine (oncovin), prednisone). Despite the use of multiple agents, the gonadotoxicity of this regimen depends on the dose of cyclophosphamide administered. As previously discussed, damage to the germinal epithelium by cyclophosphamide may be dose dependent [58, 60–63] and permanent. There is a reported 61% recovery rate by 24 months after treatment with CHOP [54].

Patients with Hodgkin's lymphoma may be treated with either ABVD (doxorubicin (adriamycin), bleomycin, vinblastine, dacarbazine) or MOPP/MVPP (mechlorethamine, vincristine (oncovin), procarbazine, prednisone). Treatment with **ABVD** does not employ the use of an alkylating agent and subsequently demonstrates less gonadotoxic effects. Patients treated with ABVD have greater sperm concentration, motility, and vitality compared to patients treated with MOPP/MVPP and CHOP [54, 56]. Although patients demonstrate temporary impairment in semen parameters, there is a greater than 90% recovery rate by 24 months after treatment [54]. In contrast, significant gonadotoxicity has been reported with **MOPP/MVPP** [16, 17, 54, 56, 59] as mechlorethamine and procarbazine are both alkylating agents. Patients report decreased libido during treatment and have lower testosterone levels with corresponding increases in FSH and LH [16]. Patients treated with this chemotherapy regimen demonstrate permanent gonadal damage and infertility [16, 17, 59]. Patients treated for childhood Hodgkin's lymphoma have persistently elevated FSH and LH levels, decreased inhibin B, and decreased sperm concentration even at a median follow-up of 15 years [17].

BEP (bleomycin, etoposide, cisplatin) is the first-line chemotherapy regimen for testicular cancer. Semen analyses in patients before and after adjuvant treatment with BEP show decreased sperm count and motility [64]. Of the toxicities of this chemotherapy regimen, gonadal cell dysfunction and Leydig cell insufficiency have been specifically linked to the cumulative dose of cisplatin [15, 60]. As a platinum-based agent, cisplatin interferes with the cell cycle by binding DNA and causing strands to cross-link. Patients treated with cisplatin-based chemotherapy have dose-dependent decreases in testosterone, corresponding rises in FSH and LH, and decreased paternity rates at follow-up ranging from 15 months to 20 years [14, 15, 60].

Gonadotoxicity from Radiation

Radiation damages cells and internal DNA beyond the point of repair to prevent replication. Radiation therapy directed at the pelvis and genitalia can cause injury to the germinal epithelium in Hodgkin's lymphoma [65] and testicular cancer [66]. Impaired spermatogenesis and semen analysis changes become increasingly

apparent by 6–12 months after radiation therapy for lymphoproliferative disorders [52] and testicular cancer [52, 53]. Gonadotoxicity may be avoided at lower levels of radiation as there is no impact on sperm count or FSH levels at radiation doses less than 20 cGy [65]. Transient dose-dependent decreases in sperm count and corresponding increases in FSH in patients with Hodgkin's lymphoma [65] and testicular seminoma [67] can occur at doses between 20 and 100 cGy. Azoospermia has been demonstrated at even higher doses of fractionated testicular radiation at 118–228 cGy [68].

The radiation dose can also affect the time to recovery [66]. Recovery is demonstrated in patients receiving lower doses of radiation by 12–30 months after treatment [65, 67, 68]. In contrast, fractionated testicular radiation at 140–300 cGy can result in permanent damage with azoospermia with no recovery at 15 months in Hodgkin's lymphoma patients [69]. The gonadotoxicity of chemotherapy and radiation can exhibit a synergistic effect as adjuvant chemotherapy has been shown to prolong the recovery period of radiation for testicular cancer [66].

Modern shielding techniques may protect against dose-dependent radiation injury. Over half of the patients regained normal semen parameters and most patients recovered some degree of spermatogenesis following treatment for early-stage seminoma with orchiectomy and radiation therapy with the use of shielding [70]. Recovery in this cohort of patients was not affected by radiation dose and was attributed to the shielding techniques employed during treatment [70].

Recovery from Gonadotoxic Treatments

The majority of patients demonstrate some form of recovery with improved semen analyses by 18 months to 2 years after chemotherapy or radiation for testicular cancer or lymphoproliferative disorders [54, 65, 67]. The influence of pretreatment semen parameters on posttreatment recovery has yet to be determined. Studies have shown that pretreatment sperm count in testicular cancer [64] and lymphoma [54] prior to treatment may be a predictor of recovery following chemoradiation [66]. Other studies, however, have demonstrated that recovery of spermatogenesis is not related to pretreatment semen parameters in patients with testicular cancer [53] [71]. Compared to men with lymphoma, men with testicular cancer may start with lower pretreatment sperm concentration but are at a lower risk of azoospermia following gonadotoxic treatment [71]. Other factors that may influence recovery include the number of chemotherapy treatments [64] and posttreatment reduction in sperm concentration [71]. There may be no difference in recovery between patients with Hodgkin's lymphoma and those with NHL [56].

Permanent testicular damage has been reported in the literature. Azoospermia at long-term follow-up has been demonstrated in 18% of childhood cancer survivors treated with chemoradiation [63] and 7% of adult patients treated for lymphoma [54]. In addition to azoospermia, patients with permanent gonadal damage demonstrate corresponding elevations in FSH and LH [17] that suggest primary testicular

dysfunction from gonadal injury. The risk of long-term azoospermia is even higher in childhood leukemia, Hodgkin's lymphoma, NHL, or testicular cancer survivors treated with concurrent radiation to the testes [63].

As gonadotoxic agents may permanently damage the germinal epithelium, fertility preservation is often recommended prior to treatment. In addition to sperm preservation, which is discussed throughout other chapters and sections in this text, manipulation of physiologic hormonal systems has been suggested as a means of protecting the integrity of the germinal epithelium. Exogenous testosterone and GnRH agonists may be administered to theoretically suppress the HPG axis through the negative feedback loop at the time of gonadotoxic treatment. By diminishing the rate of spermatogenesis, this would potentially decrease the impact of gonadotoxic agents on the germinal epithelium. Studies demonstrating the success of hormonal manipulation are limited, however, and the American Society of Clinical Oncology does not currently recommend exogenous hormone administration for male fertility preservation [72].

Sexual Dysfunction

Male sexual function depends on a combination of a functional nervous system (Fig. 1.3) and intact reproductive anatomy. Erectile function arises from parasympathetic innervation and ejaculatory function is attributed to the sympathetic nervous system. Parasympathetic nuclei at S2–S4 give rise to the pelvic nerve. The pelvic nerve signals the cavernosal nerve to initiate the release of nitric oxide and increases arterial flow to the penis. Sympathetic innervation from the hypogastric nerve arising from T10-L2 then signals the emission of sperm from the epididymis. These nerves converge at the pelvic plexus.

During emission, spermatozoa travel through the length of the vas deferens from the scrotum and through the inguinal canal before converging with semen at the ejaculatory duct. This alkaline fluid is produced by the prostate, seminal vesicles, and bulbourethral glands to provide nutrition in the form of fructose and act as a lubricant for expulsion. Pulsatile ejaculation through the penile urethra occurs with somatic innervation from the pudendal nerve arising from S2–S4.

Surgical interventions involving the retroperitoneum or pelvis risk injury to the male reproductive tract and the neurovascular structures required for sexual function. Damage to the hypogastric nerves or pelvic nerve plexus can result in erectile or ejaculatory disorders and subsequent post-testicular male infertility. Retroperitoneal lymph node dissection (RPLND) for more advanced stages of testicular cancer risk damage to the sympathetic nerve fibers and can result in ejaculatory disorders. There is a significantly higher incidence of ejaculatory disorders in patients with testicular cancer treated with primary or post-chemotherapy RPLND compared to those treated with chemotherapy alone [73]. Male sexual function before and 3 months after bilateral RPLND was assessed in patients previously treated with orchiectomy and adjuvant chemotherapy [74]. Although these patients

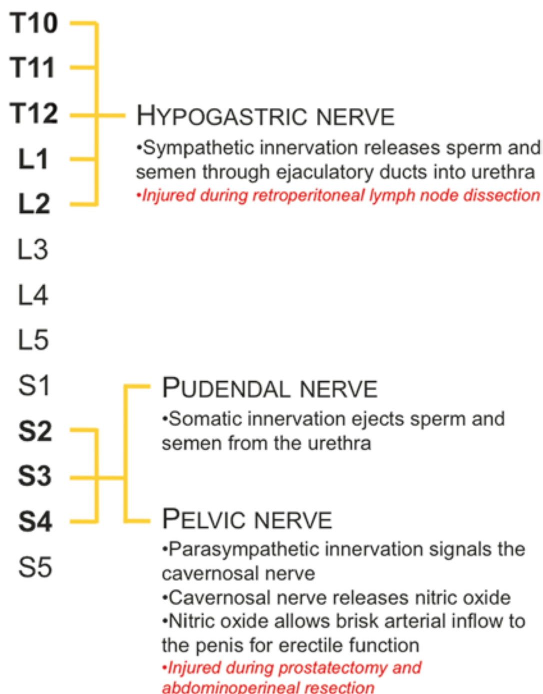

Fig. 1.3 Innervation of male sexual function. Sympathetic innervation arises from T10-L2 through the hypogastric nerve whereas parasympathetic innervation and somatic innervation arise from S2 to S4 via the pelvic and pudendal nerves, respectively. These nerves can be injured in surgeries that aim to treat cancer

reported no change in erectile function, there was significant impairment in orgasm, antegrade ejaculation, and overall sexual satisfaction [74].

While prostate and colorectal cancers tend to occur in older men, surgical intervention can negatively impact men that hope to reproduce. Radical prostatectomy with bilateral pelvic lymph node dissection can affect both urinary continence and erectile function [75, 76]. There may be a gradual return of erectile function as postoperative potency has been reported in 38% of patients at 3 months and 86% of patients at 18 months [75]. Patients can nevertheless experience permanent impotence at long-term follow-up at 18 or more months [76]. Rectal cancer is often treated with mesorectal excision through a low anterior or abdominoperineal approach. As abdominoperineal resections involve the perineum, postoperative reductions in sexual activity and ejaculatory function have been reported despite preoperative sexual function or autonomic nerve-sparing procedures [77]. Postoperative sexual dysfunction is worse with extended lateral pelvic lymph node dissection [78]. While autonomic nerve damage is identified as the main predictor of postoperative *ejaculatory* dysfunction, anastomotic leakage and perioperative blood loss may contribute to *erectile* dysfunction [79]. Inflammation from anastomotic leakage may contribute to nerve damage and this suggests that there may be other undetermined causes of nerve damage beyond mechanical injury that have yet to be elucidated.

Conclusion

Male fertility can be impaired temporarily or permanently within the setting of cancer. Circulating tumor factors and radiation directed at the head and neck can cause endocrine dysfunction at the HPG axis and reduce testosterone production. Systemic inflammation, local structural damage, and gonadotoxic agents can each decrease fertility either directly through damage to the germinal epithelium or indirectly through changes in testosterone production or the testicular environment. Surgical intervention can exacerbate infertility by causing loss of testicular tissue from orchiectomy and sexual dysfunction from structural damage. Given the complexities of cancer and the multidisciplinary approach required in its treatment, it is important to understand, evaluate, and manage cancer-induced male infertility through a systematic approach.

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Chapter 2
Impact of Cancer Treatment on Sperm Chromatin Integrity

Marij Dinkelman-Smit

Introduction

Improved cancer-specific survival, novel fertility preservation methods, and increased awareness in patients and physicians about the detrimental effects of oncological treatment on fertility have given rise to a new field of interest in reproductive medicine: oncofertility [1]. Although it is good clinical practice to offer sperm cryopreservation in male cancer patients before the onset of oncological treatment [2], cancer survivors report to have been counseled and offered fertility preservation in 30–87% of cases [3].

Cancer patients and cancer survivors who wish to conceive seek counseling to discuss spontaneous pregnancy chances, fertility preservation options, assisted reproduction techniques using cryopreserved sperm or sperm recovered posttreatment, conception timing, and safety issues related to their future offspring. In clinical practice cancer patients, their physicians and consulted fertility care providers are faced with many inconclusive aspects of reproduction following cancer treatment, devoid of certified evidence.

Sperm Chromatin Integrity

The limited prognostic value of traditional semen parameters for sperm function and fertilizing potential further complicates these issues. Assessment of the integrity of sperm DNA organization has proven to be a valuable biomarker of sperm function that gives additional insight into the quality and functionality of

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spermatozoa [4]. The term "chromatin" refers to the macromolecular complex that organizes the intranuclear DNA. The structure of chromatin is stabilized by interactions between its main compounds, DNA and DNA-binding proteins. In sperm cells, chromatin has a highly condensed structure in which DNA strands are bound to protamines, to enable safe transport of paternal DNA throughout the genital tract [5]. In humans, sperm DNA condensation is often incomplete, resulting in heterogenic sperm chromatin quality within individuals [6]. When sperm DNA packaging is incomplete, the DNA remains organized in loops that are more sensitive to damage. In infertile men sperm DNA packaging was shown to be impaired, resulting in decreased sperm chromatin integrity [4]. The structural organization of sperm DNA has proven to be a diagnostic tool in the management of male infertility, complementary to traditional microscopic analysis of sperm concentration, motility, and morphology [4]. Moreover, sperm DNA integrity assessment has been shown to be a robust sperm function biomarker with prognostic value for the outcome of both spontaneous pregnancy [7, 8] and outcome of assisted reproduction [9]. The etiology of sperm DNA damage is most probably multifactorial, but it is generally accepted to arise from a combination of compromised chromatin remodeling, oxidative stress, and abortive apoptosis. Sperm chromatin integrity assessment has been successfully used as a diagnostic tool in toxicology [10] and in male infertility intervention studies [11]. Over the past 15 years, its use as a biomarker to assess genomic integrity in sperm from males with cancer and exposure to cancer treatment has been under investigation.

A landmark paper by Thomson et al. in 2002 suggested no significant difference in sperm DNA fragmentation in 22 oligospermic and normozoospermic cancer survivors compared to 66 age-matched controls [12]. Although the study concluded that long-term survivors of Hodgkin's disease, leukemia, medulla blastoma, and sarcoma are at high risk of impaired spermatogenesis, the unaffected sperm DNA integrity in childhood cancer survivors with recovered or partially recovered spermatogenesis seemed reassuring. Since then, studies in cancer survivors with contradicting results have been published [13]. In addition, the extent of sperm DNA integrity defects in patients at the time of cancer diagnosis and following short (0–9 months) or longer (12–24 months) time intervals postexposure to gonadotoxic treatment is highly variable. One of the major drawbacks in this field is the variety of laboratory techniques available for the evaluation of sperm DNA integrity. In addition, various cancer diagnoses, widely distributed age at treatment, and a multitude of treatment regimens and follow-up protocols further prevent uniform conclusions.

The impact of cancer itself on male fertilizing potential varies greatly among individuals. For instance, one- to two-thirds of male cancer patients are confronted with impaired fertility at the time of semen cryopreservation [14]. In testicular germ cell tumors (TGCT) impaired fertility is hypothesized to be part of a spectrum in which preexistent gonadal dysfunction and TCGTs have a shared pathophysiology referred to as the testicular dysgenesis syndrome [15]. In lymphoma and leukemia patients, the underlying mechanism of impaired spermatogenesis at the time of diagnosis is thought to involve cytokines and the detrimental effects of fever, severe disease, and nightly sweats [16].

If sperm production is unaffected by oncological treatment or if sperm recovery is partial but sufficient to await spontaneous conception, patients may seek advice about the appropriate timing and safety of spontaneous conception. In general practice if spontaneous conception does not occur, assisted reproduction with cryopreserved pretreatment sperm is often preferred over assisted reproduction with fresh recovered sperm obtained after oncological treatment because of fear of imposing genotoxic gametes to the offspring. The effect, however, of both cancer itself and chemo- or radiotherapy on the genomic integrity of spermatozoa and eventually the offspring of cancer survivors is unclear. Although some epidemiological and animal studies suggest a transgenerational effect of oncological treatment, the data is controversial. Evidence-based data that is necessary for counseling on reproductive health and assessing the risk of transmitting chromosomal anomalies or de novo mutations to offspring is scarce.

The efficacy of assisted reproduction with intracytoplasmic sperm injection (ICSI) making use of available frozen sperm versus fresh sperm obtained after cessation of treatment in men with partial recovery of spermatogenesis is often debated. The available evidence suggests superior reproductive safety of assisted reproduction with pretreatment cryopreserved sperm versus posttreatment ejaculatory sperm in men with partially recovered spermatogenesis or surgically obtained sperm in post-chemotherapy.

In this overview, laboratory assays to assess sperm DNA integrity are summarized. Clinical studies that evaluated the short- and long-term effects of cancer and its treatment with chemotherapy, radiotherapy, and radioiodine treatment on sperm chromatin integrity are critically appraised. Finally, the reproductive safety of spontaneous conception in epidemiological studies among cancer survivors is discussed.

Assays to Quantify Sperm Chromatin Integrity

TUNEL

The terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) technique is a commonly reported assay to investigate sperm nuclear integrity. Essentially, this method takes advantage of the ability of the DNA repair enzyme terminal deoxynucleotidyl transferase (TdT) to incorporate fluorescently labeled nucleotides at the 3′-end of a broken DNA strand. TUNEL does not provide direct quantification of DNA breaks, but individual sperm cells are scored as DNA fragmentation positive or negative on the basis of their fluorescence intensity. Evaluation of the fluorescent intensity can be done either microscopically or by flow cytometer. The TUNEL assay is not validated for in vivo prognostic pregnancy rate in the general population.

Comet Assay

The comet assay also known as single-cell gel electrophoresis is an electrophoretic technique in which lysed sperm cells are embedded in agar and allowed to migrate in an electric field. Intact, high-molecular-weight, unbroken DNA migrates slowly and will remain in the sperm head, whereas the smaller, fragmented DNA migrates out and takes on the form of a comet. The length of the comet in 200–300 individual sperm cells is measured microscopically and sperm DNA fragmentation can thus be quantified. In the neutral comet assay DNA is not denaturated, making the assay more sensitive for the measurement of double-strand breaks. Under acid or alkaline conditions double- or single-strand DNA breaks can be detected. Few studies relating the comet assay to clinical fertility status have been published and no clinically useful thresholds for fertility potential have been established.

Chromatin Stains

Several assays identify packaging defects of sperm chromatin by staining persistent histones (aniline blue assays) or competitive binding to protamines to demonstrate poor protamination (chromomycin A3). The sperm chromatin dispersion test (SCD) is based on the principle that sperm with fragmented DNA fail to produce a characteristic halo when mixed with aqueous agarose following acid denaturation and removal of nuclear proteins.

Acridine Orange (AO) sperm DNA fragmentation assays and the sperm chromatin structure assay are based on the metachromatic properties of the DNA-binding fluorescent dye AO. When bound to intact, double-strand DNA AO emits green fluorescence, and red fluorescence when bound to single-strand, fragmented DNA. Samples are exposed to an acid solution that permeabilizes the cell membrane, allowing AO dye molecules to enter the sperm nucleus and bind to the sperm DNA. The low pH of the acid solution potentially denatures DNA in situ. DNA with abnormal chromatin structure and single- or double-strand breaks is more susceptible to denaturation. The fluorescent signals of 5000 cells are quantified by flow cytometry. The extent of compromised sperm chromatin integrity is expressed as the sperm DNA fragmentation index (DFI), reflecting the ratio of red fluorescence tot total fluorescence.

Direct Effects on Sperm DNA Integrity in Cancer Patients

Several studies have determined sperm DNA damage in cancer patients before treatment to evaluate whether cancer itself induced changes in the genomic integrity of sperm. A summary of these studies is given in Table 2.1.

Table 2.1 Summary of studies that evaluated sperm chromatin integrity at the time of semen cryopreservation before the onset of gonadotoxic treatment, compared to controls

Author	Number of patients at the time of diagnosis	Controls	Diagnosis	Sperm chromatin integrity assay	Pretreatment DNA integrity in cancer patients versus controls
Meseguer et al. [19]	75	50 fertile sperm donors, 166 men attending infertility clinic	16 Hodgkin's disease 6 NHL 6 leukemia 47 TGCT	Chromatin dispersion test	Significantly higher
Ribeiro et al. [20]	48	50 proven fertile controls	48 TGCT	TUNEL	Not significantly higher
Said et al. [17]	89	20 fertile sperm donors	39 TGCT 27 lymphoma 8 leukemia 10 colorectal cancer 3 skin cancer 2 brain cancer	SCSA	Significantly higher
McDowell et al. [18]	89	35 fertile sperm donors		SCSA	Not significantly higher

HD Hodgkin's disease, *NHL* non-Hodgkin's lymphoma, *TGCT* testicular germ cell tumors, *TUNE* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, *SCSA* sperm chromatin structure assay

Said and colleagues found significantly higher levels of sperm DNA levels assessed by SCSA in 89 patients diagnosed with TGCT, lymphoma, leukemia, colorectal cancer, skin cancer, and brain cancer compared to 20 fertile sperm donors [17]. Contradictory conclusions were drawn by McDowell et al. who used the same assay to evaluate sperm DNA integrity in 89 cancer patients, in comparison to 35 fertile controls, and found no significant differences [18].

The chromatin dispersion test detected a significant increase in DFI among patients with various malignancies including TGCT [19], contrary to TUNEL which failed to detect any significant changes in DFI among patients with TGCT compared to normal controls [20].

The general conclusions that can be drawn from these studies is that cancer itself may negatively affect the sperm DNA integrity. When the available evidence is critically reviewed, several general limitations can be observed. All studies are performed in patients referred for fertility preservation, resulting in overpresentation of the most prevalent cancer diagnosis in males of reproductive age: TGCT, lymphomas, and leukemia. The contemporary literature compares sperm DNA fragmentation levels in cancer patients before the onset of gonadotoxic treatment to proven fertile controls or subfertile men attending reproduction clinics, although population-based controls may prove to be more appropriate controls. The assays used to evalu-

ate sperm DNA integrity all require sperm concentrations of at least 2 × 106/mL excluding men with severely impaired spermatogenesis at the time of diagnosis. Finally, studied cohorts of cancer patients are relatively small. Even the two largest prospective studies among TGCT patients from the Centres d'Etudes et de Conservation des Oeufs et du Sperme humain CECOS consortium in France [21] and Gandini's Laboratory of Seminology sperm bank in Italy [22] included, respectively, 129 and 254 TGCT patients but sperm DNA fragmentation analysis at the time of diagnosis was available in only 53 and 139 patients, respectively.

Follow-Up of Sperm Chromatin Integrity in Treated Cancer Patients

Studies that evaluated the sperm DNA damage at various follow-up intervals after gonadotoxic chemotherapy or radiotherapy exposure show inconsistent results (Table 2.2). Increased DNA damage using the comet assay was reported in a prospective study by O'Flaherty et al. [23] and O'Donovan [24], but other authors [21, 22, 25–30] reported only temporary decreased sperm chromatin integrity that improved after 12–24 months posttreatment intervals, acknowledging the variable time interval between exposure with heterogeneous chemo- and radiotherapy regimens and different sperm chromatin integrity assays as the most important limiting issues. The generalized consensus is that the chromatin structure in sperm improves following chemotherapy exposure, with a transient increase in sperm DNA fragmentation at 6 months posttreatment. It has been suggested that this phenomenon can be explained by clonal expansion of quiescent type A spermatogonia that survive a chemotherapeutic assault. These quiescent spermatogonia that repopulate the testicular parenchyma after gonadotoxic exposure may be less prone to sperm DNA damage. Surviving stem spermatogonia that are able to proliferate, differentiate, and produce spermatozoa lead to the recovery or partial recovery of spermatogenesis. The clonal offspring of these unaffected spermatogonia harbor improved chromatin integrity compared to pretreatment sperm produced at the time of cancer diagnosis.

These results seem reassuring, but should be interpreted with caution. Evaluation of the fertility status in patients formerly treated for cancer is hampered by unpredictable recovery rates of spermatogenesis, selection bias, and loss to follow-up. As mentioned previously, sperm chromatin integrity assessment with SCSA can only be evaluated in patients with recovered spermatogenesis with sperm concentrations of at least 2 × 106/mL. Selection bias in former studies is further introduced by therapy-related comorbidity such as transient azoospermia, retrograde ejaculation, anorchia, and cancer-specific mortality that complicate follow-up of post-gonadotoxic exposure fertility. For example, in two recent large prospective studies, sperm chromatin integrity analysis was available at 24 months of follow-up in only 41 out of 129 TGCT patients [21], 42 out of 75 Hodgkin's and non-Hodgkin's lym-

Table 2.2 Summary of studies that evaluated sperm chromatin integrity at the time of semen cryopreservation before the onset of gonadotoxic treatment, compared to controls and posttreatment

Author	*N* Diagnosis	*N* Follow-up	Controls	Diagnosis	Sperm chromatin integrity assay	Interval between pre- and posttreatment	Pretreatment DNA integrity compared to controls	Posttreatment DNA integrity compared to pretreatment values
O'Donovan [24]	33	12	14 (proven fertile)	8 HD 3 NHD 9 leukemia 13 TGCT	Comet, chromatin condensation assay by propidium iodide	3 and 6 months	Significantly higher	Impaired following cancer treatment
Spermon et al. [30]	22	22	13 (normozoospermic attending fertility clinic)	22 TGCT	TUNEL, chromatin condensation by CMA3	18–84 months	Significantly higher	No significant change within patients Impaired compared to controls
Smit et al. [31]	127	45	22 (proven fertile)	15 HD 5 NHD 25 TGCT	SCSA	6–40 months	Not statistically higher Only in NHL patients pretreatment DFI significantly higher compared to controls	DFI decreased significantly compared to controls. DFI at follow-up was significantly higher in TGCT patients who were treated with RT compared to patients treated with BEP alone

(continued)

Table 2.2 (continued)

Author	*N* Diagnosis	*N* Follow-up	Controls	Diagnosis	Sperm chromatin integrity assay	Interval between pre- and posttreatment	Pretreatment DNA integrity compared to controls	Posttreatment DNA integrity compared to pretreatment values
O'Flaherty et al. [23]	32	32	11 (healthy male volunteers)	16 TGCT 16 HD	COMET	6, 12, 18, and 24 months	Significantly higher	Significant increase in sperm DNA damage at 6 months posttreatment, which remained elevated up to 18–24 months, compared to pretreatment and controls
Stahl et al. [25]	121	58	137 (fertile controls)	84 TGCT 18 HD 9 NHL 3 CNS 2 sarcoma 5 other	SCSA	Median 3 years	Significantly higher DFI in TGCT and HL patients, compared to controls	No significant difference in pre- and posttreatment DFI
Bujan et al. [21]	53	41	51 (fertile controls)	53 TGCT	SCSA, TUNEL	3, 6, 12, and 24 months in, respectively, 28, 36, 40, and 41 patients	Significantly higher SCSA values but not TUNEL values	No increased sperm DNA fragmentation or TUNEL values were found at 24 months posttreatment. Transient defects in chromatin condensation were shown at 6 months in patients treated with radiotherapy

Bujan et al. [27]	75	71	51 (fertile controls)	57 HL 18 NHL	SCSA, TUNEL	3, 6, 12, and 24 months in, respectively, 38, 38, 43, and 4 patients	Significantly higher	Mean values of DFI decreased from 6 to 24 months after treatment, but always remained higher compared to controls. Mean TUNEL values decreased after treatment and were similar to control values from 6 to 24 months
Paoli et al. [22]	139	82	–	139 TGCT	SCSA	3, 6, 9, 12, and 24 months in, respectively, 59, 54, 60, 75, and 75 patients	–	Impaired chromatin integrity at 3 and 6 months posttreatment, returning to baseline at 9 months and further improving at 12 and 24 months

Only the number of patients in whom sperm chromatin integrity assessment was performed is summarized
HD Hodgkin's disease, *NHL* non-Hodgkin's lymphoma, *TGCT* testicular germ cell tumors, *TUNE* terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, *SCSA* sperm chromatin structure assay

phoma patients [27], and 82 out of 254 testicular cancer patients [22] despite a well-designed study protocol and great effort of the investigators. Results from these studies show consistent results with a significant reduction of sperm DNA damage at 24 months in comparison with baseline. Paoli et al. found the greatest levels of sperm DNA damage at 3 and 6 months posttreatment [22], whereas Bujan et al. found chromatin density to be increased at 6 months posttreatment in patients with seminomas treated with radiotherapy [21].

This finding is in agreement with a study by Smit et al. that suggested a greater induction of sperm chromatin integrity defects following radiotherapy [26].

Thus far, only poor quantitative and qualitative semen parameters at the time of diagnosis and at post-cancer treatment follow-up were shown to be negatively correlated with sperm DNA damage indicating that sperm chromatin damage is more extensive in patients with impaired spermatogenesis [22]. Smit et al. have previously postulated this general association between the clinical hallmarks of the quality of spermatogenesis and sperm DNA fragmentation in a cohort of infertile men [31]. Important work on the susceptibility of individuals for increased sperm DNA fragmentation following cancer treatment was published by Zhu et al. [32]. In this study, single-nucleotide polymorphisms (SNPs) on the hMSH5 C85T allele and genotype frequencies were studied in 113 TGCT patients before and after radiotherapy to investigate individual variations in spermatogenic radiosensitivity. Posttreatment DNA fragmentation was attenuated compared to pretreatment in patients with CT + TT genotype. This indicates that the hMSH5 C85T SNP may modulate the effect of radiation on the extent of sperm DNA damage. The authors hypothesize that this may be through interference with the repair of radiation-induced DNA damage in these individuals. The study was performed in a Han Chinese population and warrants validation in a patient cohort with different genetic background. The time to follow-up was not clearly stated, but appeared to be 1 year postradiation. The association of hMSH5 C85T polymorphisms and individual variations in long-term effects of radiotherapy on sperm chromatin integrity is promising but needs to be validated in larger sample sets.

Long-Term, Transgenerational Effects on the Offspring of Male Cancer Survivors

The reproductive safety of males exposed to cancer treatment has long been questioned. It has been suggested that genetic alterations caused by gonadotoxic treatment may predispose the offspring of cancer patients to an increased risk of genetic diseases [33]. Recent epidemiological studies suggest that major congenital abnormalities are more common in the offspring of male cancer survivors [34] while other studies show no such effect [35]. Animal studies showing transgenerational transmission of genomic instability induced by chemo- or radiotherapy [36, 37] cannot be supported by studies in humans. Kryukov et al. recently reported germline whole-genome sequencing in two male cancer survivors exposed with

chemotherapy and their children conceived before and after exposure. De novo genetic events were absent in postexposure children, compared to their pre-exposure siblings [38]. Furthermore, there is no evidence for increased abnormal karyotypes in live-born children of cancer, compared to sibling families [39]. Although these studies are reassuring, they are underpowered to detect relative risks of transgenerational effects in children born to cancer patients.

Bujan et al. showed that during the first year following treatment for TGCT with either radiotherapy or BEP chemotherapy regimens, some men had sperm quality compatible with natural fertility [21]. Animal studies have raised concerns that in these cases mutagenic damage is present and can even be transmitted to the next generation [37]. In humans, the methods used to identify sperm DNA fragmentation may not be sensitive enough to detect the presence of mutational damage within functional spermatozoa. The clinical consequence of chromosome aberration, both aneuploidy and structural anomalies, is unclear. Theoretically, induction of stable aberrations such as inversions and reciprocal translocations may give rise to transgenerational effects.

Inconsistent results were obtained in small studies that evaluated sperm aneuploidy frequencies in cancer patients. For example, Robbins found transient fivefold sperm aneuploidy rates in 11 men with Hodgkin's disease treated with NOVP (novanthrone, oncovin, vinblastine, and prednisone) compared to controls. The aneuploidy effects declined to pretreatment levels after 100 days of chemotherapy exposure. In contrast, Tempest and colleagues showed elevated frequencies of sperm aneuploidy at the onset of BEP and ABVD treatment in, respectively, five TGCT and five Hodgkin's disease patients. Following 6 months of treatment, aneuploidy rates increased, where after 18 months post-chemotherapy these rates declined to pretreatment levels in most of the cases [40]. It is important to realize that only individuals with persistent spermatogenesis following chemotherapy were available for follow-up in these studies.

Recommendations

In summary, sperm chromatin defects are detected 6 months post-chemotherapy and -radiotherapy treatment, dropping to below pretreatment levels 24 months after gonadotoxic treatment exposure in individuals with recovered spermatogenesis. Increased sperm aneuploidy rates prevail up to 2 years posttreatment. These conclusions form the basis for the rationale to postpone spontaneous conception 1–2 years after cancer treatment. Future studies should be directed towards understanding the effects of oncological treatment on the male germline and its short- and long-term consequences. In addition, new insights into the nature and quality of spermatogonia that repopulate the testis following gonadotoxic treatment are needed to provide evidence-based patient counseling on safe reproduction and appropriate time interval between treatment and reproduction using ejaculated or surgically obtained sperm.

Pretreatment sperm cryopreservation should continue to be propagated in oncological practice. It remains inconclusive if these sperm harbor genetically healthier DNA compared to recovered sperm following gonadotoxic treatment. Genomic alterations with clinical implications for offspring may not be detected by the methods used to assess genomic safety, but conclusions from epidemiological studies are reassuring. As long as the spermatogonial stem cell cultures or in vivo transplantations are experimental, sperm cryopreservation is the best available method to ensure future fertility should post-chemotherapy spermatogenesis fail. In patients with post-chemotherapy azoospermia who were unable to bank sperm before their treatment, testicular sperm can be obtained by microdissection testicular sperm extraction (microTESE) in 35–47% and live birth rates following ICSI have been reported [41, 42]. The safety of microsurgically retrieved testicular sperm in cancer survivors with post-chemotherapy azoospermia is under debate.

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Chapter 3
Testicular Sperm Retrieval for Cryopreservation in Cancer Patients

Gagan Prakash and Rupin Shah

Introduction

Survival rates of younger patients with cancer have seen a significant improvement over the last few decades. Sixty percent of male patients in the United States in the age group of 15–29 years experience 20-year survival after diagnosis of invasive cancer [1].

With better survival rates the focus is now shifting to quality of life and fertility preservation. This is particularly of importance in malignancies like testicular cancer and lymphoma where the patients are in reproductive age group and chances of cure are high. Most of these patients are offered cryopreservation before the beginning of their treatment. However, despite the simplicity of this procedure only 27% of patients are successfully able to bank their sperm [2]. This is due to various reasons related to either the patient's baseline semen parameters or his inability to ejaculate. The aim of this chapter is to discuss scenarios where cryopreservation of ejaculated sample is not an option for cancer patients and testicular sperm recovery needs to be considered.

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Indications for Testicular Sperm Retrieval

Inability to Collect a Semen Sample

This could be either because the man is unaccustomed to masturbation or due to anxiety. Having learnt about their cancer recently, patients are preoccupied with their disease and treatment outcome. They may often not be able to divert their attention and masturbate. It is important that the clinician talks to the patient and counsels him for another attempt, but sometimes the patient may still fail to collect a sample.

Occasionally, the patient may be too ill to masturbate. Testicular cancer and lymphoma are often multicentric diseases. Patients with extensive nodal disease involving retroperitoneum and mediastinum, presenting with debilitating pain, dyspnea, or hemoptysis, need urgent medical attention and initiation of chemotherapy, and would not get a chance to submit an ejaculated sample.

In such cases, it may be possible to obtain a semen sample by the use of a penile vibrator or electro-ejaculator. If these methods fail, or are not available, then testicular sperm retrieval would be required.

Pretreatment Azoospermia

Sixty-four percent of men referred for sperm banking before chemotherapy have abnormal semen parameters. Twelve percent have azoospermia or severe oligozoospermia without motility, and are thus unable to bank sperm [3].

Hendry et al. studied the baseline semen parameters of 208 testicular cancer patients before they started chemotherapy and found that only 27% of patients had baseline sperm counts greater than ten million per mL [4]. Decreased spermatogenesis has also been reported in contralateral testicular biopsies at the time of orchiectomy in more than 2000 patients [5].

For most of these patients their first realization about their suboptimal sperm happens when they go for cryopreservation before initiating chemotherapy. In a small proportion of patients infertility evaluation leads to detection of testicular cancer: Jacobsen and colleagues studied 32,442 Danish men being evaluated for infertility and found them to be 1.6 times more likely than the general population to develop testicular germ cell tumors [6].

In most oncology centers, the endeavor to obtain a sample for cryopreservation stops once the ejaculate is azoospermic. For many years it was thought that the only option to father a child in a man with nonobstructive azoospermia (NOA) was by donor insemination. Devroey was the first to show that sperm could still be retrieved from the testes of some men with NOA and these could be used for assisted reproduction [7]. Since then various open and percutaneous techniques have been

developed with sperm retrieval rates ranging from as low as 11% with fine-needle aspiration to as high as 56% by microdissection TESE [8–10]. These sperm can be cryopreserved for future IVF-ICSI.

Men with Testicular Malignancy Who Are Scheduled for Orchiectomy

Often these men are faced with two problems—lack of time to collect a sample because they are busy with the medical aspects of their cancer, and a very low sperm count (related to the malignancy) because of which sometimes there may not be enough sperm to freeze. In such cases there is the opportunity for sperm retrieval from the orchiectomy specimen. After performing an orchiectomy a bench dissection can be done on the specimen and sperm can be retrieved from normal testicular tissue surrounding the tumor. This concept is called oncological testicular sperm extraction ("onco-TESE") [11].

This is possible only in smaller tumors and the division of specimen should preferably be done in consultation with the pathologist. The specimen needs to be opened in a manner that will not compromise the ability of the pathologist to stage the tumor, and then a large amount of normal testicular tissue can be obtained for sperm retrieval. The routine use of this technique is still restricted and its efficacy and long-term safety are yet to be seen. In a recent study, four out of six men with testicular germ cell tumor had sperm found on onco-TESE. Two of these were able to father a child following in vitro fertilization (IVF) with the sperm extracted through onco-TESE [12].

Prepubertal Malignancies

In this group of patients spermatogenesis has still not occurred and so sperm cryopreservation is not an option. However, the potential use of stem cells to restore fertility later in these patients is being explored as discussed in more details in Chap. 20. There have been studies in nonhuman primates where testicular tissue was extracted before the initiation of chemotherapy, preserved, and later thawed, and stem cells from this tissue were reimplanted into the testis. Spermatozoa obtained by this technique could fertilize ova in animal studies [13].

The concerns are likely reimplantation of malignant cells into testis during stem cell transplantation and damage to the testis during biopsy, thus decreasing the possibility of return of spermatogenesis on its own once the effect of chemotherapy wears off. Even though the safety and efficacy of this method to preserve fertility in

humans are yet to be proven, a survey amongst parents of prepubertal boys revealed that 76% of them were willing for tissue cryopreservation of their child's testicular tissue [14].

Techniques of Sperm Retrieval

Needle Aspiration Biopsy (NAB Technique)

This is a simple procedure that can be done under local anesthesia with a cord block. The testis is stabilized with ring and middle fingers and the scrotal skin is stretched tightly over the testis using the forefinger and thumb. The testis is punctured with an 18G scalp vein needle connected to a 10 mL syringe. As the needle is pushed into the testis the assistant creates suction by pulling the plunger of the syringe all the way to the top. While the suction is maintained the needle is slowly pulled out three-fourths and then reinserted in the same direction. This movement is repeated a few times till aspirate is seen in the tubing of the scalp vein needle. The purpose is to suck seminiferous tubules into the needle. This is not an FNAC procedure and the needle should not be pushed in multiple directions—this would cause considerable parenchymal bleeding and damage. The tubing is then clamped near the syringe and the needle is then slowly pulled out of the testis. When the needle emerges a core of testicular tissue will be pulled out with it. The assistant grasps this tissue with a non-serrated microsurgical forceps and slowly pulls the tubules out of the testis (Fig. 3.1). Two forceps are used alternately to pull the tissue till it thins out and breaks. Usually, this should provide a large chunk of testicular tissue. The tubing is then unclamped

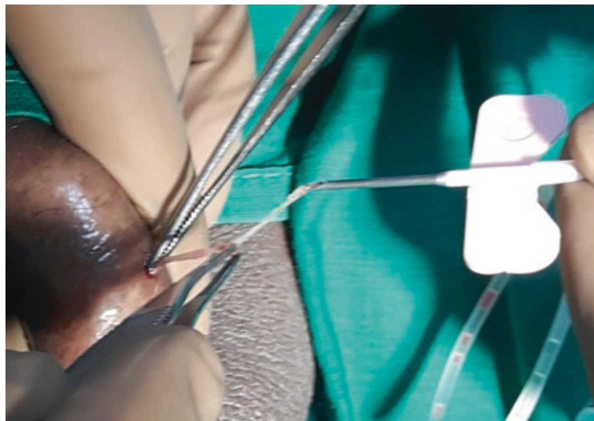

Fig. 3.1 Needle aspiration biopsy (NAB). A core of seminiferous tubules is pulled out of the testis using an 18 G scalp vein needle and fine microsurgical forceps

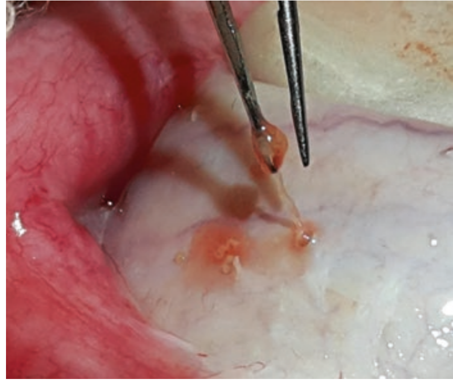

Fig. 3.2 The SST technique. A single seminiferous tubule is pulled out of a puncture hole in the tunica

and the needle is flushed with air in the syringe, delivering a second large piece of tissue. If the tissue is inadequate the procedure is repeated. It is very important to maintain firm pressure on the puncture site for 10 min to prevent bleeding. A pressure dressing is then applied.

Testicular Mapping by Single Seminiferous Tubule (SST) Technique

This is a technique [15] for obtaining multiple micro-biopsies from all over the testis without having to bivalve it as in microdissection TESE. It is much simpler, and less traumatic than microdissection TESE. It can be performed using loops making it more readily available in oncology centers where an operating microscope may not be available.

The scrotum is incised and the testis is exposed. The testis is squeezed gently and the tunica is punctured in an avascular area with a 21G needle. A loop of seminiferous tubule will pop out through the puncture hole. If it does not then the tunical opening is dilated gently with a single prong of a microsurgical forceps. Under magnification the protruding tubule is grasped with a microsurgical forceps and pulled out of the testis. Usually it is possible to pull out a long strand of seminiferous tubule through the puncture hole (Fig. 3.2). The final tissue recovered can equal an open biopsy. However, since this is only a tiny puncture hole it does not need stitching and does not harm the testicular vessels. Hence, it can be repeated over the entire surface of the testis at 1-cm intervals taking 20–30 biopsies with minimal trauma and no subsequent pain [16]. The noninvasiveness of this method, as compared to microdissection TESE, makes it very attractive in this group of seriously ill patients.

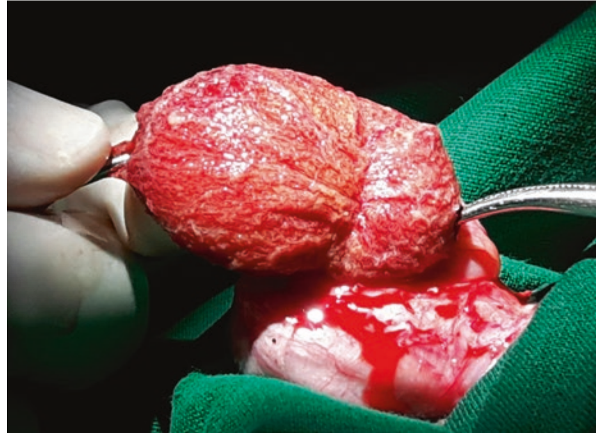

Fig. 3.3 Microdissection TESE. After the testis is bivalved the parenchma is everted over a finger and spread open for inspection of the seminiferous tubules

Microdissection TESE

This invasive technique allows for a comprehensive search of the entire testis. The testis is exposed and the tunica is incised along the transverse equator. The cut edges are grasped and slowly pulled apart, thus bivalving the testis all the way to the hilum. Vessels will be seen radiating from the hilum to the periphery, separating the lobules. The tissue is gently separated along these planes so that the parenchyma gets spread out (Fig. 3.3). The entire tissue is inspected under an operating microscope, looking for tubules that are larger than the surroundings. All these are biopsied and sent to the IVF laboratory for sperm retrieval. After careful hemostasis the parenchyma is replaced in the testis and the tunica is sutured with 5-0 prolene. This technique allows visual identification and biopsy of pockets of spermatogenesis in a poorly functioning testis.

Choice of Technique

Needle aspiration biopsy is the simplest and quickest method and avoids an incision. It is useful in those cases where a few random biopsies can be expected to yield adequate sperm. Thus, it is suitable for men who are unable to give a semen sample and in preadolescent boys. It can also be tried as the first step in men with azoospermia due to testicular failure before proceeding to more invasive sperm retrieval procedures. If the needle biopsies retrieve sufficient sperm then the open surgical procedure can be avoided.

SST testicular mapping and microdissection TESE are used in men with azoospermia or extreme oligozoospermia due to testicular failure (NOA). Since the SST mapping technique is much simpler and less traumatic than microdissection TESE it is tried first. Thus, in men with testicular failure the sperm retrieval is

started by performing three needle biopsies and sending these to the laboratory to check for sperm. If no sperm are found in the initial screening then we proceed with open SST mapping on one side. The tissue is screened for sperm as the biopsies are being taken. If no sperm are found after 20–30 mapping biopsies (as per size of testis) then the testis is bivalved and microdissection TESE is performed. If no sperm are found during initial screening the opposite testis is similarly explored. If sperm are not seen during initial evaluation the tissue is further minced, incubated, and spread into microdroplets that are examined over several hours.

Timing of Testicular Sperm Retrieval

Once chemotherapy is initiated sperm quality will decline and there are concerns about genetic damage in the remaining sperm that are available [17]. Hence, when testicular sperm retrieval is indicated it should be done before or during the first hospital visit for chemotherapy.

Further, in testicular cancer patients scheduled for orchiectomy, cryopreservation is advisable before orchiectomy as studies have shown that after orchiectomy semen parameters are worse than those before orchiectomy [18]. Similarly, if the semen is found to be azoospermic, the surgical sperm retrieval should be performed before or at the time of orchiectomy.

Complications of Surgical Sperm Retrieval

Potential complications of any testicular sperm retrieval procedure include hematoma, pain, or infection. Microdissection TESE, being more invasive, carries additional risks of intratesticular hematoma, prolonged testicular tenderness, partial testicular atrophy or reduction in testis size (due to devascularization), and fall in testosterone levels [19–21].

Outcome of Cryopreservation

Cryopreservation of normal testicular tissue is highly successful with very good sperm retrieval and pregnancy [22]. Even in men with NOA, cryopreservation of testicular tissue can be done with good success in subsequent IVF [23].

However, in some men with NOA very few sperm may be recovered and then there is a possibility that the sperm may not survive freezing-thawing. Hence, in such cases the cryopreservation must be done by an IVF laboratory well accustomed to special techniques for handling testicular tissue from men with NOA [24]. Further, such patients should be counseled for the small possibility of not finding sperm when the tissue is thawed.

Conclusion

The need for circumventing barriers to cryopreservation should be kept in mind by physicians treating young cancer patients. Appropriate counseling of patients and their care givers, and a liaison of oncologist with andrologist, will ensure that all options for sperm cryopreservation, including operative testicular sperm retrieval, are available to the patient. There are a variety of operative techniques and the least invasive one suited to the individual case should be selected.

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Chapter 4
Sperm Retrieval in Ejaculatory Dysfunction

Mohamed Arafa, Haitham Elbardisi, and Ahmad Majzoub

Introduction

Male fertility preservation is accomplished by cryopreservation of sperm from patients undergoing a treatment modality that may affect their fertility status at a later stage. Extracting sperm from the ejaculate is the most efficient method of sperm collection. However, we are commonly faced with failure of ejaculation which necessitates further steps in management.

Ejaculatory disorders are the most commonly reported sexual dysfunction interfering with sexual or emotional well-being of both partners. Apart from premature ejaculation which is extensively studied, other ejaculatory dysfunctions including anejaculation, retrograde ejaculation, and anorgasmia are still not well understood. Multiple interconnected etiologies have been identified in the pathophysiology of ejaculatory dysfunction such as psychogenic, neurogenic, iatrogenic, or congenital factors.

Cancer is a well-recognized clinical condition that has detrimental effects on fertility in general. Its influence on testicular function is either due to tissue loss in case of testicular tumors or as a consequence to different cancer treatments which lead to temporary or permanent spermatogenic arrest. Additionally, cancer treatment may lead to ejaculatory dysfunction through neurogenic or anatomic impairment secondary to retroperitoneal/pelvic surgery or bladder neck disruption, respectively. Current advances in cancer therapy improved survival especially in children and men of reproductive age. This fact highlights the importance of understanding the reproductive consequences of cancer and its therapy and the various treatment approaches that can be instilled to preserve or reverse fertility-related side effects.

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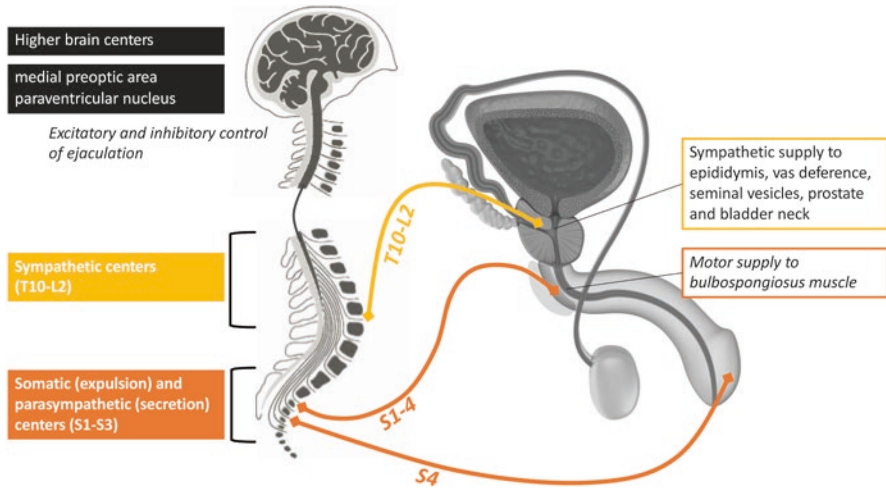

Fig. 4.1 Physiology of ejaculation. Sympathetic innervation originates from segments T10-L2 and reaches the pelvic plexus via the hypogastric nerve. Parasympathetic innervation arises from segments S2 to S4 and reaches the pelvic plexus via the pelvic nerve. The pudendal nerve (segment S4) innervates the external sphincter, bulbospongiosus, and ischiocavernosus muscles and provides sensory fibers to the dorsal nerve of the penis

This chapter aims to discuss ejaculatory dysfunction in a systematic approach, exposing its various clinical aspects from etiology to diagnosis and definitive treatment.

Physiology of Ejaculation

Ejaculation is a complex process resulting in the forcible expulsion of semen from the urethral meatus accompanied by orgasm. It is worth mentioning that orgasm and ejaculation are two different entities that should be differentiated from each other. While ejaculation is a physical procedure, orgasm is a mixture of emotional and physical sensations of sexual satisfaction experienced at the peak of sexual activity.

In order to understand the pathology of different ejaculatory dysfunctions, a brief explanation of the normal physiology is needed (Fig. 4.1). Ejaculation is composed of two successive phases: emission and expulsion which are anatomically and physiologically separate [1, 2]. These two phases comprise the efferent limb of the ejaculatory reflex. However the reflex is initiated by stimulation of sensory receptors in the mucosa of the glans penis following proper sexual stimulation. The sensory information is then transmitted via the dorsal nerve of the penis (S4) to the lumbosacral spinal cord, and is joined by sympathetic afferents from the hypogastric plexus. Together they travel up the spinal cord and are joined by visual, auditory,

and olfactory cerebral afferents. The reflex is controlled by forebrain structures that include the medial preoptic area and the paraventricular nucleus of the hypothalamus. Dopamine has been shown to stimulate ejaculation, whereas serotonin (5-hydroxytryptamine, HT) inhibits it. Nitric oxide also mediates the inhibitory neurotransmission responsible for seminal emission [3].

Seminal Emission

It is the deposition of seminal fluid to the posterior urethra. It involves muscular contraction of vas deferens, seminal vesicles, and prostate.

Bladder Neck Closure

Following emission bladder neck closes to prevent retrograde passage of semen to the urinary bladder. Both phases are under sympathetic α1 adrenergic nervous stimulation through superior and inferior hypogastric plexus (T10-L2 spinal centers).

Seminal Expulsion (Antegrade Ejaculation)

It is the forward expulsion of semen through the urethra. This is done through contraction of the bulbocavernosus muscle. The nervous control of this phase is by somatic nerve fibers from spinal segments S2–S4 and begins once the ejaculate has reached the urethra [3]. Here the external urethral sphincter relaxes while rhythmic peristaltic contractions of the periurethral and pelvic floor muscles move the ejaculate through the urethra and cause a pulsatile projectile ejaculation.

Ejaculatory Disorders

The well-known classification of ejaculatory disorders is the following:

– *Premature ejaculation:* The most common and highly debatable male sexual dysfunction. Recently defined by International Society of Sexual Medicine in 2013 as a male sexual dysfunction characterized by ejaculation which always or nearly always occurs prior to or within 1 min of vaginal penetration, and the inability to delay ejaculation on all or nearly all vaginal penetrations, and negative personal consequences, such as distress bother, frustration, and/or avoidance of sexual intimacy [4, 5]. PE can either present from the first sexual experience or following a new bothersome change in ejaculatory latency.

- *Delayed ejaculation:* Repeated or persistent difficulty in obtaining ejaculation following proper sexual stimulation [6].
- *Anejaculation (AE):* Total failure of emission of semen to the posterior urethra [6].
- *Retrograde ejaculation (RE):* Failure of antegrade ejaculation due to retrograde passage of semen to the urinary bladder caused by failure of simultaneous closure of bladder neck with ejaculation [7].
- *Low ejaculate volume:* This may be due to ejaculatory disorder like partial RE or anatomical obstruction of the ejaculatory duct (ED).

From the clinical point of view another classification for ejaculatory failure may be adopted to help in differential diagnosis:

1. *Anorgasmic Anejaculation:*

   This condition is characterized by persistent or recurrent absence of attaining orgasm after sufficient sexual stimulation. This may be situational or complete.

   (a) Situational anejaculation:

   The patient cannot ejaculate during certain conditions only, e.g., during semen sample collection for in vitro fertilization (IVF). This may be due to inability to masturbate, erectile dysfunction, or psychological stress [8].

   (b) Complete anejaculation:

   The patient here never ejaculates with either masturbation or intercourse. It can be primary or secondary. By definition, primary anorgasmia begins from the male's first sexual experiences and lasts throughout his life. However, secondary anorgasmia is preceded by a period of normal sexual experiences before the problem manifests [9].

   The main cause for this condition is psychogenic due to fear and anxiety during sexual relations. The most common triggers for this anxiety are hurting the female, impregnating the female, childhood sexual abuse, sexual trauma, repressive sexual education/religion, sexual anxiety, or general anxiety or depression [5, 9].

   Other causes include hyperprolactinemia, need for high degrees of sexual stimulation, diminished sensitive in the penile skin, and iatrogenesis due to intake of some medications especially antidepressants namely selective serotonin reuptake inhibitors, SSRIs [5, 9].

2. *Orgasmic Anejaculation:*

   In this condition, the patient reaches orgasm but doesn't ejaculate. This can be subclassified into the following:

   (a) *Failure of emission:*

   This may be caused by different neurological disorders most commonly diabetes mellitus, spinal cord injury and multiple sclerosis, surgeries including retroperitoneal lymph node dissection (RPLND), or iatrogenic causes including the intake of some medications, e.g., tamsulosin. Ejaculatory duct obstruction may also lead to failure of deposition of semen in the posterior urethra. Ejaculatory duct obstruction (EDO) may be caused by a variety of

reasons including congenital bilateral absent vas deferens, prostatic cysts, inflammatory (post-prostatitis), ED stones, and traumatic or iatrogenic injury of the ED [9, 10].

(b) *Failure of bladder neck closure:*

This will lead to retrograde ejaculation. It may be partial leading to low ejaculate volume or complete leading to anejaculation. It shares the neurological causes of failure of emission. Additionally it may be caused by iatrogenic injury of the external urethral sphincter during bladder neck surgeries or transurethral resection of the bladder.

(c) *Failure of seminal expulsion:*

This condition may be caused by urethral obstruction or failure of bulbocavernosus muscle contraction due to neurological causes.

Diagnosis of Ejaculatory Failure

The first step in the management of ejaculatory failure is to reach a diagnosis of the condition as further management plan will be fashioned accordingly. This can be done through proper history taking, clinical examination, and investigations.

History Taking

A detailed sexual history is required to clarify the actual sexual activity. Special emphasis on the history of orgasm, history of ejaculation, and its physical characteristics is important to differentiate orgasmic from anorgasmic AE. Details of sexual preference, sexual stimulus, and erectile function are also necessary to differentiate situational from complete anorgasmic AE. A thorough psychological assessment may be further needed in cases with anorgasmic AE. Looking for etiologic factors for ejaculatory dysfunction such as chronic comorbidities (such as diabetes mellitus, spinal cord aliments, neuropathies) and previous surgeries (pelvic and retroperitoneal) may help in choosing correct therapeutic approaches.

Clinical Examination

General physical examination may help in identifying features of hypogonadism. Genital examination is crucial to assess penile sensations and to look for anatomical abnormalities, e.g., congenital absence of vas deferens. Digital rectal examination may recognize dilatation of seminal vesicles or presence of prostatic abnormalities.

Investigations

Serum testosterone levels will assess the patient's gonadal state. Semen analysis showing low ejaculate volume, acidic pH, and negative fructose is diagnostic for ejaculatory duct obstruction. Postorgasmic urine analysis is needed to diagnose RE where sperm can be found in the postorgasmic urine sample differentiating it from AE. Imaging may also be needed. Transrectal ultrasound or MRI will help in the diagnosis of EDO.

Sperm retrieval in Ejaculatory Failure

The method used to retrieve sperm in cases presenting with ejaculatory failure depends mainly on the etiology. Usually the management takes stepwise approach starting with noninvasive methods ending by surgical sperm retrieval. As ejacultory failure can be a chronic condition, it is advised to do cryopreservation of the retrieved sperm to be used for future IVF trials.

Situational Anejaculation

The history guides our management in these cases. Usually these cases can be anticipated by having previous history of failure of ejaculation during semen collection procedures either for semen analysis or during IVF procedures.

If the patient has erectile dysfunction then he can benefit from using phosphodiesterase five inhibitors (PDE5i), usually short-acting PDE5i like sildenafil or vardenafil, or intracorporal injection of vasoactive agents, mostly prostaglandin E2 being the safest. For patients who cannot masturbate, coitus interruptus or sexual intercourse using spermicidal free condoms can solve the problem. Psychological stress of obtaining a semen sample can also be alleviated by instructing the patient to get the semen sample at home and deliver it to the laboratory within convenient time.

In case of failure of ejaculation despite use of these previous measures, vibratory stimulation, electroejaculation, or surgical sperm retrieval may be tried. These measures will be discussed in details later in this chapter.

Anorgasmic Complete Anejaculation

These cases are the most difficult to treat. Once a reversible cause is identified such as presence of hyperprolactinemia or hypogonadism or use of SSRI, it should be appropriately addressed initially.

Conditions associated with psychological disturbances may need prolonged psychotherapy and behavioral therapy with very limited success rates [11].

Pharmacological treatment usually has very limited success rates in these cases. Although there is no FDA-approved medication for anorgasmia, sympathomimetics may be tried [12, 13].

Penile vibratory stimulation is also a very successful method for sperm retrieval in these cases with a reported success rate of up to 72% [14]. Electroejaculation or surgical sperm retrieval can also be used if all previous measures fail [15].

Orgasmic Anejaculation due to Ejaculatory Duct Obstruction

Surgical treatment of EDO is the classical treatment in cases associated with prostatic cysts or post-inflammatory EDO. This includes transurethral resection of the ejaculatory duct (TUR-ED) with deroofing of the cyst if present. In up to 75% of patients, sperm will return to the ejaculate and 25% of them can achieve normal pregnancy. The patients should be informed that complications rate can reach up to 20% and varies from transient hematuria and hematospermia to chronic epididymitis due to reflux of urine with chances of secondary epididymal obstruction [16].

In case of failure of TUR-ED or in cases where reconstruction is not feasible (e.g., congenital bilateral absent vas deferens), surgical sperm retrieval may be used successfully including percutaneous epididymal sperm aspiration, testicular aspiration, or biopsy.

Retrograde Ejaculation

RE had been managed through different approaches throughout history. Surgical approaches aimed at restoration of bladder neck integrity included bladder sphincter and/or neck reconstruction operations [17, 18]. However, there is limited data available regarding the success rate of such procedures. Injection of collagen into the bladder neck was another method described by Reynolds et al. [19] who reported success in achieving antegrade ejaculation, two subsequent pregnancies, and one live birth. However, again limited reports are available.

Many studies have reported achieving pregnancy in RE patients by IVF using sperm recovered from postorgasmic urine [20]. However, these sperm may often be of poor quality and may be unusable due to the acidity and osmolarity of urine [21]. Therefore, prior preparation of urine is needed. Some authors instilled sperm preparation media into the bladder by a catheter prior to ejaculation; however there are many complications for catheterization and even the K-Y gel used during catheterization may be lethal to sperm [21]. Others used pharmacological treatment to alkalinize urine by using $NaHCO_3$ tablets on the days prior to sperm retrieval. $NaHCO_3$ is dissolved in 500 mL of water to adjust both the pH and osmolarity of urine [7,

22]. Results of all these trials are contradictory and insufficient, but due to the simplicity and cost-effectiveness of this procedure, it is frequently tried for sperm retrieval in men with RE.

Medical treatment aiming at restoring ejaculation in men with RE is based on either increasing the sympathetic activity or decreasing the parasympathetic activity of the bladder neck. Alpha-adrenergic agonists and antihistaminic and anticholinergic medications such as pseudoephedrine, imipramine, chlorpheniramine, milodrin, and brompheniramine were previously used. The main drawbacks of these medications are their side effects which include elevated blood pressure, restlessness, sleep disturbances, dizziness, lethargy, dry mouth, and nausea [20, 23]. A meta-analysis of studies investigating the use of alpha-agonists, anticholinergics, and antihistamines for treatment of RE reported a success rate of 50% in achieving antegrade ejaculation. Moreover, spontaneous pregnancy was reported in 34% of patients seeking fertility during treatment [20]. Arafa and El Tabie reported a superior role for using combination of pseudoephedrine 120 mg and imipramine 25 mg twice daily in achieving antegrade ejaculation in diabetic patients with RE with a success rate of 62% and spontaneous pregnancy in 12% of cases [24]. Although the overall success rate of medical treatment is still not well studied, this treatment modality has to be considered the first choice. Second-line therapies include the use of penile vibrator and sperm recovery from postorgasmic urine or electroejaculation.

Orgasmic Anejaculation due to Neurological Causes

Medical treatment can be tried but success rates are much inferior to RE although same drug regimens for both conditions can be used. One study reported successful antegrade ejaculation with sympathetic agonists in 50% of RE versus only 12% in AE [20]. On the contrary, another study evaluated the effect of midodrine, an alpha-adrenergic agonist, on 128 patients with AE and no SCI. Patients were randomized into two groups; 64 patients received 7.5–15 mg midodrine in a stepwise approach and 64 patients received placebo. Ejaculation was successfully restored in 50% of cases in the treatment group versus 0% of cases in the placebo group [25]. Reports investigating medical treatment of AE are sparse; however the general consensus disfavors the use of medical treatment as a first-line therapy in AE due to its inferior results compared to RE and other methods of sperm retrieval.

Prostatic massage (PM) can be used to stimulate antegrade ejaculation in men with AE especially secondary to spinal cord injury (SCI). Marina et al. [26] reported the first published successful sperm retrieval after PM in a paraplegic patient resulting in triplet pregnancy using intracytoplasmic sperm injection (ICSI). Fahmy et al. [27] further reported successful sperm retrieval after PM in two of three patients with psychogenic anejaculation. Since then several studies conveyed similar results in patients with AE due to different etiologies. Arafa et al. [28] published the largest study on the use of PM in 69 men with AE and SCI reporting a success rate for semen production of 31.9%. The semen produced by PM is frequently

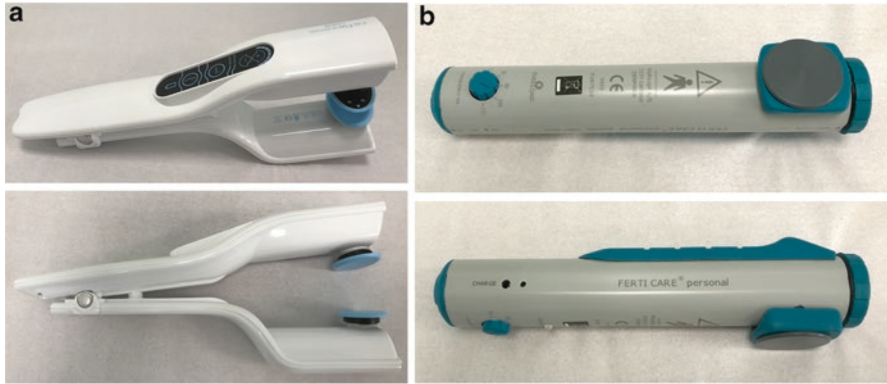

Fig. 4.2 Penile vibratory stimulator. (**a**) The Viberect® vibrator; (**b**) the Ferticare® vibrator

reported to be of low volume, normal sperm count, decreased motility and viability, and increased percentage of abnormal forms, but is generally suitable for IVF.

In case of failure of the above-mentioned methods, penile vibratory stimulation, electroejaculation, and surgical sperm retrieval may be used.

Penile Vibratory Stimulation

In the 1980s, Brindley published the first report on the use of penile vibratory stimulation (PVS) to induce ejaculation in men with SCI [29]. Since then many studies were conducted to discuss its use in various causes of ejaculatory failure [30–32]. The mechanism of action of PVS is mechanical activation of the dorsal penile nerve and with sufficient stimulation the signal will reach the ejaculatory centers of the spinal cord activating the efferent limb of the ejaculatory reflex. The success rate of PVS is highly dependent on the amplitude and frequency of the vibrator used. The best results were achieved at an amplitude of 2.5 mm and frequency of 100 Hz [33].

PVS is performed through stimulation of the dorsal and/or the ventral side of the glans penis with a medical vibrator that uses a vibrating disc (Fig. 4.2a, b). It is recommended to perform intermittent stimulation periods of 2 min, separated by rest intervals of around 30 s, until ejaculation occurs. PVS is stopped when ejaculation occurs or penile skin irritation happens. There are no studies on the recommended maximum duration for PVS use. Brackett et al. investigated the mean time from onset of stimulation to ejaculation in a group of 211 men with SCI revealing that 89% of men who achieved ejaculation did so within 2 min with a reported mean of 1.72 ± 0.15 min [34]. However, the success rate of PVS in other causes of ejaculatory failure was scarcely assessed.

The success rate of PVS can be increased by several methods. The use of two vibrators, on the dorsal and ventral surfaces of the glans to increase the stimulation

Fig. 4.3 Electroejaculation device with the rectal probe

impulses, was successful in inducing ejaculation in 20% of men with SCI who did not respond to PVS [35]. The concurrent use of oral phosphodiesterase-5 (PDE-5) inhibitors may increase ejaculatory success rate [36]. The assumed mechanism is that improved erections increase exposure of the penile sensory nerve endings, thereby amplifying the nerve signals induced by PVS.

The side effects of PVS are usually mild and related to penile skin irritation. The only serious side effect is autonomic dysreflexia that may occur in patients with spinal cord injury above T6. In these cases, the stimulation of penile skin may lead to imbalanced reflex sympathetic discharge, leading to potentially life-threatening hypertension. It is considered a medical emergency and must be recognized immediately. If left untreated, autonomic dysreflexia can cause seizures, retinal hemorrhage, pulmonary edema, renal insufficiency, myocardial infarction, cerebral hemorrhage, and, ultimately, death. Therefore, continuous monitoring of blood pressure is recommended while using PVS in these patients [37].

Electroejaculation (EEJ)

Electroejaculation works through direct introduction of an electrical current to stimulate the smooth musculature in the seminal ductal system and the accessory sex glands in order to induce seminal emission (Fig. 4.3).

The procedure is usually done under general anesthesia except in some patients with SCI where sensation is totally lost. Rectal examination with a rectoscope is done prior to the procedure to detect rectal mucosal lesions which is a contraindication to EEJ. The urinary bladder is evacuated by catheterization and sperm preparation medium is instilled into the bladder. Prior alkalinization of urine may be done

to improve the quality of sperm in the retrograde sample. The patient is placed in the lateral decubitus position and the rectal probe is introduced [38]. The electrical current is delivered in waves of 5 s of stimulation followed by a 20 s pause, during which time ejaculation occurs. Stimulation is started at voltage of 2.5–5 V and then increased gradually by 5 V in each subsequent stimulation, until a maximum of 30 V is reached [39]. The rectal probe temperature is automatically monitored and is displayed on the screen to avoid heat injury to rectal mucosa. Rectoscopy is repeated once more at the end of the procedure. The bladder is then emptied and the contents are collected in sterile containers and sent to the laboratory.

The success rate of EEJ is high especially in SCI patients reaching up to 94% [40]. However, the success rate in psychogenic AE or in cases of neurogenic AE other than SCI is not reported.

The side effects of EEJ include injury of rectal mucosa, burn to the perianal area, and anal discomfort. Autonomic dysreflexia may also occur with EEJ in SCI patients with lesions above T6; therefore continuous monitoring of blood pressure is mandatory.

Usually the quality of semen produced by EEJ is impaired. This is caused by the electrical current itself. The semen usually presents with normal or even high sperm count with severe asthenozoospermia, decreased sperm vitality, and increased percentage of abnormal forms. However, the sample is usually suitable for in vitro use whether intrauterine insemination, IVF, or ICSI [41].

Surgical Sperm Retrieval

Different procedures can be used to retrieve sperm in patients with ejaculatory failure; however as these patients usually have normal testicular function, less invasive methods are more commonly used including percutaneous epididymal sperm aspiration (PESA) or testicular sperm aspiration (TESA).

PESA can be used to retrieve sperm only in cases of EDO due to congenital bilateral absent vas deferens or after failed trial of surgical correction of EDO because there is a high possibility of epididymal obstruction following the procedure hindering future correction. It is a very simple procedure done under local anesthesia. The scrotum is cleaned with antiseptic solution and then thoroughly washed with saline. The head of the epididymis is palpated and stabilized between thumb and forefinger. It is then punctured, directly through the scrotal skin, with a 26-G needle attached to a tuberculin syringe containing 0.1 mL of sperm-washing medium. The needle is advanced into the epididymis while applying suction. Sometimes droplets of fluid can be seen entering the syringe, but usually the epididymal aspirate may be so thin and scanty that there is no visible aspirate. The contents of the syringe are gently flushed into a dish and examined for the presence of sperm.

TESA is a more invasive procedure. Under local or general anesthesia, a 22-G butterfly needle is introduced into the testicular substance while applying suction with a 20-mL syringe. The aspirated fluid is checked for sperm.

Conclusion

Normal ejaculatory function is an integral component of successful sexual activity and is required for sperm sampling and cryopreservation. The practicing clinician should be aware of the different classifications of dysfunctional ejaculation together with its etiologies before adequate workup and treatment are offered. Treatment is usually stepwise, starting with less invasive methods such as behavioral therapy, pharmacotherapy, and penile vibratory stimulation to more invasive methods such as electroejaculation or surgical sperm retrieval.

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Chapter 5
The Effect of Cancer and Its Treatment on Natural-Born Offspring

Zachary J. McDowell, Mark S. Hockenberry, and Larry I. Lipshultz

Introduction

Cancer continues to be a leading cause of morbidity and mortality in our society and does not discriminate based on age. Many patients are of reproductive age or younger. Approximately 9.2% of patients diagnosed with cancer in the United States are less than 45 years old, and 1.1% are under 20 years old [1]. As advancements in medicine lead to improvements in cancer diagnosis and treatment, more of these younger patients survive. The 5-year survival rate is now greater than 80% among all age groups [2]. In this context of increasing survival among oncology patients with reproductive potential, it is becoming increasingly important to consider future fertility in patients undergoing cancer treatment.

Although new modalities are emerging, surgery, chemotherapy, and radiation remain the foundation of cancer treatment. Unfortunately, all of these modalities can negatively affect fertility potential. The American Society of Clinical Oncology released formal recommendations for fertility preservation in 2006 and an updated version in 2013 [3]. The recommendations include discussing fertility preservation with all patients of reproductive age or their guardians if the patients are minors and referring patients who express an interest in fertility preservation to reproductive specialists.

Despite these recommendations, many patients with not only reproductive potential but also interest in future fertility are not referred for fertility preservation. Schover et al. reported that of men and women who were childless before cancer diagnosis, 76% desired children in the future [4]. Additionally, only 57% received information from their

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physicians about fertility after cancer treatment, and only 24% of childless men banked sperm before cancer treatment. Kohler et al. found that, although 86% of responding pediatric oncologists agreed with the recommendations to refer all pubertal males to a physician who specializes in fertility preservation prior to oncologic treatment, only 46% of respondents reported actually doing so more than half of the time [5]. It is also important not to make assumptions based on age about desired fertility. Salonia et al. found that up to 20% of men preparing to undergo a radical prostatectomy with an average age of 62.2 years reported desire to cryopreserve sperm before the procedure [6]. Therefore, it is imperative that the physician include a discussion of fertility preservation when counseling patients before receiving treatment for cancer.

This chapter reviews the effects of cancer and its treatment on male fertility in regard to natural conception specifically, as the use of assisted reproductive technologies and sperm cryopreservation before cancer therapy will be covered elsewhere. The literature describing the impact of cancer and its treatment on spermatogenesis, likelihood of natural conception, and discussion of potential congenital abnormalities as a result of DNA damage in sperm is discussed.

Spermatogenesis in Cancer Patients

Spermatogenesis occurs in the seminiferous tubules which account for approximately 70–80% of the testicular volume. There are an estimated 83,000,000 diploid germ cells present in the seminiferous tubules prior to pubertal development [7, 8]. Spermatogonial cells are comprised of three subtypes with specific roles: type A (dark), type A (pale), and type B. Type A (dark) spermatogonia function as quiescent, diploid reserve cells, which are active only during initial pubertal development and following stem cell depletion from exposure to gonadotoxins. Type A (pale) spermatogonia are more mitotically active and serve as permanent progenitor cells, with self-renewal occurring at each mitotic division. Type B spermatogonia also undergo frequent division as the immediate precursors to primary spermatocytes that then undergo two meiotic divisions before ultimately producing spermatids. Type A (dark) spermatogonia are therefore considered the most resistant to injury from chemotherapy or radiation, as opposed to type A (pale) and type B spermatogonia which have higher mitotic activity. These cellular qualities likely explain the transient arrest in spermatogenesis and preservation of sperm production observed following small, finite doses of chemotherapy and radiation [9–19].

Abnormal Semen Parameters in Cancer Patients

It is important to discuss abnormal semen parameters seen in men with cancer prior to receiving treatment. It is not unusual to see abnormal semen parameters in men without cancer; however, men with cancer prior to treatment are more frequently observed to have abnormal semen parameters at baseline as a result of the disease. One study reviewed all patients presenting for sperm

cryopreservation in an American sperm bank and found that over 35% of all men having sperm cryopreserved for any reason had low sperm counts, while over 50% of men presenting with testicular cancer had oligospermia [20]. Hendry et al. found that 50–70% of testicular cancer patients were subfertile or had impaired spermatogenesis before the start of chemotherapy [21]. These authors also described that impaired spermatogenesis was neither related to stage of disease nor to the duration or severity of symptoms attributed to testicular cancer. Although impaired semen parameters in pretreatment cancer patients have been most extensively study in the testicular cancer population, a clear association also exists with Hodgkin's lymphoma. Williams et al. retrospectively evaluated 717 semen samples from 409 men with 8 different types of cancer including testicular cancer and lymphoma. The investigators found that men with most types of cancer had pretreatment semen parameters that were within the fertile range in regard to density and in the intermediate range for motility, but men with testicular cancer had statistically lower semen quality compared to those with other malignancies [20].

The etiology for abnormal semen parameters in cancer patients prior to treatment is likely multifactorial. Several putative mechanisms have been proposed. These include hormones and cytokines secreted by tumors that may disrupt spermatogenesis, disturbances between the balance of subpopulations of T lymphocytes in lymphomas, hyperthermia, and central endocrine disruption—all of which may play a role in this pathologic process [22, 23]. Barr et al. described local effects of the disease on the testis itself that are likely immune mediated, resulting in significant fibrosis in testicular cancer and hyalinization of the seminiferous tubules in lymphoma [24]. Stahl and colleagues conducted a study to evaluate the DNA integrity in cryopreserved sperm from 121 cancer patients before treatment [25]. Testicular cancer and Hodgkin's lymphoma patients had a higher degree of DNA fragmentation than controls prior to treatment. The same trend was observed for other cancer diagnoses, but this trend did not reach statistical significance.

Abnormal sperm DNA condensation is adversely correlated with fertility potential. Spermon et al. evaluated DNA condensation and DNA breaks using chromomycin A3 (CMA3) and the TdT-mediated dUTP nick-end labelling assay (TUNEL) in patients who received hemi-orchiectomy for testicular cancer, but before receiving chemotherapy [26]. In all patients with testicular cancer after hemi-orchiectomy, 22% were found to have teratospermia before the start of chemotherapy, and 47% had teratospermia after chemotherapy. In contrast, the iatrogenic causes of germ cell loss through chemotherapy and radiation affecting mitosis and meiosis are well understood.

Negative Impact of Treatments on Sperm Production and Normal Ejaculation

Surgery, chemotherapy, and radiation are the mainstays of cancer treatment, and all affect spermatogenesis leading to abnormal semen parameters after treatment. Typically, the depression of spermatogenesis is transient, but occasionally it can lead to permanent sterility.

Chemotherapy

Chemotherapy exerts its effect on spermatogenesis by direct damage to spermatogonia. Depletion of spermatozoa is progressive over time after starting chemotherapy; however, sperm counts may rebound after cessation of therapy. Severe oligospermia and azoospermia frequently present in the first 6 months after the start of chemotherapy, and recovery depends on the extent of stem cell loss. Stem cell regeneration may be delayed by several years. Even in men with prolonged azoospermia, spermatozoa can be found in 30–50% of testicular sperm extractions 5 years after chemotherapy [27, 28]. The detrimental effect on spermatogenesis can vary significantly depending on what type of drug, combination of drugs, and cumulative dose are administered. Nitrogen mustard derivatives (e.g., mechlorethamine), alkylating drugs (e.g., cyclophosphamide, ifosfamide, procarbazine, and busulfan), and platinum-based drugs (e.g., cisplatin) have been shown to have the most adverse effects on sperm production [27, 29]. Cyclophosphamide has been found to have a dose-dependent effect on spermatogenesis with doses greater than 10 g/m^2 associated with an increased risk of sterility [27, 30].

Alkylating agents tend to be used in combination with other chemotherapeutic drugs which significantly increases their toxicity to spermatogenesis [31]. This negative effect is exemplified in patients treated with different regimens for Hodgkin's lymphoma. The older MOPP treatment (mechlorethamine, vincristine, prednisone, and procarbazine) was shown to cause azoospermia in 100% (47 of 47 cases) of patients 1 year after treatment [29, 32]. In contrast, one study showed that 90% of men had no change in their sperm count 1 year after treatment with the newer ABVD regimen (doxorubicin, bleomycin, vinblastine, and dacarbazine) [29, 33]. With regard to testicular cancer, in one series after orchiectomy and cisplatin-based chemotherapy, Lampe et al. reported normospermia in 64% of patients after 1 year and in 80% of patients 3–5 years after treatment. Additionally, the combination of bleomycin, etoposide, and cisplatin has demonstrated an intermediate risk of prolonged sterility affecting 20% of patients following testicular cancer treatment [27, 34, 35].

Chemotherapy when added to radiation has been shown to substantially increase the risk of permanent sterility. Anserini et al. found that cyclophosphamide with total body or thoracoabdominal radiation resulted in azoospermia for at least 4 years with only 17% of patients recovering sperm in the ejaculate thereafter [27, 36].

Radiation

Two factors that have been shown to influence the level of spermatogonia toxicity in radiation are cumulative dose and testicular exposure to radiation. Unlike conventional radiation to other organ systems where fractionated doses have been shown to be favorable for overall organ toxicity, fractionated courses

of radiation to the testis have been shown to increase toxicity when compared to a single dose. Howell et al. and Trottmann et al. reported that doses of 0.15 Gy can lead to sperm count reduction while doses of 0.35 Gy can cause reversible azoospermia [37, 38]. Additionally, doses up to 2.5 Gy can result in prolonged azoospermia with an increased chance of permanent azoospermia while doses greater than 20 Gy are generally considered to be lethal to spermatogonia [29, 37, 38]. The literature suggests that external beam radiation can lead to greater impairment of spermatogenesis when compared to localized brachytherapy or indirect scatter, e.g., from treatment of rectal cancer [29]. In addition to germinal epithelial damage, endocrine cells are affected as well. Howell et al. described a transient dose-dependent increase in FSH with concomitant decrease in sperm production with doses between 0.2 and 0.7 Gy that returned to normal values within 12–24 months [37]. Leydig cells have a higher threshold for damage, and testosterone production is not observed to be significantly impaired until doses reach greater than 14Gy [39].

Surgery

Surgery can affect sperm production and the delivery of sperm due to alterations in anatomy. Pelvic surgery can not only damage nerves but can also leave the genital tract in discontinuity, such as damage or removal of the vas deferens, seminal vesicles, or prostate [29]. Retroperitoneal lymph node dissection can damage sympathetic nerves that control normal ejaculatory processes, which can result in anejaculation or retrograde ejaculation [29]. Tumor invasion into a unilateral testicle or unilateral orchiectomy can impair sperm production by reducing seminiferous tubule mass, whereas bilateral orchiectomy obviously eliminates sperm production entirely [29].

Abnormal Spermatozoa DNA Following Cancer Treatment and Implications for Offspring Health

It is well established that cancer itself and its treatment negatively affect sperm production in men. Most literature indicates, however, that the effects of modern treatments are transient, with semen parameters normalizing within 2 years after therapy depending on the treatment modality and regimen. Given the tendency towards sperm recovery, cancer patients commonly consider use of posttreatment sperm for conception. Scant research has examined the implications for the health of the offspring in this scenario due to potential disruptions of sperm DNA, which may include abnormal chromosome number or mutations within chromosomes.

Sperm DNA Abnormalities Following Cancer Treatment

Thomas et al. conducted a study to evaluate the long-term sperm aneuploidy rates in patients 7 months to 5 years after cancer treatment [40]. The study compared 38 men with testicular cancer or lymphoma to age-matched controls with normal semen parameters and demonstrated fertility. They used fluorescence in situ hybridization to analyze segregation of chromosomes involved in common aneuploidies (13, 18, 21, X, Y). Of the 38 patients studied, 12 were found to be azoospermic. Of the remaining 26 patients, there was no significant increase found in disomy rates among the chromosomes studied. One patient with lymphoma was found to have a statistically significant increase in aneuploidy rates, but this was only 7 months after treatment with the ABVD regimen followed by retroperitoneal radiation. However two other lymphoma patients were evaluated at least 13 months after the same treatment and demonstrated no increase in aneuploidy rates. This finding may be attributed to the longer time interval between treatment and evaluation. Finally, of the patients found to have oligospermia and teratospermia, there was no increase in aneuploidy rates. A similar study by Tempest et al. also looked at aneuploidy rates of chromosomes 13, 21, X, and Y in men receiving chemotherapy for testicular cancer (BEP treatment) and Hodgkin's lymphoma (ABVD treatment). To determine whether chemotherapy caused increases in aneuploidy frequency over the 24-month study period, samples were obtained before treatment and at variable time points after the initiation of chemotherapy. Aneuploidy frequencies were significantly increased at 6 months but declined to pretreatment levels by 18 months following treatment [41].

O'Donovan et al. examined sperm quality and DNA integrity in men with testicular and blood cancers whose sperm counts were greater than 10 M/mL. Most treatment cycles consisted of six cycles of chemotherapy with an average duration of 6 months. The mean length of time to recovery of spermatogenesis was 12.5 months. The percentage of intact sperm head DNA and condensed chromatin in men following cancer treatment was less than that in the spermatozoa of fertile controls. While this study demonstrated increased sperm DNA damage following cancer treatment relative to before chemotherapy, this finding failed to reach statistical significance. The study may have been insufficiently powered to identify a difference [42]. Stahl et al. found that increased DNA fragmentation adversely correlated with fertility potential, but also failed to identify a statistically significant increase in DNA fragmentation following treatment, regardless of modality, for testicular cancer and lymphoma [25].

Effect of DNA Damage and Congenital Abnormalities

The available literature indicates that both cancer and its treatment seem to cause increased genetic damage within sperm. The concern is that this damage is transmitted to offspring and may result in fetal death, congenital abnormalities, future

malignancies, or other diseases. Marchetti and Wyrobek showed that in animal models treated for cancer, both radiation and chemotherapy induced sperm DNA damage, which was transferable to the offspring [43]. However, there is a paucity of literature showing this correlation in humans.

In one of the largest studies of its kind, Stahl et al. examined the presence of congenital abnormalities in over 1.7 million children born between 1994 and 2005 to men with and without a history of cancer of any type [44]. Data was abstracted from Danish and Swedish National Registries to investigate outcomes including birth weight, preterm delivery, and major congenital abnormalities. To assess the effect due to transient sperm DNA damage from treatment, the cohort was stratified into children born within 2 years or greater than 2 years following their father's diagnosis. Unfortunately the registries did not contain information on modality of cancer treatment in order to determine a potential differential impact of chemotherapy and radiation.

The study concluded that parental history of cancer was not associated with low birth weight or preterm delivery. With regard to congenital abnormalities, any paternal cancer history increased risk from 3.2 to 3.7 incidents per 100 offspring. Despite this finding's statistical significance, its clinical significance is uncertain. Children born within the first 2 years following their father's cancer diagnosis had an increased relative risk (1.16) of a major congenital abnormality compared with children born greater than 2 years following diagnosis. These data support the hypothesis that cancer treatments temporally increase risks to offspring but the result was not statistically significant. In contrast, however, children born to fathers with a history of childhood cancer also had an increased relative risk (1.19) of major congenital abnormality versus those born to fathers diagnosed as adults, though this result also failed to reach statistical significance. Among children with a paternal cancer history, conception with fresh posttreatment sperm versus cryopreserved pretreatment sperm yielded no difference in risk of congenital abnormalities. The study also examined the potential effect of assisted reproductive technologies (ART) on pregnancy outcomes. When compared with natural conception, ART was associated with increased risk for low birth weight and preterm delivery. Furthermore, children conceived using ART had a 20% increased risk of major congenital abnormality regardless of paternal cancer history. Overall, this study found that there is likely no clinically significant increased risk to having a child conceived by a father with a history of cancer [44].

Conclusion

The majority of men diagnosed with cancer of several etiologies, not just testicular cancer, have abnormal semen parameters prior to treatment. After receiving treatment with either chemotherapy or radiation, there is suppression of spermatogenesis and increase in DNA damage that tends to return to pretreatment levels within 18–24 months after treatment. The literature also suggests that there is likely no clinically

significant increased risk of congenital abnormalities in children conceived after cancer treatment, even if the father may have increased DNA damage as a result of treatment. For this reason, we recommend that patients have semen analysis performed and wait at least 18 months after cancer treatment before trying to conceive a child.

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Chapter 6
ART Success and Long-Term Outcomes on Offspring of Cancer Survivors

Peter T.K. Chan and Maria Belén Herrero

Introduction

According to the latest statistics, the relative 5-year survival rate for all childhood cancers combined is approximately 84 and 87% among adolescent and young adult patients [1, 2]. It has been well documented in the literature that cancer and cancer therapies including surgeries, radiotherapy, and chemotherapy can compromise the fertility status of these survivors through various mechanisms ranging from alteration of body image and other psychosocial issues and sexual dysfunction to their negative impact on the quantity and quality of gametes [3–7]. In reality, many cancer survivors could maintain their fertility potential, depending on their baseline, pre-cancer fertility status, types and staging of cancers, nature and levels (e.g., dosage or intensity and duration of chemo- and radiotherapy) of treatment received, and their general mental and physical health statuses. Indeed, fertility is long recognized as one of the most important cancer survivorship issues, with over 75% of childless young cancer survivors stating their desire to have children and 80% viewing themselves very positively as potential parents [8]. It is also reported that fertility was consistently listed as one of the top three life goals among young cancer survivors [9]. Evidently young cancer survivors are concerned about their offspring's health [10, 11]. In the following section we discuss the reproductive outcomes of cancer survivors' offspring in three aspects, namely, the need of and access to assisted reproductive technologies, perinatal outcomes, and congenital anomalies.

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The Use of Assisted Reproductive Technologies

Assisted reproductive technologies (ART's), including intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI), have helped many couples who failed to achieve natural pregnancy to become biological parents. In the past two decades, there has been a significant increase in the number of reproductive centers worldwide. Simultaneously, with the increase in the efficacy and our understanding on the safety of these technologies [12, 13], their acceptance by societies and access by infertile couples have increased tremendously in recent years.

When experiencing infertility, both female and male cancer survivors with gonadal dysfunction post-cancer therapies may benefit from ARTs to use their fresh gametes for reproduction. In addition, fertility preservation, which involves cryopreservation of oocytes and sperm whenever feasible, is now part of the standard cancer management for postpubertal cancer patients [14]. ICSI is generally required when using cryopreserved gametes for reproduction. Thus, it is not surprising to see that some cancer survivors, particularly male survivors for whom sperm banking as a means of fertility preservation is much more widely available in most centers than oocyte harvesting for cryopreservation, have a significantly increased usage of IVF/ICSI compared to age-matched subjects in the population, as reported recently (adjusted OR 1.83, 95% CI 1.35–2.49) [15, 16].

With regard to the outcomes of IVF/ICSI using cryopreserved sperm, we recently reported that the usage rate of cryopreserved sperm is significantly lower among cancer survivors compared to noncancer patients (e.g., for infertility treatment) (11% vs. 31%), and that in the cancer survivor group a slightly higher number of IVF cycles were needed before achieving a pregnancy. Nonetheless, the live-birth rate of offspring with ICSI among male cancer survivors was comparable to that of noncancer patients [17]. Specifically, the average success rate of achieving parenthood using cryopreserved sperm was 62.1%, which was at least comparable to the infertile patient population: oligospermic and testicular sperm extraction (TESE) patients (40% and 48.6%, respectively). This provides evidence that cancer patients can bank sperm as effectively as men banking for infertility reasons. However, the cost of ARTs remains a significant barrier both to fertility preservation and to subsequent use of the technologies for procreation. As we recently demonstrated, in the absence of fees for sperm banking and subsequent storage, young cancer patients are willing to come for significantly more sperm banking sessions to preserve their fertility prior to cancer treatment, despite the fact that they are under significant level of stress and time constraint to begin treatment [18]. The result is that a great quantity of sperm would be available for their future use, potentially leading to a higher chance of procreation success.

There exist data among noncancer infertile couples indicating an increased risk of adverse progeny outcomes among those who were conceived by ARTs. These risks include neonatal death, low birth weight, preterm birth, genetic and epigenetic conditions, congenital malformation, developmental anomalies, and cancer risk [19–24]. It should however be pointed out that the increased risks of adverse progeny out-

comes with ARTs were not confirmed in other studies [25]. Further, it is possible that some of these adverse outcomes may be related to abnormal gametes health (e.g., impaired sperm chromatin integrity) of the infertile parents undergoing ARTs rather than from the manipulation techniques of ARTs.

Various mechanisms have been proposed on how ARTs may increase the risks of adverse progeny outcomes. Interrupted gene and epigenetic regulation, tumor suppression, overcoming the natural selection, and survival mechanism present in spontaneous conception may leave gametes and embryos with higher risk for insults and higher risk to contain defects that could lead to developmental abnormalities leading to adverse outcomes [26–30]. It is also speculated that the exogenous hormone administration may affect the fetus during the critical period of growth and cell differentiation, thereby increasing the risk of endocrine-sensitive cancer later in life [31].

Unfortunately, with regard to the offspring of cancer survivors, there is a lack of data evaluating their long-term health risks specifically in the context of ARTs in the literature. In fact, most registry studies (see below) on the outcomes of cancer survivors' offspring failed to make a distinction on the mode of conception (i.e., whether through natural intercourse or with various forms of ARTs with or without the use of donor gametes). This limits our ability to properly counsel cancer survivors on the health risks of their offspring with the use of ARTs. Given that cancer survivors are at risk to have impaired gamete quality both from cancer and cancer therapies [3], there is an urgent need to have further data to better define the long-term health risks of offspring of cancer survivors conceived with ARTs.

Adverse Perinatal Outcomes

Several groups have reported perinatal outcomes of offspring of cancer survivors. Female childhood cancer survivors who received abdominal, pelvic, or total body irradiation appeared to be at risk to have stillbirth and neonatal death [32] in addition to offspring with preterm birth (<37 weeks of gestation), hypertension, gestational diabetes mellitus, anemia, and low birth weight (<2500 g) [33–39]. The exact biological mechanisms remain to be fully established, particularly with regard to the risks of hypertension, gestational diabetes, and anemia. Chronic radiation-induced renal injury and uterine damage leading to myometrial fibrosis and impaired vascular development of the uterus are among the proposed mechanisms. Such risks are less consistent among female cancer survivors with cancer occurred in adulthood, with some investigators reporting increased risks of preterm birth, low birth weight, and perinatal death [15, 36, 40–42] while others not observing an increase in such risks [15, 43–48]. The outcomes for offspring of male cancer survivors, on the other hand, are reassuring, with most studies reporting no significant increase in perinatal adverse events [15, 32, 49, 50]. Taken together, extra vigilance may be required during pregnancy of women who survived childhood cancer treated with radiotherapy.

Congenital Anomalies Among Offspring of Cancer Survivors

Whereas the nature, mechanisms, and extents of gamete damage from cytotoxic anticancer therapies are important research questions, for cancer survivors one of the most important clinical questions is the health risks to their offspring after cancer. There exists an extensive volume of evidence in the current literature in animal models, as we reviewed recently [3], supporting the negative impacts of chemo- and radiotherapy affecting the health of offspring [51, 52]. In human, on the contrary, earlier studies exploring the risk for congenital anomalies in offspring of cancer survivors did not find an increased risk for these outcomes [43, 44, 53–55]. However, studies from other investigators [40] including a recent large register-based study, combining Danish and Swedish data [56], reported an elevated risk for both all congenital anomalies and major congenital anomalies in almost 9000 offspring of male cancer survivors compared with a healthy population control. Other investigators have weighed in to further explore this controversy on the potential risk of congenital anomalies among offspring of young cancer survivors. The following is a description of the findings of a selected group of recent large-scale registry studies from various countries.

Data from Childhood Cancer Survivor Study, a multicenter cohort study which evaluated 4699 children from 1128 male and 1627 female childhood cancer survivors who received chemo- and radiotherapy, reported no significant increase in the risk of congenital anomalies (including, in addition to congenital malformation, cytogenetic abnormalities such as Down syndrome and single-gene defects such as achondroplasia) in their offspring [57]. The investigators found no association between congenital anomalies and radiation doses specifically to the ovaries or testes. Even a higher dose of an alkylating agent was not associated with an increased risk of congenital anomalies. In contrast, a Norwegian study using their cancer and medical birth registries reported an increased risk of major congenital anomalies only among offspring of ovarian cancer survivors (adjusted OR 3.23, CI 1.15–9.09) but not among other types of cancer combined [15].

Using data collected from 1953 to 2004 from registries (e.g., national cancer, population birth, and hospital discharge registries) on close to 7000 offspring of cancer survivors with congenital anomalies and over 35,000 offspring of these survivors' siblings, another recent population-based cohort study from Finland reported insignificant increase in the prevalence of congenital anomalies in offspring of cancer survivors of 3.2% compared to 2.7% in offspring of the survivors' siblings [58]. The prevalence of anomaly observed was consistent with that reported in other similar epidemiological studies [43, 53, 56, 57]. Further, a similar observation was noted regardless whether the cancer was diagnosed and treated during childhood, adolescence, or young adulthood (20–34 years). The only anomalies that reached a statistically significant increase in adjusted prevalence ratio (2.1, 95% CI 1.1–3.99) among offspring of cancer survivors was non-extremity-related skeletal system anomalies (e.g., abdominal wall defects, craniosynostosis, diaphragmatic hernia). However, it should be noted that this group of diagnoses contains a large variety of anomalies

and frequency of cases was low (0.2% in survivors' offspring vs. 0.1% in siblings' offspring). Interestingly, offspring of survivors diagnosed and treated with cancer in the earlier decades between 1955 and 1964 were at a significantly elevated risk (prevalence ration 2.77, 95% CI 1.26–6.11) for congenital anomalies in comparison with offspring of their siblings. On the other hands, no significant difference in the risk for congenital anomalies was detected in offspring of survivors diagnosed and treated for cancer in the more recent decades. While the exact reasons of this finding remained unclear, presumably it is in part related to reduced toxicity of more recent cancer treatment modalities and improved prenatal screening.

Some Insights into the Controversy on Offspring Health Risks from Cancer Survivors

The inconsistency of the risk of congenital anomalies in human compared to animal studies can be explained by several differences in the synthesis of evidence. For instance, unlike studies in animal models, epidemiological data from human studies are mostly observational as it is rarely feasible to conduct such studies with a prospective interventional design. The differences among various human studies in samples sizes and power, study designs, definitions of cases or study outcomes, and inherent selection biases may lead to the different conclusions generated. To reduce the risk of bias, with regard to the choice of comparison group, many investigators consider siblings as a better choice than general healthy subjects in the population, as the former group allowed controlling for shared unmeasured familial confounders such as genetic background, early lifestyle, and social/economical (e.g., healthcare access) factors. Thus, the use of siblings as a comparison group allows for a proper evaluation of additional risk attributable to cancer and cancer therapies rather than risk originating from various confounders.

Determining the extent of genetic risks on offspring of cancer survivors is challenging, given the rarity of individual genetic disorders and insufficiency of detailed information on cancer treatment exposures on each subject. Indeed, often these data sets included a large variety of cancer diagnoses at various stages, comorbidities, treatment regimens, and duration. Since both early-onset cancer and congenital anomalies are rare outcomes, large epidemiological studies are needed to explore the risk for congenital anomalies in offspring of cancer survivors. But even with large data sets, analyses of events occurring at low frequency could be prone to bias [59]. Thus, the detected risks, particularly those derived from subanalyses with small numbers, may be due to chance and their clinical significance must be interpreted with caution.

There are additional biases readers should keep in mind when using epidemiological data to evaluate the health of offspring of cancer survivors. For instance, many of these survivors are under heightened health surveillance. Thus their children may be diagnosed with more congenital anomalies. The types of anomalies

registered and reported should also be examined in depth. What one may consider minor, non-dysfunctional, and non-life-threatening anomalies in offspring from comparison group may be registered in offspring from the study group that can result in biased conclusions.

A few caveats must be noted before concluding that the results from most of these new registry studies are reassuring with regard to the risks of congenital anomalies in offspring of cancer survivors. First, most registry studies did not make a distinction whether the offspring was conceived naturally or with fertility treatments. Stahl et al. reported a significantly higher risk of birth abnormalities in offspring of men with a history of cancer (relative risk 1.17, 95% CI = 1.02–1.31) not only with natural conception but also with assisted reproduction [56]. Presumably, a higher proportion of offspring from some cancer survivor groups may have been conceived with fertility treatment and assisted reproduction, as we discussed earlier [15, 16]. The observed increased risks among offspring of cancer survivors using ARTs may thus also be attributed to both the use of damaged gametes and gamete and embryo manipulations in ARTs.

Perhaps more importantly, these data do not address adequately other important reproductive outcomes such as time required to achieve pregnancy, risks of lower number of offspring, or rate of miscarriage, particularly early (<20 weeks) miscarriage. Based on animal studies, these are some of the important reproductive outcome measures that one might predict to be affected. Several studies have reported that cancer survivors have a significantly reduced probability of having children [16, 40, 54, 60, 61]. The reasons for this may be secondary to psychosocial factors such as reduced marriage rate [16], but it may also be secondary to their impaired reproductive health. While infertility presenting as failure to achieve pregnancy is devastating, those infertile couples who have achieved pregnancy but experienced miscarriage and perinatal loss may also have significant emotional distress that put them at risk to have posttraumatic stress [62]. Thus, in addition to focusing just on the health of live offspring born to parents who are cancer survivors, information on their risks to experience prolonged time to achieve pregnancy, having lower number of children, miscarriage, perinatal death, and still birth are important when counseling cancer survivors on their reproductive prospects.

Conclusions

Based on currently available evidence, cytotoxic cancer therapies will have negative impacts on some cancer survivors in terms of function of their reproductive organs and quantity and quality of their gametes leading potentially to adverse reproductive outcomes including clinical infertility, increased use of assisted reproduction, and potential adverse perinatal outcomes. Even for cancer survivors who managed to achieve live births naturally or via assisted reproduction, the potential risks of adverse outcomes including congenital malformations, genetic diseases, and low

birth weights cannot be completely eliminated. Further large-scale prospective longitudinal studies on cancer survivor cohorts and multicenter cancer registry studies, with a focus on the extents of these risks and on the long-term developmental well-beings of cancer survivors' offspring, are needed to allow formulation of proper counseling to young cancer survivors. Meanwhile, pre-cancer treatment fertility preservation counseling remains the key to minimize the potential risks of adverse outcomes in the reproductive status of these patients.

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Part II
Fertility Preservation: Special Conditions

Chapter 7
Fertility Preservation in Adolescents with Cancer

Lenore Omesi and Jennifer Levine

Introduction

More than 15,000 children in the United States are diagnosed with cancer annually [1]. With survival rates now exceeding 80%, it is estimated that by the year 2020, half a million individuals will be survivors of a childhood malignancy [2]. Sixty percent of these survivors will experience late effects including infertility in up to 46% of male survivors [1, 3]. Young cancer survivors have reported their personal interests in having children, including expressing the belief that their cancer diagnosis may make them better parents in the future [4]. In addition, attention to fertility preservation presents an opportunity to focus on cure and survival [5]. With advances in the field of assisted reproduction, mechanisms exist to help mitigate the late reproductive effects of cancer-directed treatment [6]. However, numerous barriers have been identified that prevent systematic implementation of fertility preservation in at-risk populations, resulting in inadequate provision of fertility preservation services [7–10]. Consequently, guidelines have been developed to assist practitioners in providing patients with accurate information about risk, options for fertility preservation, and referrals to the appropriate providers at diagnosis and throughout survivorship [11–13]. Although risks and options for adolescent males do not necessarily differ significantly from their older counterparts, addressing fertility preservation requires taking into account their potentially different developmental,

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Table 7.1 Considerations regarding fertility preservation in adolescent males with cancer

	Adolescent
Emotional	• Transition from abstract to concrete thinking • Creation of self-identity • Development of sexual preferences
Physical	• Physical development affects capacity to bank sperm
Psychosocial	• Less concern with the future, may not be thinking about parenting • Various degrees of knowledge about masturbation
Ethical	• Parents/guardians make decisions for minors • Adolescent's decision may be different than parent/guardian • Adolescent's notions about masturbation may conflict with familial values
Legal	• Adolescent's ability to provide assent but not consent • Disposition of cryopreserve sperm or tissue in future
Financial	• Adolescents are dependent on guardian/parent to pay for fertility preservation

physical, and psychosocial status. These unique attributes are discussed further in depth and can be summarized in Table 7.1.

Cancer-Related Risks for Male Infertility

Evaluations of male patients have demonstrated that diminished fertility or infertility can exist at the time of a cancer diagnosis prior to treatment [14, 15]. Testicular cancer is known to be associated with pretreatment decreased sperm concentration, motility, and total sperm count [16]. A recent assessment by Paoli et al. identified impaired spermatogenesis in 25% of adolescent and young adult males diagnosed with Hodgkin lymphoma at the time of diagnosis [17]. In addition, constitutional symptoms such as fever and anorexia have been associated with impaired semen parameters [18, 19]. Cancer-directed therapies can permanently affect male fertility by damaging self-renewing spermatogonial stem cells. Among chemotherapy agents, alkylators, including cyclophosphamide, ifosfamide, procarbazine, melphalan, and busulfan, consistently exert the most deleterious effect on male fertility, in a dose-dependent fashion. For example, a total cumulative dose of 19 g/m^2 of cyclophosphamide consistently results in azoospermia, while abnormal semen parameters are seen after exposure to a total cumulative dose of 5–7.5 g/m^2 and impaired spermatogenesis is unlikely with a cumulative dose less than 4000 mg/m^2 [20–25]. These agents are commonly used as the backbone of therapy for lymphomas, sarcomas, and germ cell tumors and as conditioning for stem cell transplants. Additionally, the use of multiple alkylating agents in a single regimen results in an additive effect on gonadal toxicity.

Radiotherapy to the spinal or pelvic regions, as well as whole-body irradiation, can also impair fertility. The extent to which the testis is affected is dependent on the dose, fractionation schedule, and field. The testicular germinal epithelium is highly

sensitive to radiation. Doses of radiation to the testicles at 0.1 Gy can impair spermatogenesis, with irreversible damage after a single testicular dose exceeding 4–6 Gy. Small fractions of testicular radiation over time are more detrimental than an equivalent single-dose exposure. Patients with central nervous system malignancies who undergo cranial irradiation, typically at doses of 30 Gy, may also be infertile due to disruption of the hypothalamic-pituitary-gonadal axis [24]. The effects of surgical intervention on male fertility are related to the removal of male reproductive organs and nearby structures that are also vital to reproduction [26]. Some testicular malignancies, i.e., testicular rhabdomyosarcoma, involve retroperitoneal surgeries such as lymph node dissection, which may damage the nerves responsible for ejaculation [18, 27]. Immunotherapy is now becoming an integral part of cancer therapy for many malignancies, but data is scant on the effect of immunotherapy on male fertility and further studies are needed to monitor the effects of these therapies in the long term [11].

Sperm Banking in Male Adolescents

Sperm banking via masturbation at diagnosis is the standard-of-care fertility preservation method for postpubertal cancer patients whose treatment-related exposures place them at high risk of permanent azoospermia post-therapy [28, 29]. Current recommendations also suggest consideration of sperm banking for all postpubertal males, regardless of risk [30]. The rationale for this recommendation is based on the fact that exposure to chemotherapy with a low risk for permanent azoospermia can still cause temporary azoospermia. This temporary azoospermia may preclude the opportunity for males to sperm bank in the setting of relapse where a patient may be exposed to additional gonadotoxic therapy, and consequently permanent azoospermia. While the primary recommendation for sperm banking via masturbation is the same in the adolescent and adult population, the process of introducing and accomplishing sperm banking in the adolescent patient introduces very different challenges, particularly in younger adolescents.

Factors Affecting Capacity to Sperm Bank

An initial aspect of fertility preservation with adolescent males is an assessment of the patient's pubertal status and capacity to bank sperm. Capacity refers to the individual's ability to intellectually understand the implications of fertility preservation as well as physically provide sperm.

The period of adolescence is marked by rapid psychosocial growth, development of identity, and formation of sexuality. The beginning of adolescence is further characterized by the developmental transition from concrete to abstract thinking. For adolescents in the concrete thinking phase, it may be challenging for them to con-

template making decisions about matters such as maintaining the opportunity for future parenthood [31]. One case series showed that young men rejected the idea of sperm banking based on a common adolescent feeling of invincibility, believing that they would either not become infertile or would not care if they did. This was accompanied by regret later on in life [32]. Klosky et al. examined future fertility as a priority for adolescents at diagnosis and found that only half of those adolescents surveyed reported having children as a top life priority [33].

Emotional development, physical maturity, and the current state of illness all impact an individual adolescent's ability to produce sperm for cryopreservation. Concepts such as sexuality, reproduction, and masturbation may not have been previously discussed within the family unit and may be embarrassing to the patient [5]. Raising these issues to maintain fertility may exacerbate stress and anxiety and impact the ability to masturbate. Indeed, depending on the patient's age, one should expect varying levels of knowledge and experience with masturbation and ejaculation; a thorough history should elicit this information from the adolescent. The physical exam must evaluate development of secondary sexual characteristics, including testicular volume, penile size, and pubic and axillary hair. In order to masturbate to ejaculation, a patient generally needs to be Tanner stage III or higher [18, 34, 35]. Finally, even in a patient who is emotionally and physically mature enough for sperm banking, the patient's state of illness, including any underlying pain, physical discomfort, immobility, or critical state due to the underlying malignancy, may hinder their capacity to masturbate.

Initiating the Discussion and Decision Making

A new diagnosis of cancer presents an extremely stressful situation for an adolescent and his family. In addition to facing the prospect of a life-threatening illness, the patient and his family are often exposed to a whirlwind of logistics involved in diagnostic tests and the establishment of a treatment plan. Very often, the window for fertility preservation is small due to the pressing need to start cancer therapy. This presents added pressure for introducing the risks cancer treatment poses to future fertility and the options for fertility preservation. A thoughtful introduction of sperm banking must acknowledge the stress of the situation as well as the factors for capacity outlined above. While the initiation of the topic of fertility and sperm banking presents unique challenges in this setting, it is also a topic about which patients and families want information and which they may already have concerns [34, 36].

Because of potential time constraints, the topic of fertility preservation may need to be raised while diagnostic procedures and treatment planning are still under way. Preferably, the introduction of the infertility risks and fertility preservation options is initiated with the patient and his parents together although, depending on an assessment of family dynamics, there may be consideration of presenting this information separately [5]. Ideally, there should be an opportunity for the patient to speak privately with the physician and medical care team as he may feel uncomfortable discussing masturbation with a parent or guardian present. Patients

may also have concerns about their own sexuality and gender identity which may or may not overlap with their own and/or their families' religious beliefs and culture, which may prohibit masturbation. In addition, parents may be incorrect in their assumptions about whether or not their child is able to masturbate [37]. During this conversation, the child can decide who they would like present in the room when making fertility preservation decisions and when receiving information about the actual process. Crawshaw et al. identified that almost half of patients preferred to have the initial discussions without their parents present, but were pleased with their parents' role in the decision-making process [5, 38]. Ginsberg et al. found 58.3% of adolescent cancer patients and 79.5% of parents reporting that the decision to bank (or not bank) sperm was made conjointly [34]. In addition, the role of parents must account for logistical aspects of the process, including payment, communication with the medical team, and making arrangements with the sperm banking clinic [39]. Studies have found that the single most important reason for undergoing fertility preservation was a desire for children in the future [35]. Reasons for declining sperm banking were fears of delaying therapy and worries about the consequences of children conceived from frozen sperm [40].

Logistical Issues

Explaining the Process

An explicit and detailed explanation of the sperm collection process should be provided to the adolescent. As stated above, it is important to clearly assess the patient's knowledge and experience with masturbation, including establishing that the patient understands what is meant by masturbation, as they may be more familiar with alternative terms. The discussion should include what types of age-appropriate stimulatory materials, e.g., magazines or videos, have been used by the patient in the past and whether they believe the presence of such materials will be necessary in the current situation. At the time of sperm collection, that patient should be instructed to wash and dry hands, and then masturbate to ejaculation into a sterile cup. It is important to advise patients that they cannot use saliva or lubricants to masturbate since this may impact sperm quality, although mineral oil limited to the shaft of the penis can be used. If it is feasible to delay the start of therapy, patients should be directed to masturbate to ejaculation and then abstain from ejaculation for 48 h prior to sperm collection.

It is also important to let the adolescent know that they may not succeed in masturbation and/or ejaculation and provide reassurance if this were to occur. Informing them that pain and illness may be contributing to their inability to do so may also help. It is important to reinforce that they should not be embarrassed or distressed if they are not successful. In addition, it is possible that, even if they successfully ejaculate, there may be no viable sperm and that this may be related either to age, disease, or occasionally underlying conditions, such as Klinefelter's [41, 42].

Providing them with knowledge about alternate methods may also alleviate the stress associated with the process.

Establishing a Safe Space

It is critical that the patient have a private space wherever sperm banking is attempted. When patients must remain admitted to the hospital, a designated room should be identified if they are not in a single room. All curtains should be pulled down and doors covered. When possible, doors should be locked and "Do not disturb" signs can be hung to ensure privacy. A member of the medical team can be designated to remain outside the door and prevent interruptions.

It is equally as important to ensure the patients' comfort if they are outpatient and it is possible for them to go to a sperm banking facility. When possible, institutions should establish relationships with sperm banks that are experienced with adolescents, as well as oncology patients. When these relationships do not exist it is incumbent upon the medical practitioners to alert the sperm banking facility about the patient's age and reason for sperm banking. When personnel are knowledgeable about this group, they can provide more sensitive and personalized care and can prevent a great deal of embarrassment for the patient. Patients should be advised that they will be given a small private room for the collection. If feasible, parents and practitioners should allow the adolescent to choose who they want to accompany them to the sperm bank. One study suggested that adolescent boys may be more successful at masturbation if a parent does not accompany them to the sperm bank [29, 43].

Demonstrations of Success

A recent large ($N = 4345$) retrospective study of the French national sperm banking network demonstrated a 93% success rate for providing a sperm sample by masturbation among a population of primarily newly diagnosed adolescent oncology patients, median age 18 years. Of this group 83% had sperm frozen; reasons for not being able to freeze sperm included very small semen volume, low motility, and oligo- or azoospermia. Increases in age were associated with greater success, 81% in 11–14-year-old age group vs. 95% in 18–20-year-old age group [42]. In a smaller but similar study from the UK with a slightly younger population (mean age 16.1 years), 66% of subjects successfully banked sperm, 10% were unable to provide a sample, and 13% had an ejaculate that did not contain sperm. Semen volume and number of ampules stored increased with an increase of age; there was no difference in parameters based on disease type [44]. Edge et al. showed that those who were unsuccessful in fertility preservation were younger males and those who described more anxiety at diagnosis. They had greater difficulty in talking about fertility and less understanding of the process [41, 45]. It should be noted that patients as young

as age 12–13 successfully provide sperm samples via masturbation, suggesting that age itself does not preclude the capacity to sperm bank [46–48].

Alternate Methods for Sperm Collection

If masturbation is not possible, alternate options exist for obtaining sperm. Electroejaculation (EEJ) involves electrical stimulation using a transrectal probe to trigger ejaculation and collection of sperm. Other methods include microsurgical epididymal sperm aspiration or testicular sperm extraction (TESE). These methods can also be used when sperm counts are low or in the case of obstructive azoospermia. Disadvantages to these procedures are that they require general anesthesia and are invasive, although they can be combined with other necessary procedures (i.e., placement of central line) that also require sedation [35]. These methods may be particularly applicable for younger adolescents who may have greater difficulty producing sperm [42, 49].

An alternate experimental method involves cryopreserving testicular tissue prior to exposure to cancer therapy. This is done with the goal of later germ cell transplantation into the patient's own testes or matured in vitro and used in conjunction with intracytoplasmic sperm injection (ICSI) to fertilize an embryo [50]. This is the only option that is available for prepubertal males as they do not produce mature spermatozoa. As an experimental methodology this intervention should only be offered under an IRB-approved protocol. Ginsberg et al. have identified that parents are willing to participate in this experimental procedure. Those who consented to the procedure endorsed beliefs that scientific advances in reproductive medicine would be successful in using the testicular tissue. They also felt that fertility was important and worth trying to preserve. These parents also reported wanting to mitigate the potential psychological trauma of infertility due to cancer therapy. After the procedure was completed, all families felt that they had made the correct decision for their child. Those who declined the procedure did so over concerns with the risk of biopsy. They were also more overwhelmed at the time of decision. The experimental nature of the procedure was not a factor for those who consented or refused the procedure. Interestingly, neither group stated that their decision was significantly influenced by religion, ethics, or finances. This was supported by data that showed no significant differences between those who consented or refused in relation to any of these factors [30].

Ethical and Legal Issues

In the young adult population, ethical and legal issues in the setting of fertility preservation are related to the disposition of cryopreserved gametes in the event of patient death and the use of cryopreserved embryos either in death or dissolution of

a relationship. Because adolescents have not yet reached the age of majority and consequently are not able to consent to procedures, additional ethical and legal issues can arise. In most circumstances, unless they are emancipated, parents or legal guardians are making the majority of the medical decisions for their children [10]. Many adolescents do have a clear understanding of the issues related to fertility preservation and may wish to voice their reproductive choice. These choices may contradict their parents' wishes and pose a dilemma for families and practitioners [5]. This conflict can arise in the setting of religious beliefs especially when there is prohibition against masturbation. While both points of view should be respected, it is also important to acknowledge that the child's religious views may differ or change with time [29, 51]. When conflicts arise the assistance of a child psychologist or psychiatrist may also aid in the process, both in understanding the child's level of comprehension about sexual reproductions and helping with the assent process [51].

Testicular tissue cryopreservation presents especially controversial issues if there is disagreement between parent and child about undergoing the procedure because it is invasive and experimental, with a need for anesthesia. While the decision about harvesting immature germ cells may be made by a parent or guardian, the decision of how to use the gametes in future should be made by the patient at the time of adulthood [51]. The disposition of cryopreserved sperm or tissue must also be addressed at the time of cryopreservation. For adolescents, the sperm or tissue must either be discarded or designated for research if death were to occur. It is deemed unethical to use children's cryopreserved sperm for posthumous reproduction [18]. At the time of attaining majority, the contract related to sperm disposition must be re-addressed if this was not clearly articulated in the original contract.

Financial Issues

The average cost of sperm banking in the United States has been reported as a range from $1000 to $1500 for analysis, freezing, and the initial year of storage, plus $300–$500 per year for additional years of storage [18, 29, 52]. The average cost of testicular sperm extraction is $6000–$16000 [52]. The wide range in cost is attributable to various factors, including hospital fees, anesthesia, and equipment, and likely varies with region. The younger the patient is at the time of sperm collection generally translates into a longer time frame in which they need to pay for the annual storage of sperm, thereby increasing the overall cost. Compounding these expenses is the utilization of sperm at the time of desired conception when the adolescent reaches adulthood. This includes fees for thawing sperm. The cost of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) may be more than $20,000 [18, 53]. To minimize cost, it is reasonable to consider performing a semen analysis following the time period of greatest risk for relapse to assess fertility and to consider disposing of cryopreserved sperm in the setting of normal sperm production. Although considered a standard of supportive care, many healthcare

insurance policies do not provide coverage for fertility preservation costs for patients with an oncologic diagnosis leading to inequitable access to care.

The average cost of testicular tissue freezing is approximately $2500 for surgery, plus $300–$500 per year for storage. The costs may often be reduced or waived due to the experimental nature of the procedure if grants are available to pay for part or all of the procedure. Conversely, because of the experimental nature, insurance companies will rarely cover this expense, particularly as a self-standing procedure. This technique is offered under IRB protocol and only at a few locations across the country [52].

Some institutions may have access to charitable organizations that can assist families with payment for fertility preservation. There are several advocacy programs, including Livestrong Sharing Hope, that may also be able to provide financial assistance. Sperm banks also may be able to offer monthly payment plans to make the fees more affordable. Referring physicians should become familiar with these organizations and what they offer, as well as local sperm banks, to expedite the process as much as possible [18].

The Role of the Physician

Guidelines that endorse sperm banking prior to gonadotoxic cancer-directed therapy have been endorsed by the American Society of Clinical Oncology (ASCO), National Comprehensive Cancer Network (NCCN), American Society for Reproductive Medicine (ASRM), and the American Academy of Pediatrics (AAP) [28]. In the context specifically of adolescents, the AAP recommend development of a fertility counseling team, with inclusion of the oncologist, fertility specialist, ethicist, and mental health professional [29]. Unfortunately, despite these guidelines, overall referrals of adolescent cancer patients for sperm banks remain under 50% [54, 55]. After release of the ASCO guidelines, pediatric oncologists were surveyed to assess their attitudes towards fertility preservation and practice. Study results revealed a majority of respondents lacking familiarity with the 2006 ASCO recommendations. Physicians overall reported an interest in providing fertility preservation options to their patients. However, over 60% of the respondents reported that they only used the ASCO recommendations in healthcare decision making a quarter of the time or less [10].

Physicians often feel challenged by the task of discussing fertility, given the sensitive nature of the topic. In one UK study, Anderson et al. followed over 1000 children to assess the practices of pediatric oncologists with regard to fertility preservation. This study found that the effect of cancer treatment on fertility was discussed with only 63% of patients, and that discussions took place more commonly with boys than girls. While the majority of postpubertal boys who were assessed to be at medium or high risk of infertility were referred for semen cryopreservation, only 39% of those in early puberty were referred [56]. Physicians have identified young age, concern that techniques were unproven, patient prognosis, and

inadequate funding and/or facilities as reasons for lack of discussion [56–58]. Yet the role of the physician cannot be overstated as acceptance of fertility preservation procedures has been linked to the quality of information that is provided to the patient from the medical team [5, 45, 59, 60]. Various recommendations have been made to assist physicians in overcoming the barriers they face when it comes to having these vital conversations about fertility preservation, including the addition of fertility preservation questions in medical intake forms. The integration of other medical staff, including social workers, nurses, and non-oncology physicians well versed in fertility preservation, may also be helpful.

Standardizing the Process

Establishing an oncofertility program with attention to issues specific to adolescents increases the likelihood that existing guidelines will be executed in a standardized fashion. The multidisciplinary team of such a program is ideally composed of pediatric oncologists, reproductive endocrinologists, urologists, social workers, and nurses, led by a coordinator with expertise in the area of oncofertility. External relationships with sperm banks or other reproductive center when necessary should be made [61]. Written educational materials or dependable Internet resources should be provided that are appropriate for the child's developmental age and parental reading level such as those found at "myoncofertility.org" and "livestrong.org" [58]. Processes for assisting with the financial support should be easily accessible.

Maximizing the efficacy of a fertility preservation process may require an iterative process, particularly as an individual institution may face issues idiosyncratic to the patient population or medical practices in a given region. Shnorhavorian et al. used continuous process improvement involving a comprehensive group of practitioners involved in fertility preservation, with the aim of standardizing the process of sperm cryopreservation. They implemented workshops that discussed barriers, patient and parental experiences, and costs and gathered information about sperm banking facilities. Using this, they designed a new standard working process for patients and providers. After implementation of this, they showed an eightfold increase in the proportion of patients attempting to bank sperm prior to cancer therapy. Furthermore, they found that telling patients and families that sperm banking was offered to all male adolescent patients gave validity to the process and increased receptiveness [62].

Summary

Fertility preservation prior to the start of gonadotoxic therapy in newly diagnosed adolescent cancer patients is both feasible and desired. However, the issues in this population differ from those in the adult population, particularly in younger

adolescents, and therefore must be addressed to maximize success. Physicians and other members of the healthcare team need accurate information about risks of fertility and fertility preservation options, and act as a liaison to fertility specialists and sperm banks to facilitate the process. When discussing fertility preservation with adolescents, their development and unique characteristics of adolescence must be taken into account. While masturbation and sperm banking are the easiest and most common methods, alternate methods exist as well. Throughout the process, the ethical and legal matters as they pertain to adolescents must always be considered. As cancer care and survival outcomes continue to improve, fertility preservation is evolving as one of the cornerstones of improving late effects in this population.

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Chapter 8
Testis-Sparing Surgery: Balancing Cancer Control with Fertility Preservation

Muhammad A. Bulbul and Bassel G. Bachir

Introduction

Testicular cancer accounts for about 1–1.5% of all male neoplasms and 5% of urological tumors, with an estimated incidence of 3–10 new cases per 100,000 males/per year in Western countries [1]. Unfortunately, for reasons unknown to us, this rate has also been increasing over the last 10 years in North America and Western Europe. Malignant germ cell tumors represent the vast majority of palpable, symptomatic testicular masses, and radical orchiectomy is still considered the standard of care for the surgical management of these lesions [2].

The axiom that testis harboring any suspicious mass needs to be removed had to be challenged since complete orchiectomy could be too "costly" from a hormonal and reproductive point of view [3, 4].

Due to pathological, psychological, and hormonal issues, the concept of testicular sparing surgery (TSS), in selected cases, emerged as an alternative to radical orchiectomy. It has the advantages of preserving reproductive and hormonal functionality and decreasing physical and psychological morbidity especially for benign lesions [3, 5]. It is inherently understood that TSS should never compromise the desired oncological cancer control. The widespread use of high-frequency ultrasonography has led to a marked increase in the number of incidentally detected non-palpable testicular masses [6]. There is data confirming that most of these small testicular masses are benign and a blanket approach for complete orchiectomy in this setting could represent an "over-kill" policy [6]. Frozen section examination (FSE) has achieved higher diagnostic accuracy, and thus if employed to confirm a benign lesion could obviate the need for an immediate radical orchiectomy [7, 8].

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Testicular Cancer and Treatment Effect on Fertility

There is sufficient evidence that the mere presence of testicular tumors has a negative effect on spermatogenesis that could be reversible after tumor removal. The exact mechanism of suppression of spermatogenesis is unclear but it could be due to unknown gonadotoxins secreted by the tumor. Poor semen quality could also be due to constitutional symptoms including fever and weight loss. In addition, treatment of testis tumors whether with chemotherapy, radiation, or surgery inherently has negative effects on the germinal cells and spermatogenesis [9].

Chemotherapy

Chemotherapy works by killing rapidly dividing cancer cells. Many other cells in the body are also rapidly dividing including those lining the gastrointestinal tract, hair follicles, and germ cells including sperm and oocytes. Thus the most common side effects of chemotherapy are diarrhea, mouth sores, hair loss, and infertility [9]. The effects are dependent on the type and number of chemotherapy drugs as well as the cumulative dose used. Alkylating agents are the worst offenders. Sperm banking and cryopreservation are a routine practice requirement in patients with testis tumors needing chemotherapy. Leydig cells are unlikely to be affected by chemotherapy. The recovery of the testis may take 18 months to 5 years or more after completion of therapy and may never bounce back to normal levels to initiate a natural pregnancy.

Radiation Therapy

Germ cells can be damaged by radiation even in low doses. The damage could be reversible but difficult to predict. Doses as low as 0.1 Gy can result in decreased sperm count but doses of 1.5–4 Gy can result in permanent sterility [10]. Leydig cells can also be affected by radiation but are not as sensitive as germ cells and require a much higher dose, with damage occurring at 30 Gy [10]. Pediatric cancer patients may suffer from hypogonadism after high-dose radiation. The role of shielding is crucial as to be expected [11, 12].

Surgery

Orchiectomy will remove almost half the potential sperm production of the patient. TSS will preserve some normal-functioning testicular tissue without compromising cancer control to maximize the chances of adequate reproductive potential.

Retroperitoneal lymph node dissection, when indicated, might have an effect on erection, ejaculation, and subsequent fertility, with up to 50% of patients unable to ejaculate after surgery [13]. It is therefore imperative that patients undergoing retroperitoneal or pelvic surgery be counseled on the potential risks to fertility, and offered the option of sperm cryopreservation.

Presentation and Initial Evaluation

1. Palpable mass
   Testicular masses are either discovered by the patient himself or on routine physical examination. All solid testicular masses and most scrotal masses will require a scrotal ultrasound to confirm the nature and the extent of the mass.
2. Testicular mass seen on imaging
   The routine use of scrotal imaging, mostly ultrasonography, has identified a variety of small non-palpable testicular masses both solid and cystic. The majority of such masses are benign [6]. For patients with small non-palpable testicular masses who undergo TSS, the incidence of benign pathology ranges from 33% up to 100% in different series.

In addition to testicular evaluation complete physical examination needs to be performed. Serum testicular markers, alpha-feto protein, beta-HCG, and LDH are obtained. Semen analysis along with complete hormonal profile is requested as well that will assess the fertility status of the patient and serve as a baseline for future follow-up.

Surgical Management of the Testis with a Solid Mass

Radical orchiectomy continues to be the gold standard of care in the management of solid testicular masses since most of these are malignant germ cell tumors [2, 11]. This axiom stood the test of time and tabooed any attempt at even biopsy of any testicular mass let alone opening the testis and resecting the tumor. The concept of seeding and development of anti-sperm antibodies has not been challenged yet never confirmed [12, 14].

Due to the improvement in oncologic outcome and growing attention devoted to functional issues of cancer survivorship, the management of testis tumors is evolving in favor of conservative surgery mirroring the current trend of organ preservation in the treatment of several other cancers as for breast and kidney.

There is growing awareness of the potential advantages of testis preservation over traditional extirpative surgery in terms of health-related quality of life, namely preservation of fertility; preservation of endocrine function, thereby avoiding the risk of late-onset hypogonadism; and preservation of male body image. The issue

has therefore emerged whether or not the entire testis needs to be sacrificed without exception in every case of a known or suspected malignancy. TSS, in selected cases, forced itself as an alternative to radical orchiectomy [7, 12, 15–23].

Indications for TSS

1. Benign tumors:
   The undisputed indication for TSS is a histologically confirmed benign lesion. Leydig cell tumors with typical signs of gynecomastia, infertility, and endocrine abnormalities; tunica albuginea cysts; dermoid cysts; and adenomatoid and Sertoli cell tumors all can be safely treated with TSS [12, 16, 18].
2. Malignant tumors:
   TSS can be considered only for selected patients with malignant tumors with:

   (a) Solitary testis or atrophic contralateral testicle
   (b) Bilateral synchronous tumors
   (c) Bilateral metachronous tumors
   (d) Testis with a lesion diameter of <2 cm and no invasion of the rete testis, with normal preoperative serum (LH) levels [24–28].

   In all these cases, the excision of the mass must be accompanied by multiple biopsies of the surrounding tissue and frozen section obtained to confirm the absence of malignancy.
3. Non-palpable tumors:
   The incidental detection of asymptomatic, non-palpable, and small testis masses by scrotal ultrasound is an increasingly encountered scenario. Several series are available, reporting on the management of such patients with TSS as an alternative to radical orchiectomy since many of these lesions are benign [6, 29–33].
4. Testicular masses in the pediatric age group

Operative Technique

(a) Inguinal approach should be performed to expose the cord up to the internal ring and the testicle delivered without scrotal violation. Adequate control of the cord should be secured. The testicle should be left attached to the gubernaculum, which can be clamped. The tunica vaginalis is opened to expose the testicle.
(b) Identification of the mass is done if palpable to plan the incision in the tunica albuginea to allow for good testicular margins. Intraoperative ultrasound should be used in case of small deep lesions, using a 7.5–8 MHz linear probe.

(c) Though cord clamping and cooling could be done, it is not necessary before opening the tunica albuginea since we have learned from bivalving testes for ICSI that the bleeding is very manageable, thus avoiding any ischemia injury to an already fragile testis.
(d) The mass should be removed with an adequate testicular margin and the mass sent for quick frozen sectioning. Separate bed biopsies should be sent for pathology to check for any testicular intraepithelial neoplasia (TIN), which may be present in up to 82% of patients [34]. The microsurgical approach and use of an operating microscope also has the advantage of enhancing vision and allowing for better visualization of small masses.
(e) After removal of the mass and obtaining the biopsies, hemostasis is performed and tunica is approximated with continuous 4-0 absorbable sutures. If the frozen section showed that it is a benign mass the testicle is placed back in the scrotum and the incision closed routinely.
(f) If the frozen section showed a malignant tumor then the decision has to be made whether to remove the testicle or keep it. The decision should have been made before the surgery and with discussion with the patient. A functionally solitary testis is the major driving force to keep the testis to avoid future hormone replacement. Testicular tissue might be obtained and frozen for future ICSI.

Outcome of TSS

Oncological

Germ Cell Tumors

Seppelt is credited with the first TSS ever performed for a malignancy. The case, which dates back to 1982, reported the management of a metachronous contralateral seminoma after radical orchiectomy [35].

Two years later Richie reported on performing radical orchiectomy of one testis and hemi-orchiectomy of the contralateral testis in a man with bilateral seminoma. This approach was labelled as “unorthodox” by the author himself, but it stimulated the research of subsequent investigators [24]. Since then, in fact, two series and several individual case reports (Table 8.1) on TSS for GCTs have appeared in the literature [7, 34].

The German testicular cancer study group compiled the largest case series and presented the updated results in 2006. A total of 101 men with seminomatous and non-seminomatous bilateral tumors or tumors in solitary testes were treated with TSS at eight centers. During surgery, multiple biopsies of the surgical bed were taken to disclose concomitant foci of TIN, and local adjuvant radiotherapy with an 18-Gy dose was offered to all patients with TIN. A total of 85 patients had TIN, and 80 underwent local radiotherapy. After a mean follow-up of 80 months, cancer-specific survival was excellent (100 of 101) and was coupled with low local recurrence rate (6 of 101) [27].

Table 8.1 Oncological outcome after TSS: reported studies

Year	Authors	Country	Number treated with TSS	Mean size of tumor mass US Dmax (range)	Histological findings (% on TSS procedures)	Outcome after TSS
2014	Leonhartsberger et al. [36]	Austria	33 in 30	14.8 mm (2–30 mm)	Stromal cell tumor: 19 (57.57%) Metachronous bilateral: GCT: 6 (18.18%)	Disease-free survival: 100%
					Bilateral synchronous: Seminoma: 2 (6.06%)	
					Benign lesions: 6 (18.18%)	
2015	Bojanic et al. [37]	Serbia	26	>20 mm	Seminoma: 16 (61.53%) nonseminoma: 9 (34.61%) Leydigoma: 1 (3.84%)	Local recurrence: 7 (26.92%)
						Radical orchiectomy: 5 (19.23%)
						Overall survival: 100%
2013	Bozzini et al. [38]	Italy	22	11.4 mm (5–31 mm)	Leydig cell tumur: 20 (90.90%)	Local recurrence or distant: 0 (0%)
					Nonmalignant stromal: Tumor: 1 (4.54%)	Disease-free survival: 100%
					B cell lymphoma: 1 (4.54%)	
2011	Lawrentschuk et al. [39]	Canada	27	Benign 10 mm (5–28 mm) malignant 11 mm (6-27 mm)	Benign seminoma: 8 (36.3%) Nonseminomatous GCT: 2 (7.4%)	No perioperative complications
					Malignant seminoma: 11 (40.7%) Nonseminomatous GCT: 3 (13.6%) mixed: 1 (4.54%) Teratoma: 2 (7.4%)	Observation in 12 of 17 cases (70.59%)
						Local recurrence: 2 (11.76%)
						Retroperitoneal lymph node dissection: 1 (5.88%)

2009	Suardi et al. [40]	Italy	28	13.3 mm	Leydig cell tumor: 28 (100%)	Patient died from the disease during the follow-up: 0 (0%)
						Local or distant recurrence: 0 (0%)
2006	Heidenreich et al. [27]	Germany	100	15 mm (5–30 mm)	Seminoma: 57 (56.4%)	1 patient died
					Embryonal carcinoma: 20 (19.8%)	100 patients are NED (99%)
					Mature teratoma: 15 (14.8%)	
					Mixed/combined TGCT: 9 (8.9%)	

A uniform experience of all reports is that TSS for GCTs does require adjuvant radiotherapy to the remaining testis. Virtually all GCTs are associated with the presence of TIN in the adjacent parenchyma. Irradiation does eradicate TIN, thus preventing newly arising GCT, but it does also destroy all of the remaining germ cells causing permanent sterility. Nevertheless, in patients desiring to preserve fertility (or father), radiotherapy can safely be postponed provided that proper counselling is given and close monitoring is provided [34]. Leydig cells seem to be more resistant, yet many patients undergoing local radiotherapy experience some grade of endocrine function impairment. Optimal dose of local radiotherapy remains controversial. 20 Gy applied in 10 fractions within 2 weeks remains the standard scheme of adjuvant radiotherapy [11].

Dermoid cyst and mature teratoma represent true germ cell neoplasms. The presence of TIN in the accompanying parenchyma must be considered yet adjuvant radiation does not appear to be mandatory, as again these patients can be monitored closely with adequate counselling [41, 42].

Sex cord/gonadal stromal tumors account for 3–5% of all testis tumors, with Leydig cell tumors representing 75–80% of them. Less than 10% of all these tumors follow a malignant course. In contrast to GCTs, Leydig cell tumors may sometimes be suspected preoperatively because of typical heralding symptoms/signs (gynecomastia, infertility, endocrine abnormalities) or ultrasonographic features [43].

Functional Outcome

The loss of testis parenchyma has potential negative consequences on long-term exocrine and endocrine function [34]. The impact of unilateral orchidectomy has not been widely addressed in the literature so far. Some evidence indicates that the loss of one testis is associated with impaired spermatogenesis and altered endocrine function [44].

Consequently, it appears reasonable to preserve as much testicular parenchyma as possible by pursing TSS whenever possible, provided that cancer control is not jeopardized. These considerations hold particularly true for patients with malignant GCTs because a significant proportion of them have impaired spermatogenesis at the time of diagnosis [45]. In the largest TSS series reported so far with a mean follow-up of 80 months, 84 of 101 patients treated with TSS for GCTs had a normal postoperative testosterone [27].

In the series by Steiner et al. all patients had normal preoperative testosterone levels. Approximately 60 months after TSS, all patients but one had a normal testosterone level [7].

Grouping together the remaining individual case reports, providing functional data after TSS with a follow-up reaching 93 months, it emerges that most patients did not require androgen supplementation and had a satisfactory sexual function but were infertile [5, 46].

Conclusion

Organ-sparing surgery is a concept/reality that its time has come. Testicular sparing surgery should be embraced, in the appropriate patient, in the management of selected solid testicular masses replacing the dogmatic axiom of radical removal of the whole testicle. The psychological, hormonal, and fertility benefits are obvious. The utilization of intraoperative frozen section will guide and strengthen our indications. The indications are benign tumors, mass in a solitary testicle, bilateral tumors, and non-palpable ultrasound-detected small testicular mass.

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Chapter 9
Fertility Preservation in Hypogonadal Men

Robert J. Carrasquillo and Ranjith Ramasamy

Introduction

Testicular failure is defined as the impairment or loss of both endocrine function of the testis (production of testosterone, or T) and exocrine function (production of spermatozoa). Testicular failure can result from pathology of the testis itself, or disorder at any point in the hypothalamo-pituitary-gonadal axis. Primary testicular failure is characterized by normal/low T in the presence of elevated follicle-stimulating hormone (FSH) indicating intact feedback loops to promote spermatogenesis and testosterone production in the central nervous system. Etiologies may be acquired or congenital, with congenital causes being most common.

Conversely, low serum FSH, luteinizing hormone (LH), and T correspond to a state of hypogonadotropic hypogonadism (HH). This state may arise as a result of congenital gonadotropin-releasing hormone (GnRH) deficiency, and neurologic and systemic diseases affecting the normal hypothalamo-pituitary axis, or it may be idiopathic (IHH). Treatment of the underlying disorder, when identifiable, may allow for restoration of the normal hormonal axis with subsequent improvements in endogenous testosterone production and spermatogenesis. When necessary, medical treatment for fertility preservation can be successful in these cases of ondary testicular failure, whereas in idiopathic primary failure, our lack of knowledge regarding the pathogenesis leaves us with no targets for medical therapy [1].

Recent evidence suggests that the prevalence of hypogonadal men in the United States is substantial, approaching 39% in men aged 45 years or older in a multicenter study of men presenting to primary care centers [2]. Additionally, the use of exogenous testosterone in this same age group has increased exponentially in the last decade, as much as threefold, as revealed by a large-scale population study [3]. The

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widespread use of testosterone supplementation therapy (TST) on the part of urologists and primary care providers in men with hypogonadism for the treatment of symptoms including decreased libido, fatigue and exercise capacity, depression, and erectile dysfunction has significant implications on potential fertility. This is due to the known negative feedback inhibition of exogenous testosterone on the normal intratesticular testosterone production necessary for spermatogenesis. The same phenomenon is observed in active individuals and athletes using anabolic steroids. The prevalence of anabolic steroid use is significant, ranging from 1 to 3 million people in the United States, which creates a unique population of men with anabolic steroid-induced hypogonadism who may also present with fertility concerns [4].

This chapter reviews currently available medical treatment to restore spermatogenesis in hypogonadal men naïve to TST as well as patients currently on TST. Recommendations and treatment algorithms will also be provided for the maintenance of spermatogenesis in men considering initiation of TST.

Fertility Preservation in the Hypogonadal Man Naïve to Androgen Supplementation

Selective Estrogen Receptor Modulators (SERMs)

SERMs are a class of agents with estrogen receptor agonist or antagonist activity, such as clomiphene citrate and tamoxifen. While their use has been well established in the stimulation of ovulatory cycles, treatment of osteoporosis, and breast cancer in women, the utility of these agents for the treatment of male infertility in the setting of ondary hypogonadism remains off-label. They are an attractive method of treatment given their low cost, east of administration, and favorable side effect profile. Clomiphene citrate has antiestrogenic effects on the hypothalamus and pituitary, blocking the negative feedback inhibition of estrogen in the central nervous system and promoting increased LH and FSH retion which drives endogenous testosterone production and spermatogenesis in the testis [5]. Its safety and efficacy have previously been established. In 2012, Katz et al. published their prospective study on the efficacy of clomiphene citrate in men with confirmed hypogonadism and baseline serum testosterone <300 ng/dL. Eighty-six men with an average age of 29 years were treated with 25 mg of clomiphene citrate administered every other day over an average period of 19 months to a goal serum testosterone range of 500–600 ng/dL. As needed to meet this goal, the dose was uptitrated to 50 mg. Their results confirmed an increase in serum testosterone and gonadotropins with symptomatic improvement based on a validated questionnaire for the assessment of male hypogonadism. No major side effects were reported, but may include rare vision disturbances or breast tenderness [6].

With regard to infertility, the efficacy of clomiphene and tamoxifen has been assessed in multiple clinical trials in conjunction with other agents, yet the efficacy of

these drugs alone remains undetermined. A 2010 randomized controlled trial of daily clomiphene citrate with the antioxidant vitamin E (25 mg and 400 mg, respectively) in men with idiopathic oligoasthenozoospermia showed superiority of this regimen over placebo in improving semen analysis parameters including total count, progressive motility, and rates of unassisted pregnancy (36.7% vs. 13.3%, $P = 0.04$) [7]. Hussein et al. published a multicenter case series of 42 men with non-obstructive azoospermia (NOA) treated with dose-titrated clomiphene citrate to achieve serum testosterone between 600 and 800 ng/dL, with periodic semen analyses during the treatment period. With treatment, 64% of patients produced sperm in numbers sufficient for intracytoplasmic sperm injection (ICSI), ranging from 1 to 16 million/mL (mean density 3.8 million/mL) [8]. Notably, the lack of a control group in this study limits our ability to attribute a treatment-related effect on fertility. A 2013 meta-analysis of recent randomized controlled trials investigating the use of either clomiphene citrate or tamoxifen for treatment of idiopathic male infertility with oligo- and/or asthenoteratozoospermia demonstrated a statistically significant increase in pregnancy rates compared to controls (pooled OR 2.42, 95% CI 1.47–3.94, $P = 0.00004$) as well as sperm concentration by a mean difference of 5.24 million ($P = 0.001$) and motility by a mean difference of 4.55 ($P = 0.03$) [9].

Enclomiphene citrate is a more potent trans-isomer of clomiphene citrate with similar antiestrogenic effects in the central nervous system. A recent parallel randomized placebo-controlled multicenter study comparing the use of enclomiphene citrate and topical testosterone (Androgel® 1.62%) in overweight men aged 18–60 years with ondary hypogonadism demonstrated an increase in serum T and serum gonadotropins, as well as normalization of sperm concentration in the enclomiphene citrate group. An expected increase in serum T and decrease in gonadotropins and sperm concentrations were seen in the topical testosterone group [10]. Thus enclomiphene citrate may represent an alternative oral option once approved by the FDA, though as of yet no head-to-head studies comparing clomiphene with its trans-isomer demonstrate superiority and enclomiphene remains investigational.

Aromatase Inhibitors

The aromatase inhibitors (AI), such as anastrozole, testolactone, or letrozole, increase endogenous T levels by inhibiting the peripheral conversion of androgens to estrogens. By doing so, there is less feedback inhibition by estrogens on the hypothalamic-pituitary axis and thus increased gonadotropins [11]. The negative consequences of elevated serum estrogen in combination with low serum testosterone on spermatogenesis have been demonstrated in vivo [12]. The administration of AI can restore a normal T/E_2 ratio and has been shown to improve sperm concentration and motility in oligozoospermic men, though these studies were not placebo controlled and randomized by design [11, 13, 14]. The mean serum T/E_2 ratio in fertile men is 14.5 ± 1.2; conversely in men with NOA and Klinefelter's syndrome, the ratio is 6.9 ± 0.6 and 4.4 ± 0.5, respectively [11, 13]. The patients who benefit

most from such therapy carry a diagnosis of NOA or idiopathic oligoasthenospermia and low T, and have a T/E_2 ratio of <10 [11, 13, 15, 16]. While the use of AI in this indication remains off-label and testolactone is commercially unavailable in the United States, a subset of infertile men with elevated serum estradiol appear to benefit from the use of AI (anastrozole 1 mg daily or letrozole 2.5 mg daily). These medications are generally well tolerated with rare side effects including nausea, decreased libido, and decreased bone mineral density [17, 18]. As suppression of estradiol production to near-undetectable levels with daily dosing may have consequences for bone health and sex drive, we recommend dosing of anastrozole at 1 mg twice weekly for a pretreatment estradiol level between 60 and 80 pg/mL, and 1 mg thrice weekly for a pretreatment estradiol level >80 pg/mL.

Gonadotropins

To review, hypogonadotropic hypogonadism may be idiopathic or due to congenital deficiency of GnRH (Kallman syndrome), central nervous system neoplasm, or systemic disease such as sarcoidosis or hemochromatosis. While treatment of the underlying problem may improve fertility, most men will benefit from gonadotropin replacement to restore normal spermatogenesis [19]. It is understood that the pulsatile release of GnRH from the hypothalamus will in turn stimulate the release of gonadotropins (LH and FSH) from the anterior pituitary promoting testosterone production in the testis and spermatogenesis. This pulsatile retion can be recapitulated by the use of GnRH subcutaneous infusion pump at a dose of 5–20 mcg every 1–2 h, but given the inherent inconvenience, it is largely only available at specialty centers for clinical trials [20]. Pulsatile GnRH replacement therapy is initiated with a starting dose of 25 ng/kg/pulse every 2 h subcutaneously via portable infusion pump with dose adjustment to obtain mid-normal testosterone. Doses up to 200 ng/kg may be needed to induce virilization, at which point it may be reduced [21]. As pulsatile GnRH replacement and recombinant gonadotropins appear equivalent in improving semen analysis parameters and pregnancy rates [22, 23], the mainstay of therapy is gonadotropin replacement.

While gonadotropins were previously extracted from urine, high-quality recombinant human chorionic gonadotropin (hCG), FSH, and LH as well as purified urinary gonadotropins are available for use with no differences in safety or clinical efficacy observed among them [24]. Conventional therapy for gonadotropin deficiency involves the subcutaneous administration of hCG to replace physiologic LH at 1500–3000 IU two or three times weekly, with or without menopausal FSH (75 IU two or three times weekly) or recombinant human FSH (100–150 IU two or three times weekly) (rhFSH). hCG is first administered to correct LH deficiency and the dose is adjusted to achieve nadir T at 48 h post-injection in the normal range. Following administration of hCG for 4–6 months, if no sperm are detected on semen analysis, recombinant or purified FSH can be co-administered, with improvement in semen parameters taking up to 1–2 years [25].

The efficacy of combined hCG and rhFSH has been established and a prospective observational study by Saleh and Agarwal demonstrated an increase in average testicular volume from 4.1 to 12.4 mL and total motile sperm count from zero to 4.8 million [26]. Another study of men with HH treated initially with hCG identified 81 men who had responded in regard to testosterone level but remained azoospermic. Of these, 84% achieved spermatogenesis and 69% achieved a sperm concentration >15 million/mL after the addition of rhFSH [25]. A multi-institutional phase III randomized efficacy and safety study confirmed that weekly rhFSH of 450 IU dosing, in combination with hCG, was adequate to induce spermatogenesis in many men with HH and azoospermia who had failed on hCG alone [27]. Predictors of a good response to gonadotropin therapy include postpubertal onset of gonadotropin deficiency and testicular volume >8 mL indicating less severed gonadotropin deficiency [28, 29]. The addition of FSH to hCG is shown to be most efficacious in restoration of spermatogenesis in patients with prepubertal onset HH, whereas in men with postpubertal onset, hCG alone appears to be sufficient [30].

There is little evidence for the use of gonadotropins in men with idiopathic infertility in the absence of HH; however, there is preliminary evidence suggesting that rhFSH may be of clinical benefit in limited circumstances. One clinical trial randomized 112 men with idiopathic oligozoospermia to treatment with 100 IU of rhFSH every other day for 3 months versus no treatment. The treatment cohort overall showed no benefit, but on subgroup analysis, 30 men (48.4%) with cytologic evidence for hypospermatogenesis without maturation defect on fine-needle aspiration demonstrated improvement in semen parameters and a significantly higher spontaneous pregnancy rate compared to nonresponders and non-treated patients (5/30 [16.7%] vs. 1/32 [3.1%] and 2/50 [4.0%], respectively) [31]. Early evidence also suggests a specific role for rhFSH therapy in men with primary spermatogenic failure who also harbor certain FSH receptor polymorphisms. In one study, patients were randomized to 3 months of rhFSH at 150 IU three times weekly ($n = 70$) and no treatment ($n = 30$). When the 70 treated subjects were divided by genotype, only those men with a serine at position 680 demonstrated a statistical improvement in seminal parameters [32]. Further studies are certainly needed to validate the clinical use of rhFSH in these circumstances.

Antioxidants

The presence of reactive oxygen species (ROS) in seminal fluid has been associated with sperm dysfunction, sperm DNA damage, and impaired fertility, prompting clinicians to offer men antioxidant supplementation [33, 34]. Few clinical trials have suggested that antioxidant therapy may confer improvements in sperm function and DNA integrity. A recent Cochrane Database systematic review analyzed data from 48 randomized controlled trials (RCT) comparing single and combined antioxidants with placebo, no treatment, or other antioxidant in a total population of 4179 men with infertility [35]. Duration of trials ranged from 3 to 26 weeks with follow-up

ranging from 3 weeks to 2 years and the age of men enrolled ranged from 20 to 52 years. Most men had low motility and sperm concentration. The authors indicated that the review was limited by the fact that 25 of the 48 trials reported on sperm parameters as their primary outcome with only 3 of those trials also reporting on live birth or clinical pregnancy. Additional limitations were poor reporting of and inconsistency of study design, imprecision, small sample size in many of the trials included, and lack of adverse event reporting resulting in a designation of the evidence in favor of antioxidant therapy as "very low" to "low." The authors concluded that antioxidants may increase live birth rates (OR 4.21, 95% CI 2.08–8.51, $P < 0.0001$, from 4 RCTs with 277 men) but this was based on only 44 live births from 277 couples in four small studies. As for clinical pregnancy, they suggested that antioxidants may increase pregnancy rates (OR 3.43, 95% CI 1.92–6.11, $P < 0.0001$, from 7 RCTs with 522 men) but again the quality of the evidence was low. There remain no specific recommendations on the use of antioxidants for the treatment of male infertility.

Dopamine Agonists

Men who present with infertility and hyperprolactinemia should be considered to harbor a prolactin-reting micro- or macroadenoma until proven otherwise and diagnostic evaluation for pituitary adenoma should ensue. Elevated serum prolactin inhibits the pulsatile retion of GnRH-inducing hypogonadotropic hypogonadism and infertility, and space-occupying tumors may also lead to symptomatology such as headache or visual field defects due to compression at the optic chiasm. In this context, dopamine agonists such as bromocriptine or cabergoline are indicated for the treatment of both the adenoma and infertility with some evidence suggesting that cabergoline is superior in suppressing prolactin production with normalization of prolactin in 70% of patients who are bromocriptine resistant [36, 37]. Cabergoline is thus the preferred choice and administered at a dose of 0.25–1.0 mg twice weekly. Reversal of infertility is seen in 53% of cases, with those that fail therapy potentially dopamine agonist resistant and thus candidates for surgical retion of the adenoma [38].

Medical Therapy to Optimize Surgical Sperm Retrieval

It is understood that spermatogenesis depends on a local hormonal milieu of high intratesticular T and FSH for Sertoli cell stimulation, and as up to 70% of men with NOA will harbor focal spermatogenesis, optimization of the hormonal profile can be beneficial for maximal surgical sperm retrieval [39]. As previously described in this tion, the use of SERMs, AI, and gonadotropins can increase intratesticular T levels and normalize serum estrogen. A retrospective study of Klinefelter's patients with NOA who received clomiphene, AI, or hCG prior to microTESE with a rebound

of serum testosterone to 250 ng/dL or greater had a 22% higher sperm retrieval rate compared to patients who did not meet that threshold testosterone level [40]. Another study in men without Klinefelter's syndrome but with NOA and hypogonadism demonstrated that these men do respond to medical therapy (SERM, AI, or gonadotropin) with an increase in T levels, but in this context neither pre- nor post-treatment T levels appear to correlate with overall sperm retrieval, clinical pregnancy, or live birth rates [41]. Despite these findings and the lack of well-designed RCTs to assess the use of medical therapy to optimize sperm retrieval, limited data suggest a benefit. One prospective study on the use of clomiphene citrate showed a statistically significant increase in the likelihood of sperm retrieval and favorable testis biopsy patterns in men with maturation arrest or hypospermatogenesis on pre-treatment biopsy [8]. Additionally, the use of hCG and rhFSH is documented to improve posttreatment sperm retrieval in men with NOA and who failed initial microTESE [42, 43], as well as in men who failed initial therapy with clomiphene to normalize serum T levels before microTESE [44]. Future RCTs will be needed to further clarify the benefit these drugs may provide in surgical sperm retrieval.

Conclusions

In this chapter we have summarized available medical therapies for the treatment of hypogonadal men with infertility (see Table 9.1). The goals of therapy are based on our knowledge of the hypothalamic-pituitary-gonadal axis and the importance of optimizing serum LH for endogenous testosterone production, serum FSH for spermatogenesis, and reduction of serum estrogens. Medical therapies should not be used indiscriminately or empirically in men with idiopathic NOA, and certainly not in men with known genetic factors (abnormal karyotype or Y chromosomal microdeletion), as it can delay definitive therapy with assisted reproduction. Further high-quality studies including RCTs are needed to clarify the efficacy of these agents for improving seminal parameters, clinical pregnancy, live birth rates, and surgical sperm retrieval.

Fertility Preservation in the Hypogonadal Man on Androgen Supplementation

Increasing numbers of men in the United States are initiating TST for the treatment of hypogonadism [3]. The majority of prescriptions for testosterone supplements come from endocrinologists (23.73%), followed by general practitioners (16.95%), and thirdly urologists (15.25%) [45]. Perhaps more alarming is the finding that up to 25% of urologists surveyed by the American Urological Association reported using testosterone therapy as a treatment for the indication of infertility despite the known

Table 9.1 Summary of the reviewed available medical treatments for fertility preservation

Medication	Administration	Dosage/frequency	Special considerations
Selective estrogen receptor modulators (SERM)	Oral	Clomiphene citrate 25–50 mg daily, tamoxifen 20 mg daily	Generally well tolerated. Off-label use for male infertility. More potent isomer enclomiphene citrate currently in phase III trials
Aromatase inhibitors (AI)	Oral	Anastrozole 1 mg daily, letrozole 2.5 mg daily	Indicated for men with T/E_2 ratio of <10. Consider twice or thrice weekly dosing for bone health and libido. Side effects include nausea, decreased libido, bone demineralization. Off-label use for male infertility
GnRH	Subcutaneous infusion pump	25–200 ng/kg per pulse every 2 h	Not commonly used outside of clinical trials due to inconvenience of administration
Human chorionic gonadotropin (hCG)	Subcutaneous/intramuscular	1500–3000 IU two to three times per week	FDA approved for fertility preservation in ondary hypogonadism
Recombinant human follicle-stimulating hormone (rhFSH)	Subcutaneous/intramuscular	75 IU two to three times per week	FDA approved for fertility preservation in ondary hypogonadism
Dopamine agonists	Oral	Cabergoline 0.25–1 mg two times per week, bromocriptine 2.5–5.0 mg two times per week	Cabergoline is preferred. Surgical retion of pituitary adenoma indicated for dopamine agonist resistance. Off-label use for male infertility

contraceptive effect of testosterone supplementation [46]. Exogenous testosterone induces negative feedback inhibition on the hypothalamic-pituitary-gonadal axis, thus leading to atrophy of the germinal epithelium in otherwise normal men and suppressing spermatogenesis, with azoospermia inducible by 10 weeks of testosterone use [47]. Testicular atrophy is common with loss in volume due to both suppressed spermatogenesis and decreased Leydig cell function. While otherwise

healthy men may demonstrate rebound of spermatogenesis after 6–18 months of abstinence from exogenous testosterone [47], up to 4–10% of patients with impaired spermatogenesis prior to TST may remain azoospermic after cessation of therapy, with significant implications for their future fertility [48]. Thus, in any patient who desires to maintain fertility and is considering TST, a semen analysis should be obtained prior to initiation of treatment to rule out idiopathic infertility or an undiagnosed hypogonadal state (e.g., Klinefelter's syndrome). Also previously discussed is the increasing population of men on anabolic-androgenic steroids (AAS) [4], many of whom are in their reproductive years, who may present with subfertility as a result of steroid-induced hypogonadism.

In the previous tion, hCG therapy was discussed as a means of replacing LH in hypogonadal men to promote restoration of intratesticular testosterone production. Intramuscular hCG has also been shown to reduce the impact of exogenous testosterone on intratesticular T levels, though data is scarce on its use in men previously on TST/AAS. A RCT was conducted with 29 healthy men receiving 200 mg per week of testosterone enanthate, who were also randomized to receive intramuscular saline placebo, 125, 250, or 500 IU hCG every other day for 3 weeks. Intratesticular testosterone levels and gonadotropins were assessed at days 0 and 21. Intratesticular T levels were suppressed by 94% in the T enanthate/placebo group, 25% in the T enanthate/125 IU hCG treatment group, and 7% in the T enanthate/250 IU hCG treatment group, and were actually increased 26% from baseline levels in the T enanthate/500 IU hCG treatment group [49]. Endogenous LH and FSH levels were not surprisingly suppressed to 5% and 3% of baseline, respectively, in the T enanthate/placebo group. This demonstrated that even supraphysiologic doses of TST can be countered by low-dose hCG to maintain normal levels of intratesticular testosterone. The effect on spermatogenesis was later shown in a retrospective study conducted on 26 hypogonadal men treated with TST via transdermal patches or intramuscular injections, as well as low-dose hCG. Serum total and free T, serum estradiol, semen parameters, and pregnancy rates were assessed. Pretreatment semen parameters included an average volume of 2.9 mL, concentration of 35.2 million/mL, motility of 49.0%, and forward progression of 2.3. There were no observed changes in semen parameters regardless of T formulation over more than 1 year of follow-up, none of the men became azoospermic during the treatment course, and 9 of 26 contributed to a pregnancy with their partners [50]. A recent multi-institutional series of men previously on TST with subsequent azoospermia or severe oligospermia were treated with hCG 3000 IU every other day and supplemented with either FSH, clomiphene citrate, tamoxifen, or anastrozole. Patients on these hCG-based combination therapies demonstrated a recovery of spermatogenesis to a mean density of 22 million/mL in 4 months [51]. These studies suggest a beneficial role for hCG therapy in hypogonadal men who desire both symptomatic relief via TST and preservation of fertility potential during their reproductive years. Data is even more limited on the use of hCG therapy for men with hypogonadism ondary to AAS use. Case reports have documented that hCG alone at doses of

2000 IU three times weekly to 10,000 IU once weekly can restore spermatogenesis and lead to clinical pregnancy [52–54]. hCG and FSH combination therapy (10,000 IU weekly and 75 IU daily, respectively) has also been reported with clinical success in restoring spermatogenesis [55].

The role of SERMs was previously discussed in the treatment of symptomatic hypogonadism via suppression of estrogenic negative feedback inhibition on the hypothalamic-pituitary-gonadal axis, thus promoting increased gonadotropins and downstream intratesticular testosterone production. Data on the use of clomiphene citrate for restoration of spermatogenesis is scarce. Case reports on the use of high-dose clomiphene (100 mg daily) in men with AAS-induced hypogonadism documented restoration of the normal hormonal axis within 2–3 months, but spermatogenesis was not assessed [56, 57]. Also, as mentioned in the previous paragraph, clomiphene in combination with hCG has demonstrated efficacy in recovery of spermatogenesis in men previously on TST [51]. Enclomiphene citrate is a more potent and shorter acting trans-isomer of clomiphene citrate that was evaluated in a randomized, open-label, controlled, phase IIB study designed to assess fertility in 12 men with ondary hypogonadism previously treated with 1% testosterone gel for a minimum of 6 months. After cessation of TST, morning total T values averaged 165 ± 66 pg/dL. The treatment group was then given 25 mg enclomiphene citrate and the control group received 1% testosterone gel with results compared at 3 and 6 months including serum total T, FSH, LH, and semen parameters. In follow-up, only enclomiphene citrate therapy was observed to restore both serum T levels and sperm counts while also elevating LH and FSH in the treatment group [58]. A later randomized, phase IIB, placebo-controlled, parallel, multicenter study of 73 men with ondary hypogonadism was conducted using two oral doses of enclomiphene citrate or 1% topical T gel. All men had either discontinued prior TST for at least 6 months or never been treated. This particular study population was notable for more severe hypogonadism and lower baseline serum T levels than prior studies. Again, enclomiphene was demonstrated to reverse low serum T and gonadotropins compared to placebo while preserving sperm production compared to the TST treatment group [59]. These findings have since been further validated in a phase III RCT [10]. As of this time, enclomiphene is not yet FDA approved for the treatment of male hypogonadism and further phase III studies are pending.

There are no prospective trials in the literature evaluating the use of aromatase inhibitors in men with hypogonadism ondary to TST or AAS use. The previously mentioned retrospective series by Wenker et al. evaluating hCG-based combination therapies (including AI) in men with azoo- or oligospermia following TST demonstrated a 98% success rate at restoring spermatogenesis with no differences noted between supplemental therapy administered with hCG and the type of TST used [51]. Patients who stand to benefit most from therapy with these agents will have low serum T and have a T/E_2 ratio of <10 [11, 13, 15, 16]; thus their role in restoration and maintenance of spermatogenesis in men previously on TST/AAS or who wish to continue TST/AAS will be limited and likely adjunctive.

Recovery of Spermatogenesis with Recent or Current TST or AAS Use

Several options may be presented to the patient who presents with NOA or oligospermia and reported recent or current use of exogenous androgen. Firstly, primary hypogonadism should be ruled out. If the patient and his partner are able to comply, he may cease TST/AAS and await spontaneous recovery of spermatogenesis with probability of recovery approaching 90% at 12 months and 100% at 24 months [48, 60, 61]. Alternatively, for more rapid recovery, following discontinuation of androgen we recommend 2000 IU hCG intramuscularly every other day for 3 or more months with dose titration as needed, with clomiphene citrate 50 mg every other day for 3 months. Co-administration of clomiphene may counter the suppression of endogenous FSH that is observed with higher doses of hCG therapy. At follow-up, hormonal evaluation and semen analysis are repeated. If estradiol is elevated or the T/E_2 ratio is <10, anastrozole may be implemented at 1 mg twice weekly. If FSH remains low, clomiphene may be discontinued in favor of rhFSH at 75 IU every other day for 3 months (see Fig. 9.1). As many men may not tolerate cessation due to recurrent symptoms, may not wish to discontinue treatment, may not be willing to wait for spontaneous recovery, or may not

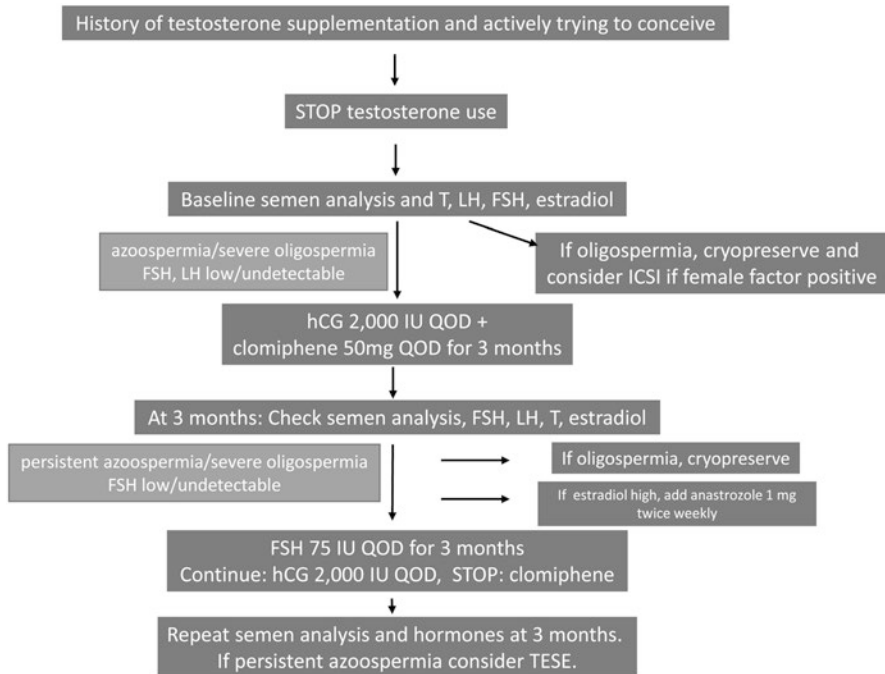

Fig. 9.1 Algorithm for fertility restoration in the hypogonadal man previously on testosterone supplementation therapy. *T* testosterone, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *hCG* human chorionic gonadotropin

be willing to accept the uncertainty of successful spontaneous recovery, restorative treatment may be offered in conjunction with androgen.

Maintenance of Spermatogenesis Prior to Initiation of TST or AAS Use

The maintenance of adequate intratesticular T levels is essential to sustain spermatogenesis. For those men who desire to maintain fertility and also treat symptomatic hypogonadism or engage in the use of AAS, we propose the following algorithm based on historical evidence (see Table 9.2) [62]. In men seeking treatment for hypogonadism, the first question to answer is whether they desire fertility or not. If not, the patient may begin treatment with 1500 IU hCG weekly to maintain testicular size, or alternatively cycle on/off TST with a 4-week treatment cycle of 3000 IU hCG administered every other day at 6-month intervals to enhance the response to TST. If the patient desires to maintain fertility at the outset, then a baseline semen analysis should be obtained and a decision made as to the timing of desired pregnancy. For patients who desire a pregnancy within 6 months, TST should be discontinued immediately and therapy initiated with 3000 IU hCG every other day, with or without 25 mg daily clomiphene citrate, and a semen analysis obtained every 2 months. If semen parameters do not improve sufficiently and FSH remains suppressed, rhFSH at 75 IU every other day may be added with discontinuation of clomiphene citrate. If the patient and his partner anticipate desired pregnancy in 6–12 months, TST may be started or continued with 500 IU hCG given every other day with or without clomiphene citrate at the aforementioned dose. For those patients desiring pregnancy in greater than 1 year, we recommend the patient cycles off TST every 6 months with a 4-week treatment cycle of 3000 IU hCG every other day. Other potential options include enclomiphene citrate which has been shown to recover spermatogenesis in men previously on TST, but not yet studied in men

Table 9.2 Summary of recommendations for maintenance of spermatogenesis with TST or AAS use

Timing of desired pregnancy	Treatment recommendation
<6 months	• *Stop* TST/AAS • *Start* 3000 IU hCG every other day ± clomiphene citrate 25 mg oral daily • Semen analysis every 2 months • No FSH response: discontinue clomiphene and add rhFSH 75 IU every other day
6–12 months	• *Continue* TST/AAS • *Start* 500 IU hCG every other day ± clomiphene citrate 25 mg oral daily
>12 months	• *Continue* TST/AAS • *Cycle off* TST/AAS every 6 months with a 4-week cycle of 3000 IU hCG every other day

desiring fertility while actively on TST; and as described in this tion, further phase III studies are pending. Additionally, AI such as anastrozole or letrozole may be considered in men with T/E_2 ratio of <10 as previously stated.

Conclusions

In this tion we have reviewed the potential role of medical therapy in the restoration and maintenance of spermatogenesis and fertility in men before, during, and after the use of TST or androgenic steroids. Knowledge of male reproductive endocrinology, the available treatment options, and their limitations is essential in counseling patients who wish to pursue treatment of hypogonadism or the use of androgenic steroids while also desiring fertility preservation. As epidemiologic data suggest, the use of TST and AAS is on the rise and role of the provider in warning patients of the potential fertility consequences and educating patients on the current state of the art for fertility preservation will grow ever more critical.

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Chapter 10
Sperm Preservation in Transgender Patients

Chloë De Roo and Guy T'Sjoen

Introduction

Gender-confirming treatment options for transgender people comprise both hormone therapy and surgical interventions [1]. Unfortunately, both options have a negative effect on fertility [1, 2]. Healthcare professionals should address the consequences for future fertility with their patients before treatments are started.

Although there are only few studies that have investigated the desire of transgender people to have children, they all conclude that approximately half of transgender women and half of transgender men wish to have children [3–5]. The relevance of this topic is also reflected in the fact that transgender people with children score significantly higher on self-perceived positive mental health status and vitality in quality-of-life surveys than transgender people without children [5]. Additionally, in transgender women, parenting has been identified as a protective factor for suicide [6]. Apart from an apparent child wish, a small majority of transgender men and transgender women would actually have their gametes frozen, or would have seriously considered doing it, if the technique had been available [5]. Especially lesbian and bisexual transgender women were interested in using their own frozen sperm to fulfil a future child wish [5]. Regardless of their personal desire, the majority of transgender people clearly expressed the opinion that fertility preservation techniques should be discussed and offered [3]. On the other hand it is striking that some transgender people, in order not to postpone their transition process, are

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willing to sacrifice their fertility [3]. Most transgender people are in favour of transitioning without delay, and hence fertility preservation might appear not important enough to postpone transition [4].

The Seventh version of the World Professional Association for Transgender Health (WPATH) Standards of Care recommends to discuss fertility options with patients prior to any treatment or medical intervention, especially before genital reconstructive surgery [1]. The impact of each treatment on fertility as well as the fertility preservation option available that will allow the possibility of having future genetically related children should be addressed. This chapter provides the most recent insights into the effects of therapy on fertility, fertility preservation options, success rates, future use of stored gametes, and transgender parenting. It is of interest for all healthcare professionals working with transgender people and could be used as a tool in order to correctly inform their patients regarding the possible fertility preservation options available. It is of note that certain treatments and possibilities cited below might not be possible in every country due to national legislation.

The Effect of Hormone Treatment and Gender-Confirming Surgery on Fertility

Genital Reconstructive Surgery in Transgender Women

Penectomy and orchidectomy in transgender women lead to irreversible sterility.

Cross-Sex Hormone Treatment in Transgender Women

Intratesticular testosterone levels correlate strongly to the serum testosterone levels [7]. Clinically, prolonged oestrogen treatment results in reduction of the testicular volume and weight [7, 8]. Additionally, oestrogens have a suppressive effect on sperm motility and density in a [9] dose-dependent manner [8]. Feminizing hormonal therapy induces hypospermatogenesis, ultimately leading to azoospermia [10]. Hamada et al. (2014) clearly demonstrated a poor semen quality in transgender women following feminizing therapy. Their results show a high incidence of oligozoospermia, asthenozoospermia, and teratozoospermia, related to cross-sex hormone treatment in transgender women [6, 11]. Payer et al. [8] described the histological effects on Leydig cells of treatment with oestrogens alone or with medroxyprogesterone acetate in transgender women. Three morphologies were distinguished: Leydig cells very similar to untreated Leydig cells (type 1), the absence of Leydig cells in the presence of cells with increased microfilaments and abundant smooth endoplasmic reticulum and lipid drops (type 2), and complete absence of any cell type but with varying amounts of microfilaments and pigmentation (type 3)

[8]. The persisting spermatogonia show the typical features of the so-called pale type-A spermatogonia [13]. Because of the low mitotic rate of these cells, the pale type-A spermatogonia survive disturbances of the endocrine balance, as well as other noxious stimuli such as radio- and chemotherapy [13]. A more recent testicular histology study on 108 transgender women by Schneider et al. (2015) confirmed a heterogeneous effect, from complete spermatogenesis in approximately half of the patients, over meiotic arrest, spermatogonial arrest, Sertoli-cell-only to tubular shadows [7].

The impact is—to a certain extent—heterogeneous, possibly due to individual sensitivity and response to cross-sex hormone therapy [7] and is reversible upon cessation of oestrogen therapy based on the presence of the spermatogonial stem cells [13]. The effect is therefore highly dependent on the therapy compliance as well [7]. If semen samples of such poor quality are used, assisted reproduction techniques, such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), are needed [12].

Current Fertility Preservation Options for Transgender Women

The fertility preservation options for transgender women include the freezing of sperm [14] collected through ejaculation or direct testicular extraction and the freezing of immature testicular tissue. An overview of fertility preservation options in transgender women is provided in Table 10.1.

Sperm Cryopreservation

Human sperm cryopreservation is a procedure to preserve sperm cells through freezing. For human sperm, the longest reported successful storage is 40 years [15]. This procedure is the simplest and most reliable method of male fertility preservation [16]. The sperm is obtained through masturbation, electroejaculation, or penile vibratory stimulation. Transgender women may find it difficult to masturbate in order to produce a semen sample for preservation. However, electroejaculation requires anaesthesia. Having semen samples stored reminds transgender women of their (male) past and may make some transgender women not feel as a complete woman [3, 4]. Depending on the sperm quality, the cryopreserved sperm can be used for future intrauterine insemination or to perform IVF/ICSI in the case of a female partner [10]. The need for IVF/ICSI, however, creates the necessity to start controlled ovarian stimulation in the female partner, followed by the aspiration of the egg cell. The embryo obtained can subsequently be transferred into the partner’s uterus. In the case of a male partner, a donor oocyte and a surrogate mother are both necessary.

Table 10.1 Fertility preservation options in transgender women

Technique	Description	Considerations	Future use
Sperm cryopreservation	Cryopreservation of ejaculated sperm through masturbation or electroejaculation	– Established technique – Masturbation – Post-pubertal – Anaesthesia in case of electroejaculation	*Male partner* Need of a donor oocyte and surrogate mother
			Female partner Intrauterine insemination or IVF/ICSI depending on sperm quality followed by embryo transfer in partner
Surgical sperm extraction	Percutaneous aspiration of sperm from testis or epididymis	– Established technique – No masturbation – Surgical procedure – Post-pubertal	*Male partner* Need of a donor oocyte and surrogate mother
			Female partner IVF/ICSI treatment followed by embryo transfer in partner
Immature testicular tissue cryopreservation	Surgical biopsy of testicular tissue	– Experimental – Pre-pubertal or post-pubertal – Possible at the moment of genital reconstructive surgery	*Male partner* In vitro maturation and need of a donor oocyte and surrogate mother (not possible at the time of publication)
			Female partner In vitro maturation and IVF/ICSI followed by embryo transfer in partner (not possible at the time of publication)

Surgical Sperm Extraction

In cases of surgical sperm extraction, a percutaneous aspiration of sperm from the testis or the epididymis is performed [16]. Again, this is an established method in daily IVF practice. Although a solution for transgender women for whom masturbation is a burden, one must not forget that this is a surgical procedure [16]. The obtained spermatozoa can be used for future IVF or ICSI procedures in the case of a female partner. Again, in the case of a male partner, an oocyte donor and surrogate mother are both necessary in order to fulfil their child wish [12].

Testicular Tissue Cryopreservation

Testicular tissue cryopreservation means the freezing of the testicular tissue. For this technique a surgical biopsy of testicular tissue from pre- or post-pubertal transgender women is performed [16]. This option overcomes the need for masturbation and is possible in pre-pubertal boys [16]. It is a surgical procedure that can be combined with genital reconstructive surgery. Compared to the other two options, this is an experimental method. For future use, an in vitro maturation procedure, which is currently not clinically possible, or transplantation is necessary followed by assisted reproduction techniques. Transplantation can, however, restore the male endocrine environment, which clearly is an undesired effect for transgender women.

Future Fertility Preservation Options for Transgender Patients

Current research focuses on optimizing the in vitro maturation of spermatogonial stem cells [17]. An optimized culture model would allow the use of the currently banked testicular tissue without the need for transplantation. This would solve the side effect of having the unwanted hormones due to the transplanted tissue.

Apart from a testicular biopsy in transgender women to obtain spermatogonial stem cells, research to obtain artificial gametes through stem cells is ongoing [12, 18]. Obtaining functional gametes from induced pluripotent stem cells has been proven successful in mice [18]. Producing oocytes and sperm cells in human, starting from pluripotent stem cells derived from a skin biopsy (as an example), would be an important breakthrough [12]. This would be a possibility for those patients who cannot or have not stored their own gametes and currently need oocyte or sperm donation to fulfil their future genetically related child wish [2].

Transgender Gestation

In transgender women, being pregnant and giving birth are still not possible. The Swedish research unit of Brännström and his colleagues conducted a series of uterus transplants and reported a first live birth in 2014 [19]. This opens the possibility for assisted gestation for transgender women [20]. However, there are important medical concerns regarding uterus transplantation if introduced to transgender people [2, 6]. A challenging surgical procedure would be needed in order to change the anatomy of the male pelvis with the intention to perform a successful uterus transplantation. Moreover, immunosuppressive therapy would be necessary and is possibly contraindicated during a pregnancy [2], but that in itself would not be any different from a uterus transplantation for other indications resulting in the absence of the uterus or the presence of a non-functional uterus [19].

Transgender Parenting and Children

The above-mentioned options clearly show the opportunities [21] for transgender patients with a present or future genetically related child wish. All these possibilities, however, are strictly regulated by national legislations. Apart from legislation, some healthcare professionals still need to be convinced about the necessity and the ethical acceptability to preserve fertility in this patient group [10]. The underlying question is whether transgender parenting has a negative influence on the gender identity and the sexual orientation of a child [2, 22]. Few studies have addressed this question and conclusive evidence is scarce. Although the results from these studies are reassuring, long-term follow-up studies are undoubtedly needed. None of the studies published so far showed that children suffer to such an extent that would warrant a prohibition of transgender parenting [23]. Being transgender as a reason to interrupt contact between the transgender parent and his or her children, as is the case in some countries, is documented to be harmful for the children [2, 24]. It is shown that a child having a transgender parent may experience more transient and mild harassment than those who do not have a transgender parent [2, 24].

Children who were younger at the time of their parent's transitioning showed better adaptation and maintained healthier relationships with both the transitioning and the other parent in a study by White and Ettner (2007). A less conflicted relationship between child and parents is also predicted by a positive relationship between the two parents [22, 25]. In cases of transitioning of the transgender parent before the birth of a child, it is important to disclose the transgender identity of the parent early in childhood, rather than later in the life of the child. The possibility that specific circumstances concerning the birth of the child are disclosed by someone else than the parents should be avoided as this can be tremendously traumatic for the child [26].

Discussion and Conclusion

Fertility and fertility preservation are important topics to discuss before planning gender confirming treatment. Patients should be clearly informed before starting cross-sex hormone treatment or genital reconstructive surgery. The first information on fertility preservation should be given by healthcare professionals at transgender health services. Following this, patients should be referred to a specialized fertility centre, where their available options can be discussed in more detail. Information on success rates of each technique especially is highly patient specific. In the case of sperm cryopreservation or surgical sperm extraction, success rates are similar to preservation in cisgender patients. However, reproductive techniques as well as pregnancy results depend on the age and fertility-related medical history of the partner. All the other aforementioned techniques are still experimental; therefore referring a patient to a specialized fertility centre in order to have correct and balanced information is a necessity.

The current available options for fertility preservation are very promising. One must, however, realize that the banking of gametes cannot guarantee future treatment. If a transgender person has a wish for a genetically related child (individually or as a couple) pre- or post-transitioning, they should undergo the screening procedure according to the protocol of the centres for assisted reproduction. Furthermore, not all theoretical reproductive options are possible at this time and not all forms of medically assisted reproduction are available in every country. Additionally, medically assisted reproduction, although considered to be safe, is not without health risks and it is often expensive.

The use of the cryopreserved gametes will therefore depend on their quality, success rate of the technique, and choice of partner, as well as a specialized fertility centre's policy and national legislation. Fertility in transgender patients also raises the need for appropriate and adapted care before conception, during pregnancy, and after giving birth.

We conclude that transgender patients should be counselled on reproductive issues by professionals prior to initiating gender-confirming treatment, which adversely affect fertility in order to have a clear overview of the effects of treatment, the preservation possibilities, and what to expect from it. One cannot forget that other options, like adoption and sperm donation in case of a female partner, should be discussed as well. We advise referring transgender people to specialized centres for assisted reproduction to discuss fertility-preserving possibilities. Even though a patient does not have a clear view on his or her future child wish, it is very important that patients have access to clear and detailed information so that a well-informed choice can be made.

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Chapter 11
Postmortem Sperm Retrieval: Ethical, Legal, and Logistical Considerations

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Introduction

As a result of advancements in assisted reproductive technology (ART), sperm procured after death can be cryopreserved and used for posthumous reproduction via in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI). Postmortem sperm retrieval (PMSR) was first described in 1980 by Rothman and was followed by the first reported live births from posthumous reproduction in the 1990s [1, 2]. Requests for PMSR are infrequent, but have been increasing worldwide [2]. A study published by Hurwitz and Batzer found a 60% increase in demand for PMSR between 1997 and 2002 in the United States, and there have been reports of requests for PMSR in developing countries like India [2, 3]. Despite growing demand, PMSR is rarely performed because of the complex medical, legal, and ethical aspects of these cases.

Debate about what constitutes consent from the decedent heralds the complex legal and ethical issues surrounding PMSR, which also include questions about who can rightfully request sperm retrieval, gamete ownership, inheritance rights, etc. [4]. Internationally, the legality of PMSR is widely variable, often leaving requests for

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PMSR to be handled on an institutional level [3, 5, 6]. Policies for PMSR are uncommon, even at major academic medical centers [7]. Without established protocols in place, physicians and hospital ethics committees considering PMSR requests are forced to make challenging ethical and legal decisions in a narrow window of time, since PMSR must be performed within 24–36 h of death [8, 9]. Logistical considerations, for example deciding which sperm retrieval procedure to use, scheduling the appropriate surgeon, and coordinating with a laboratory equipped to perform sperm processing and cryopreservation, further increase the complexity of these situations and must be resolved quickly.

In this chapter, we summarize the legal, ethical, and practical considerations for PMSR, including a review of sperm procurement procedures and points hospitals should consider when developing institutional policies. Three fictionalized case studies are included to help guide the legal and ethical discussions and illustrate some of the diverse scenarios from which PMSR requests may arise.

Case Studies

Case 1

A motor vehicle accident left a male in his mid-20s in a neurologically stable persistent vegetative state. At the time of the accident the patient was engaged to be married, and his fiancée continued to wait for his recovery. The patient's father inquired about acquiring sperm for insemination of the fiancée. The fiancée did not inquire about sperm retrieval, and the father stated that he preferred, for her sake, not to involve her in such discussion until more time had passed following accident. The father intended to pay for cryopreservation and storage.

Case 2

A man in his late 20s was the victim of an assault-induced head injury and met the criteria for brain death. At the time of the assault the patient was married with no children. The wife requested sperm retrieval. The wife and many family members gave a very consistent report that the couple had been planning to start their family in the next few months. There was also convincing evidence that the patient's family would assist in the care of any children to allow the wife to work to support her family.

Case 3

A man in his mid-40s sustained a severe head injury and was declared dead by neurologic criteria. At the time of the injury, the patient's wife of a second marriage was in her late 20s. This marriage was childless. The patient had two children from his

first marriage. The wife stated that she and the patient had been planning to start their own family and inquired about obtaining sperm to inseminate herself. But according to other family members, the patient had stated repeatedly to others that he did not plan to have any more children.

Legal Considerations

Not surprisingly, there are significant legal issues surrounding PMSR. Although this section focuses on the legal landscape within the United States, where standard legal sources (statutes, regulations, and case law) offer little guidance and existing legislation is widely variable, it is worth noting that other countries have sought to provide greater legal clarity. Some countries, like France, Germany, Canada, and Sweden, have total bans on posthumous reproduction [5, 6, 10]. The United Kingdom requires written informed consent from the decedent [11]. In Israel, where perhaps the most liberal laws governing PMSR can be found, legislation establishes a presumption of consent from the deceased, and PMSR can be performed freely at the request of the surviving spouse or de facto party (after a 6-month waiting period) [5].

In this section, we draw upon broader legal concepts to offer guidance to institutions and physicians seeking to devise policies for PMSR when governing legislation is otherwise lacking. Consent and authorization are the leading legal concerns for PMSR. Additional issues to consider include payment for sperm retrieval and storage, ownership of the sperm, consent for IVF, and treatment of posthumously conceived children in the realms of benefits and estate law.

Consent or Authorization

Most posthumous reproduction involves men who, prior to their deaths, discussed or formally consented to cryopreservation and posthumous use of their sperm, for example, soldiers deploying to war. Requests for PMSR, on the other hand, typically arise following sudden severe illness or death of a reproductively aged man who had not previously discussed or formally consented to either PMSR or the posthumous use of his sperm.

The legal analysis when considering PMSR for a sperm donor who is legally incapacitated (e.g., persistent vegetative state) is different than for an individual who is legally dead (e.g., brain death) [12]. This difference influences the initial question to be addressed: whether to pursue informed consent from a surrogate decision maker or authorization from legally empowered next of kin. Ascertaining the wishes of the sperm donor will be paramount in either situation since without the ability to demonstrate donor intent, retrieval of sperm for use in artificial conception *may* be deemed by courts to be an unconstitutional invasion of privacy. However, to date no actions have been brought against physicians or hospitals for performing PMSR without informed consent or authorization from the decedent. Regardless of whether

consent or authorization is deemed to have been established, a physician is not obligated to perform PMSR if they feel that it is not ethically justifiable.

An additional point to keep in mind is that at any point an individual or entity with an interest in the outcome may invoke participation from the court system. For example, family members opposed to PMSR may look to block the procedure, or the hospital or sperm bank may demand the reassurance of a court order before moving forward. Thus, even when there appears to be a clearly identifiable legal decision maker, best practice is to always reasonably ascertain and document the wishes of the donor and to reach agreement among family members whenever possible.

A full explanation about how sperm retrieval will be performed is required before consent or authorization on behalf of the incapacitated patient can be granted, as is the case whenever informed consent is obtained. In the context of PMSR, the choices of *after-death* decision makers can be affected by their understanding of the retrieval procedure. For example, in one case a man was declared brain dead after being struck by lightning. His wife inquired about sperm retrieval, and the providers were willing to proceed with electroejaculation; however, once the wife learned that electrical stimulation would be used to induce ejaculation, she refused the procedure, because an electrical storm had caused her husband's death [13].

Informed Consent: Incapacitated Patient

When a patient is incapacitated, medical decisions are made by a surrogate decision maker. The surrogate may be designated in a document such as an advance directive, living will, or durable power of attorney for health care. If the patient has not previously designated a surrogate decision maker, state law (and often hospital policy, if no state law exists) will provide the designation, typically in the order of next of kin. In Case 1, for example, if the patient had not executed a document designating his fiancée as the surrogate, it is likely that the father would be the legal decision maker under state law and able to consent to sperm retrieval. Documents appointing surrogate decision makers should be verified for authenticity and scope of decision-making authority, and questions should be directed to the hospital's legal counsel when there is any uncertainty.

Authorization: Postmortem

Once an individual has been declared dead, as in Cases 2 and 3, the next of kin are typically designated under state law to make decisions concerning the disposition of the body and administration of the individual's estate. From a legal standpoint, these individuals may be able to authorize PMSR and claim ownership of the decedent's sperm.

In this context, sperm retrieval has been authorized by analogy to organ donation in at least three probate court cases in the United States [14–16]. Since sperm is included in the definition of "tissue" under the Uniform Anatomical Gift Act, the

argument goes, individuals authorized to make anatomical gifts after death—in a PMSR case, typically the parents or spouse—may authorize retrieval of sperm [17]. In one case, a probate judge in Texas authorized PMSR at the request of the mother of a 21-year-old deceased man who was not survived by a spouse or partner [14]. In two other cases, sperm retrieval was granted at the request of the parents of a deceased man for the purpose of impregnating a surviving fiancée [15, 16].

Donor's Wishes: Documentation, Court Involvement

Because of the importance of the donor's intent when *use* of cryopreserved sperm is disputed, it is important to ascertain and document, as clearly as possible, the wishes of the deceased individual at the time the sperm is retrieved. For example, in Case 1, even though the father might have the legal authority to request sperm retrieval, the fiancée may be in the best position to offer evidence of the donor's intentions regarding procreation. Although the father's desire to insulate the fiancée from a potentially emotionally charged discussion is understandable, consideration should be given to encouraging her participation. Case 2 offers an example of universal agreement among family members regarding the decedent's intentions to start a family; even here, where no conflict is apparent, the agreement of all relevant parties should be documented at the time that PMSR is performed. In Case 3, there is open disagreement between the wife (who is most likely the legal decision maker) and other family members regarding the donor's intention to have more children. It would be tempting under this scenario for the involved providers to demand the parties have the issue clarified in court before agreeing to move forward, but it is unlikely a court would be able or willing to render a decision in time for retrieval to remain a viable option. Given the time constraints associated with PMSR, if the physician and hospital are satisfied that the legal decision maker is acting in good faith, they may choose to proceed, since the family can turn to the courts to challenge the *use* of the sperm for posthumous reproduction at a later date. In cases where a spouse requests retrieval, rather than a parent or a fiancée, hospitals and sperm banks are less likely to demand a court order before performing the procedure [18].

It goes without saying, if the providers are uncertain of the donor's wishes, or if a court has issued a temporary restraining order *against* PMSR, the procedure must not be performed until further order from the court.

Payment for Sperm Retrieval Procedure

Sperm retrieval procedures are unlikely to be covered by insurance. As with any other elective procedure that will be paid for out of pocket, the provider should inform the requesting party, before performing the procedure, of the anticipated costs, including not only the cost of the procedure but also the estimated costs of storage and IVF. Such disclosures are best practice and may also be required by state law [19].

Ownership, Storage, and IVF

After the sperm is retrieved, there are other legally significant steps before the retrieved sperm may be used for posthumous reproduction. Typically, legal determinations will be sought regarding ownership and use of the sperm in one of the two situations: when family members disagree or when a sperm bank or IVF provider requires it. Legal decisions involving the disposition of cryopreserved sperm, embryos, and other gametic material—usually arising upon a couple's divorce or separation—have become increasingly frequent in recent decades [20].

As noted above, next of kin may claim *ownership* of the decedent's sperm under the Uniform Anatomical Gift Act. But the organ donation analogy is irrelevant when a court considers a dispute over subsequent *use* of the sperm for the purpose of posthumous reproduction. Using a man's sperm for the purpose of reproduction implicates his constitutional right of procreational autonomy [21]. The right of procreational autonomy includes both the right to procreate and the right to avoid procreation [22]. When courts determine the disposition of pre-embryos, which include sperm and ova from two different individuals, various legal analyses are used to determine and balance the rights of both parties who have contributed gametic material [23]. But sperm that is preserved alone is treated differently than a frozen embryo. In this situation, since the spouse or partner has not yet contributed ova to the sperm, her procreational autonomy is not implicated, and only the intent of the deceased donor is relevant to determining how the sperm may be used [24].

Estate Law

Issues of estate disbursement and the financial effects of PMSR and posthumous conception on the donor's current heirs may serve a central role in defining the legal implications of a sperm retrieval case. The donor's heirs are among the persons most likely to bring cases to the judicial system in an effort to prevent sperm retrieval and use [25].

Dilution of the estate by producing more heirs is one possible financial effect of posthumous procreation. Additionally, there is the question of social security survivor benefits for a posthumously conceived child. These issues will turn on whether the state where the child is born considers the deceased donor to be the legal father of the child [26]. A recent slew of social security cases interpreting intestacy law of various states consistently found that posthumously conceived children were not recognized as heirs of their deceased biological fathers under the relevant state laws [27].

Partially in response to the negative social security decisions, states have begun enacting or amending their laws to address the status of posthumously conceived children. Uniform acts, including the Uniform Parentage Act and the Uniform Probate Code, include provisions providing inheritance rights for posthumously conceived children in certain situations [28, 29]. Many states have adopted versions of the uniform provisions, and at least 15 states now have laws addressing posthu-

mous conception [30]. These laws are unlikely to support paternity in the case of a posthumously conceived child born from posthumously *retrieved* sperm; rather they are drafted to accommodate more common situations like cancer patients who cryopreserved sperm before undergoing gonadotoxic chemotherapy. Existing laws universally require strong evidence, typically in the written form, that the deceased individual consented to be treated as the father of a child conceived after his death.

Ethical Considerations

It would be impossible to fully and completely answer or even discuss all the ethical questions raised by PMSR in this chapter alone. At the heart of the debate is controversy about whether the final objective of PMSR—posthumous reproduction—is ethical; and if so, should authorization from surviving parties for the retrieval procedure be accepted without clear consent by the decedent for posthumous reproduction? The decision to proceed with PMSR must take into consideration the rights, duties, wishes, and safety of all parties involved including the decedent, his surviving spouse and family members, and any children conceived posthumously. In this section we aim to highlight, in the broadest sense, the primary ethical questions raised by PMSR.

Ethical Considerations for the Decedent

Would the decedent have consented to PMSR or the use of his sperm for posthumous reproduction? The dominant ethical consideration with regard to the decedent is consent. When written consent has been documented, the consensus of professional societies, including the Ethics Committee of the American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE), is in favor of PMSR [31, 32]. Unfortunately, previously documented consent for PMSR is vanishingly rare, and lawmakers worldwide have been cautious in protecting the rights of the decedent with legislation that begins without the presumption of consent [5].

The most conservative viewpoint contends that a procedure which does not benefit the patient and which is not medically necessary should never be performed [11]. In this context, emergency surgery without consent is appropriate since the patient stands to benefit from the operation; however PMSR does not benefit the decedent, so would be considered unacceptable. There are situations in medicine when authorization from a proxy is reasonable and widely accepted even when there is no benefit to the patient—for example, organ donation. This line of reasoning cannot be applied unconditionally to PMSR, however, since the surrogate consenting on behalf of the decedent has a conflict of interest. When a proxy consents to organ donation, the beneficiary is an unrelated third party, but it is the proxy who benefits directly if PMSR is performed [3]. Furthermore, an organ donor does not

have any legal, moral, or financial obligation to the recipient. The intention of PMSR, on the other hand, is to create a new human life.

In the absence of documented consent, the debate shifts to the question of implied consent which may be authorized by a legal surrogate based on the wishes of the decedent. Expert opinion regarding implied consent is inconsistent. ESHRE and the Mayo Clinic Reproductive Medicine Advisory Board only consider written consent sufficient for PMSR [32, 33]. The ASRM Ethics Committee allows physicians to consider PMSR based on implied consent only when requested by the surviving spouse or life partner [31]. A more liberal position, posed by Tremellen and Savulescu, is that the default assumption should be presumed consent unless there is evidence that the decedent would be opposed to PMSR, for example if he had previously undergone a vasectomy [11]. This standpoint reasons that the autonomy of the decedent should not indiscriminately take precedent over the wishes and welfare of surviving loved ones. They support their position by noting that 85.2% of men who cryopreserve sperm consent to posthumous reproduction and 84.9% of men surveyed at an infertility clinic responded that they would agree to PMSR [34, 35]. They also suggest that the decedent does benefit from PMSR, since he is satisfying his widow's wishes. There are limitations to this position, since respondents in the studies cited by Tremellen and Savulescu were all pursuing parenthood, so their attitudes may not be generalizable to the entire population. Furthermore, approximately 25% of women incorrectly assumed that their male partners would be in favor of PMSR [34].

Ethical Considerations for the Surviving Family

Should requests for PMSR from anyone other than a surviving spouse be considered? In the context of PMSR the decedent's widow is unquestionably at the apex of the moral hierarchy for surviving family members. Although not a unanimous opinion, many medical experts recommend only considering requests for PMSR from a surviving spouse or life partner for several reasons [9, 31, 32]. First, there should be a reasonable chance that gametes will be used if they are procured, and they cannot be used for reproduction with another family member. They argue that sperm retrieved in the context of PMSR should not be donated or sold to a third party, which could theoretically create an incentive for surrogates to consent to PMSR for their own financial gain [9]. Second, the desire of surviving parents to "continue the bloodline" or to become grandparents does not give them rights to their son's procreational autonomy [6, 31]. Finally, if a surviving spouse did not request PMSR yet sperm was procured at the behest of a family member, she may feel pressured or obligated to attempt posthumous reproduction [9]. Similarly, requests for PMSR from a surviving spouse should not be acted upon unilaterally when other family members express dissent. In cases without a widow, next of kin may have legal grounds to authorize PMSR, but use of the sperm for posthumous reproduction encroaches on the decedent's procreational autonomy and would likely face legal hurdles.

Requests for PMSR from a surviving spouse still raise their own ethical questions. Has she been coerced to carry out a deathbed promise from her husband to carry on

his family name? Is posthumous reproduction a misguided attempt to clone or commemorate her husband? In cases of brain death, is there misguided hope for a miraculous recovery? However unlikely these scenarios may be, the stress and grief associated with sudden loss of a spouse can certainly cloud decision making and judgment and should not be underestimated. To allow time for thoughtful consideration about the decision to initiate a pregnancy, there is broad consensus to require a mandatory 6–12-month waiting period before attempting posthumous reproduction [9, 10, 31, 32]. The importance of a waiting period is underscored by the fact that only a fraction of sperm procured via PMSR is used for posthumous reproduction [9].

Ethical Considerations for the Conceived Child

When the conceived child's well-being is considered, is it ethical to attempt conception using sperm procured through PMSR? Whether PMSR is ethically justifiable is based on the assumption that posthumous reproduction, a controversial practice in its own right, is ethical [36]. Some question the ethics of conceiving a child who will be fatherless, although use of donor sperm for ART is acceptable in most modern societies, and there is evidence that children conceived with donor sperm and raised by a supportive mother are equally healthy and happy [11, 37]. The medical risk to children conceived with postmortem sperm is unknown, although in one study Robson et al. did report four children who were born healthy [38].

Logistical Considerations

Once the decision has been made to proceed with PMSR, several logistical issues must be considered. While there are not any nationally or internationally recognized guidelines for PMSR, the ASRM, ESHRE, Mayo Clinic, as well as other professional societies, prominent institutions, and experienced andrologists, have published their own recommendations for PMSR policies [31–33]. Having institutional policies in place, in concordance with national and state laws, helps physicians and hospitals efficiently and consistently navigate requests for PMSR when they arise [9, 31, 39, 40]. Institutions are encouraged to consider these practical considerations when developing policies for PMSR.

Consent or Authorization

Consent or authorization is required to perform PMSR [9, 31, 32]. What constitutes consent is variable and should be defined by each institution in advance. Documented consent from the decedent is ideal but uncommon. Requests made with inferred

consent may be considered with support from a non-benefiting third party, such as a physician. In these cases, efforts should be made to obtain supporting evidence that the decedent would have been in favor of PMSR, for example, if the couple was actively trying to conceive, undergoing fertility treatment, or had expressed plans to conceive in the immediate future, etc. [5, 31, 39, 40].

Inclusion Criteria

Most experts agree that only requests for PMSR from a surviving spouse or life partner should be considered [9, 31, 40]. In cases where the decedent has not given legal decision-making authority to a life partner, such as a fiancée or other long-term partner, hospitals should consider requests from next of kin (often a parent) on behalf of the surviving partner who is present and in agreement with the request. PMSR should be performed within 36 h, but ideally within 24 h of cardiac death since sperm quality likely deteriorates quickly following tissue ischemia [8, 9, 39, 41]. When possible, PMSR should be performed prior to withdrawal of cardiopulmonary life support. The decedent should be of legal age to consent to procedures [40]. Hospitals should consider whether PMSR is permissible only after cardiac death or prior to termination of life support in cases of brain death, a persistent vegetative state, or terminal illness.

Exclusion Criteria

Requests for PMSR should be denied when disagreement between surviving family members, including a surviving life partner, suggests a lack of consent on the part of the decedent [31, 40]. Other exclusion criteria include death caused by communicable diseases including HIV, hepatitis B, and hepatitis C; plan for use of sperm by a third party; and for patients who had previously undergone vasectomy [9, 32, 39, 41].

Sperm Designee

The sperm designee must be identified prior to PMSR. The designee should consent in writing to take sole ownership of the sperm and acknowledge that it cannot be transferred to a third party, take financial responsibility for all aspects of the retrieval procedure as well as processing and storage of sperm, contact and contract with a sperm storage facility within a reasonable traveling distance, and acknowledge that the quality and viability of sperm cannot be guaranteed [39, 40].

Confirm Laboratory Availability

All methods of PMSR require sperm processing and cryopreservation by a specialized andrology laboratory. As such, availability of an appropriate laboratory is critical. If a qualified andrology laboratory is not available, PMSR should not be performed.

Surgeon Selection

Urologists with expertise in sperm retrieval procedures are ideal PMSR surgeons; however few urologists have this type of training. General urologists, general surgeons with knowledge of pelvic anatomy, and even pathologists could conceivably perform less technically specialized PMSR procedures.

Procedure Selection

The type of retrieval procedure to perform should be selected on a case-by-case basis depending on the patient's clinical situation and the surgeon's expertise and experience [6, 13]. The optimal method for performing PMSR has not been determined since studies comparing the efficacy of different procedures have not been performed. Sperm retrieval procedures should aim to obtain enough cryopreserved sperm for 5–6 IVF/ICSI cycles [41]. The algorithm proposed by the authors in Fig. 11.1 may help identify the most appropriate retrieval procedure for a variety of clinical situations, including when there is suspicion for impaired spermatogenesis based on physical exam findings or past medical history. A review of different PMSR procedures, including their indications, benefits, and drawbacks, can be found later in this chapter.

Waiting Period

A mandatory 6–12-month waiting period is recommended before proceeding with posthumous reproduction [9, 31, 39, 40]. Grief counseling should be considered for the surviving spouse during this bereavement period [6, 32, 40].

Miscellaneous

When available, involvement of a hospital ethics committee is prudent. Hospitals may stipulate that they release their responsibility for the sperm following its procurement. No hospital employee should be forced to participate in PMSR.

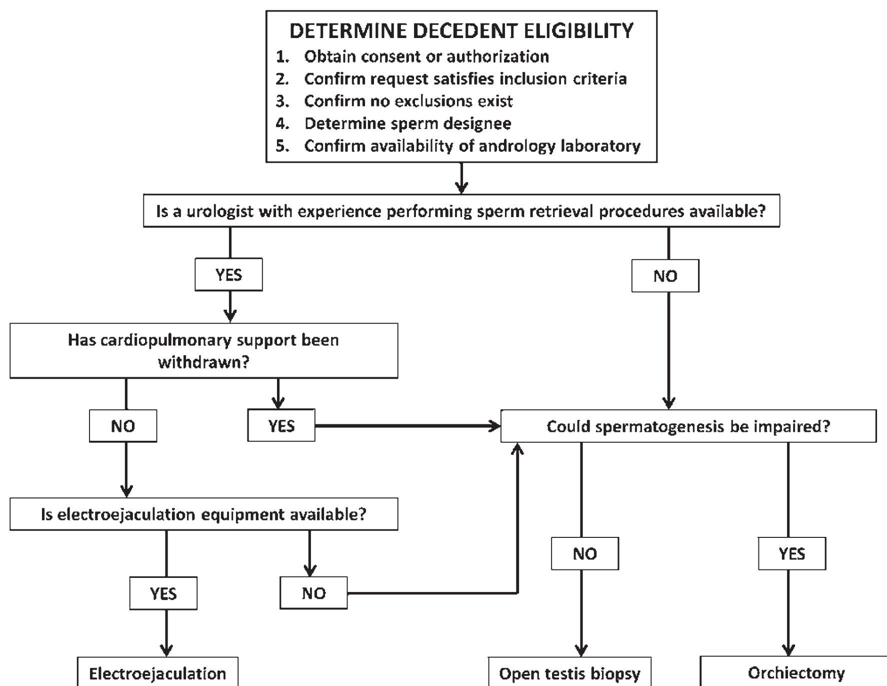

Fig. 11.1 Postmortem sperm retrieval algorithm

PMSR Procedures

PMSR has been performed successfully using several techniques including electroejaculation (EEJ), testis biopsy, vasal or epididymal aspiration, and simple orchiectomy [6, 13]. All procedures can be carried out antemortem or postmortem with the exception of EEJ, which relies on an intact ejaculatory reflex, so it can only be performed prior to the cessation of cardiac activity [8, 13, 40]. The authors recommend obtaining a generous tissue sample since the decedent's fertility status is usually unknown, there is no risk of causing hypogonadism or infertility in the future, multiple cycles of IVF/ICSI are often required to achieve a pregnancy, and there will only be one opportunity to retrieve sperm.

Electroejaculation

EEJ is the preferred method of sperm retrieval prior to the cessation of cardiac activity. EEJ delivers wavelike electrical stimulation to pelvic nerves via a rectal probe to trigger ejaculation [42]. EEJ is minimally invasive and can yield a large quantity of motile sperm, making it an appealing option for cryopreservation. There are two

major drawbacks to EEJ. It requires availability of an FDA-approved Seager Electroejaculator (Dalzell Medical Systems, The Plains, VA), as well as a specialized urologist with experience performing EEJ.

Testis Biopsy

The authors recommend open testicular biopsy for PMSR following cardiac death. Open biopsy yields a large volume of testicular tissue and can be performed without specialized instruments by general urologists, general surgeons, or a pathologist during an autopsy. Testicular tissue can also be stored in sperm-friendly medium overnight, which minimizes a potential barrier to cryopreservation since processing can occur during "social" work hours [41]. Microscopic testis biopsy is favored by others, who argue that open biopsy removes an excessive amount of tissue. Percutaneous testis biopsy requires availability of a specialized urologist and, even in experienced hands, only yields a small volume of tissue.

Simple Orchiectomy

Orchiectomy removes the entire testicle and epididymis en bloc through a scrotal incision. If there is concern for impaired spermatogenesis this approach may be prudent to maximize the volume of testicular tissue procured. Similar to open testis biopsy, orchiectomy can be performed by a general urologist, general surgeon, or pathologist without specialized instruments.

Vasal or Epididymal Aspiration

Aspiration of the epididymis or vas deferens for PMSR has also been described, and can yield motile sperm for cryopreservation [9]. Proponents of vasal aspiration favor this approach since it is minimally intrusive and prevents mixture of sperm with the decomposing body [9]. General urologists who perform vasectomies will be comfortable with the vasal dissection and manipulation, although they are unlikely to have experience with the actual aspiration. Epididymal aspiration is technically difficult and requires surgical expertise.

Conclusions

PMSR is a controversial practice which raises a myriad of ethical questions, yet it is being requested with increasing frequency worldwide [2, 39]. Successful PMSR requires swiftly navigating the logistical issues summarized in this chapter and has

been described using a variety of retrieval procedures. No ethical calculus or system of judgement could conclusively resolve the ethical questions surrounding PMSR given the intense circumstances under which requests arise and when the interests of all involved parties are considered. The legal precedent for PMSR is highly variable and in many places nonexistent. In the absence of any official international or national guidelines, experts have called on medical centers to develop their own institutional policies to help guide physicians and hospital ethics committees considering requests for PMSR.

Conflicts of Interest No authors have any commercial interests or financial disclosures.

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22. Davis v Davis, 842 SW2d, at 601.
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Part III
Sperm Banking: Technical Issues, Standards and Maintenance

Chapter 12
The First Visit: Consult and Workup Before Sperm Banking

Alan Scott Polackwich Jr. and Maurilio Garcia-Gil

Introduction

Any initial patient consult centers around developing the patient-clinician relationship and the patient narrative that will steer their care. Consults that involve male fertility and fertility preservation will have three additional important goals of identification of conditions that may affect the health of the patient, identification of conditions that may affect the health of the offspring, and finally prediction of any problems that may occur with cryopreservation. Certain clinical scenarios may portend a loss or decline in a male patient's fertility, obliging the clinician to explore fertility preservation with the patient. Prior to any male fertility preservation therapy or assisted reproductive technology (ART), it is critical to fully assess the factors impacting the patient's individual fertility to better craft a treatment plan and counsel the patient on expectations.

History

Medical History

As with any consultation, a detailed general medical history should be obtained [1]. Medical conditions can cause sexual dysfunction (interfere with sperm delivery: erectile dysfunction (ED), anejaculation, retrograde ejaculation) or impair the production and function of the sperm.

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General Medical History

Developmental History

Organization of the general medical history is typically simplified by using a chronological approach. Beginning with the patient's developmental history can uncover syndromic disorders such as those of testosterone production and function. In one study of almost 35,000 children, 1 in 426 births had abnormalities of the sex chromosomes. Of these, Klinefelter's syndrome was the most common, affecting 1 in 576 boys, which can present as delayed puberty or incomplete masculinization [2]. Absent or significantly delayed puberty can suggest a failure of testosterone production, such as that seen in Kallmann syndrome, fertile eunuch syndrome, or other such causes of hypogonadotropic hypogonadism. Patients with poor access to health care earlier in life may initially present with these conditions as infertility in the second or third decade of life [3–5].

Congenital disorders without any direct androgenic symptoms may also affect male fertility. Rarely, sperm motility may be affected by congenital syndromes that present with pulmonary complaints. Young's and Kartagener's syndrome are associated with sinus pulmonary infections, and can have occult effects on fertility associated with tubular obstruction by thickened secretions and nonfunctional cilia, respectively [6, 7]. Patients with personal or family history of cystic fibrosis (CF) should be screened for congenital bilateral absence of the vas deferens (CBAVD), and vice versa, due to effects on both fertility and the patient's global health [8, 9].

These conditions may necessitate medication prior to cryopreservation (e.g., rFSH and HCG in patients with Kallmann syndrome) or may make cryopreservation with ejaculated sperm impossible (CBAVD, many patients with Klinefelter's) requiring advanced procedures to obtain tissue.

Chronic Conditions

Acquired medical conditions, both chronic and acute, can affect fertility by disrupting sexual function or sperm function. A common example is diabetes mellitus (DM) which is increasing in prevalence, including among children and adolescents, occurring at a rate of 5300 per 100,000 in some studies of adolescents [10]. Increased DM in this age group is expected to result in more diabetes-associated ejaculatory and erectile dysfunction, as well as direct changes to sperm function and production in their reproductive years [11, 12]. Such issues may interfere with collection of specimens and can be anticipated with a few simple questions concerning erections and ejaculatory quantity.

Transient Conditions

Transient conditions, especially those with fever, can have significant effect on sperm production. Clinicians should directly inquire for these as they may not be initially seen as significant by the patient. Acute febrile illnesses have been demonstrated to

debilitate all aspects of sperm production and function, taking 2–3 months for sperm parameters such as concentration, motility, and DNA fragmentation to recover [13–15]. Thus, if possible, patients should be counseled to delay preserving a specimen immediately after a major illness to optimize the stored specimen.

Genitourinary Illnesses

Genitourinary specific illnesses can affect fertility by disrupting the blood-testis barrier, allowing infiltration by the immune system. Loss of this barrier can lead to decreased sperm function or even necrospermia. In a study of 60 men, leukocytes in ejaculate were positively correlated to markers of oxidative stress and apoptosis [16].

Epididymoorchitis can affect fertility both by breaking down the blood-testis barrier and by causing epididymal scarring and subsequent obstruction necessitating reconstruction or assistive reproductive technology (ART) [17]. Many patients and practitioners may falsely attribute a groin pain to epididymoorchitis. History including a combination of erythema, induration, swelling, and fever is more likely to be from epididymoorchitis. Before attributing the patient's infertility to epididymoorchitis, genital pain from musculoskeletal, gastrointestinal, or neurologic sources should be ruled out [18].

Chronic prostatitis can be subtler but can also affect male fertility. A meta-analysis found chronic prostatitis to be associated with significantly decreased sperm concentration, decreased progressive motility, and increased abnormal sperm morphology, suggesting that inflammatory conditions of the genitourinary tract should be detected and treated [19]. Chronic prostatitis can also impair successful intercourse by causing sexual dysfunction [20]. The UPOINT Classification System has been validated and shown to be effective for identifying and evaluating patients using the signs of chronic prostatitis, including pelvic pain, dysuria, pain with defecation, pain with sitting, urinary frequency, and/or urgency [21]. In the patient with minimal to no symptoms, analysis of expressed prostatic secretions may find the cause of leukocytospermia [22].

Reproductive History

Taking a thorough history can yield valuable information about the reproductive system. In evaluating fertility, the most important question is whether or not the patient has previously caused a pregnancy or fathered a child. This suggests that at least at one time, the patient had normal sperm production and should not have issues with cryopreservation. Then, the clinician's task is to identify changes in health or exposure since that time that may have changed that status.

When thinking about cryopreservation, identification of infertile couples who may be more likely to have substandard semen analyses is important. The currently accepted definition of infertility is the failure to achieve clinical pregnancy after

12 months or more of regular unprotected sexual intercourse, though some authors extend this interval to 2 years to account for the ability of some couples to conceive despite relatively low monthly fecundity [23]. Activation of infertility evaluation and treatment should be based on the couple, rather than preset norms. Waiting for 2 years before evaluation or treatment may allow for significant declines due to aging and oocyte quality in treatment success rates [24].

It is critical to definitively determine how long a couple has been attempting to conceive without birth control. As there is no biologic difference between "trying" to conceive and having frequent unprotected intercourse, the question should be well defined by the clinician. A couple could report consciously "trying" to conceive for 6 months, but not report that they never used birth control for the previous 5 years. An increased duration of infertility, recognized by the couple or not, has been associated with decreased chance of live birth with in vitro fertilization (IVF) [25].

Frequency of intercourse can have an impact on time to pregnancy. Couples reporting a long infertile period should be queried as to their frequency of intercourse. The optimal time for intercourse is at least every other day during the 7 days in the post-ovulatory period [26, 27]. Timing of ovulation can be estimated with noninvasive methods such as urine dipstick kits, basal body temperatures, calendar prediction, analysis of cervical mucus, or ovarian ultrasound [27]. Psychological stress, whether from mundane events or from the physical, emotional, and social effects of a cancer diagnosis, can both strain the couple's relationship and impair spermatogenesis [28–30]. After the substantial stress of Hurricane Katrina, local men experienced significant decreases in sperm count [31]. Furthermore, the psychological stress of infertility can worsen sexual dysfunction in men, potentiating more stress and infertility [32].

The physical act of intercourse is an intimate and individual process for the couple. No sexual positions are recommended for optimizing fertility. The couple should be encouraged to do what is comfortable for both parties. Lubricants do not appear to influence fertility rates in fertile couples, but it is not recommended for couples with difficulty conceiving as use of lubricants has been found to impair sperm function [33, 34]. All lubricants, including saliva, negatively impact sperm function to some degree [35], with the most impact coming from the common "non-spermicidal" water-based lubricants such as KY™, Surgilube™, and Astroglide™ [36]. Oil- or glycerin-based lubricants are less problematic for sperm function [37]. However, patients should be counseled to avoid lubricants if possible during procurement of a sample for preservation.

The number of previously lost pregnancies can point to possible dysfunction. Pregnancy loss can be due to issues with male or female partner. In male factor causes, this could be due to translocations denying offspring a complete genome [38, 39], or poor sperm DNA integrity with increased DNA fragmentation [40–42]. Identifying these types of issues is especially important to prevent failed IUI/IVF attempts with these preserved specimens.

Determining whether patients may have a reproductive issue is central when evaluating them for cryopreservation. It is important to cryopreserve optimal specimens. If these issues are identified prior to preservation, one can avoid patients attempting to cryopreserve a specimen only to find that there are minimal usable sperm in the collected specimen.

Social History

A patient's behavior and use of alcohol, smoking, and illicit drugs can negatively impact fertility. Alcohol can impair sperm production with chronic use associated to liver disease which can disrupt hormonal metabolism and significantly disrupt fertility [43–45]. Even short-term use of alcohol has been found to reduce sperm production as measured by sperm concentration, total motile count, and morphology with as few as five drinks per week. The effect on sperm production increased with greater alcohol use, with greater than 40 units of alcohol per week decreasing total motile count by 33% and decreasing sex hormone-binding globulin (SHBG) resulting in increased free testosterone [46].

By-products of tobacco smoke are detectable in semen and have been related to changes to sperm morphology [47]. Mean sperm DNA fragmentation is higher in smokers at a rate of 32% compared to 25.9% in nonsmokers [48]. Even prenatal exposure may decrease sperm counts in offspring when they reach adolescence [49]. Even among patients utilizing ICSI, there are demonstrated decreases in clinical pregnancy rates for men who smoke [50].

Surgical History

The surgical history is important as some surgeries can interfere with sperm delivery, while others can affect sperm production itself. Impaired spermatogenesis can be caused by surgeries of the pituitary which may cause hypogonadotropic hypogonadism, as well as other long-term sequelae such as osteoporosis [51].

Surgeries that affect sperm delivery include those that change the anatomy of ejaculation, such as any prostate surgery or resection [52], or those that disrupt sympathetic innervation to the bladder neck or genitourinary system that can cause retrograde ejaculation or even anejaculation [53]. Hernia repairs can damage the vasculature of the testicle, risking atrophy [54]. Hernia repairs in both pediatric and adult populations can also risk obstruction of the vas deferens by either direct injury or fibrotic response to mesh, potentially affecting spermatogenesis [55, 56].

Pediatric inguinal hernia repairs are commonly associated with orchidopexy for undescended testicles. However, patients may only be aware of the hernia repair and not the simultaneous orchidopexy. Cryptorchid testis has baseline decreased function, even after orchidopexy, such that testicles are less metabolically active on PET scan 20 years after orchidopexy [57]. This loss of metabolic activity represents loss of spermatogonia and potentially oligospermia [58]. Cryptorchidism is associated with a relative risk of 2.9 for development of testicular metachronous cancer, which can cause infertility both directly and through its treatment [59]. When patients present for preservation, this represents a good opportunity to screen and teach these higher risk patients about self-exams.

Medications

Medications can significantly affect fertility by affecting the hypothalamic-pituitary axis and gonadotoxicity or indirectly interfering with sperm production.

Disruption of the hypothalamic-pituitary axis can be caused by many medications. Testosterone supplementation is known to suppress endogenous testosterone production, yet they are found to be prescribed in 30% of patients trying to obtain a pregnancy [60]. Exogenous testosterone, through negative feedback, can decrease endogenous production of LH, FSH, and testosterone. Exogenous testosterone's blocking of endogenous testosterone production can prevent the locally high levels of testosterone required in the testicle for spermatogenesis [61, 62].

Testosterone metabolism is also disrupted by medications such as 5-alpha reductase inhibitors, opioids, and ketoconazole. Full-dose 5-alpha reductase inhibitors, but typically not the low doses used for hair restoration, can cause transient decreases in sperm counts [63, 64]. Chronic opioid use can decrease sperm production by decreasing free testosterone and increasing prolactin [65, 66]. Likewise, ketoconazole has antiandrogen effects that can significantly decrease sperm concentration and motility [67].

Limited in vitro studies have demonstrated direct impairment of sperm function by medications such as the antibiotics cotrimoxazole, erythromycin, high-dose amoxicillin, tetracycline, and chloroquine [68]. Psychotropic drugs such as lithium have been shown in animal and in vitro studies to inhibit sperm motility [69, 70]. In humans, paroxetine has been shown to increase sperm DNA fragmentation [71].

Common medications that impede sperm delivery include alpha-blockers, specifically tamsulosin [11, 72, 73]. Typically patients with good response in urinary function to tamsulosin can resolve their retrograde ejaculation and preserve their urinary response by switching to alfuzosin.

Cessation of these medications when possible prior to cryopreservation gives a patient the best chance at an optimal specimen.

Physical Exam

Overview

When evaluating patients for cryopreservation, a directed exam can quickly identify potential issues and avoid unexpected problems with preservation. Male exams are best initiated in the standing position. This allows for easy access for all parts of the exam and for evaluation of varicoceles which are difficult to detect when supine.

In evaluating overall appearance, the clinician scans for normal gross genital anatomy, proper development, and pubertal stage. The first indication of abnormality will be sparse genital hair or abnormally small genitals. Because the genitourinary system is testosterone dependent, altered testosterone metabolism can cause both developmental issues with poor virilization and interference with spermatogenesis.

Scars in the scrotum or inguinal region may indicate a pediatric operation that may be unknown to the patient, such as hernia repair, hydrocelectomy, or orchiopexy. Laparoscopic scars may indicate orchiopexy of an abdominal testicle. Due to the age these are typically performed, patients may be aware of a surgical history without being aware of laterality or specific operation performed.

Penis

The penile exam can uncover issues unknown to the patient or too intimate to previously reveal to a clinician. With respect to fertility, penile disorders can be divided into two categories: those that prevent intercourse and those that impair intravaginal sperm delivery.

The meatus should be the first aspect of the penis examined after retracting the foreskin. Difficult or painful retraction of the foreskin can interfere with successful intercourse. Orthotopic placement of the meatus should be verified. Glandular hypospadias should not interfere with semen deposition, but more proximal hypospadias will be progressively more problematic [74].

Patients with erectile dysfunction will typically have a normal genitourinary exam, though ED can be associated with other disorders such as Peyronie's disease. In such cases, one may be able to palpate penile plaques along the penis. Those with a history of pelvic trauma may have vascular thrills detected along the corpora, suggestive of arteriovenous shunting which can disrupt erections [75].

Testes

The testicular exam is going to be the most useful in predicting preservation issues, as many problems of either production or obstruction can be predicted by exam. The testicular exam first focuses on size, because 80% of testicular volume is comprised of cells associated with spermatogenesis [76], giving significant insight into the level of function. There are multiple methods of measuring testicle size, including measuring with a caliper, use of an orchidometer, or testicular ultrasound, which is considered the gold standard, though each method has some intra-observer variability [77, 78]. Depending on the volume equation used, median testicular size varies from 12.7 to 18.9 mL on ultrasound [79], with volumes less than 12 cm^3 correlated with abnormal semen parameters [76]. Small testicles may suggest syndromic issues with testosterone metabolism or spermatogenesis such as Kallmann or Klinefelter's syndrome [5, 80], or atrophy after vascular injury from torsion or inguinal hernia repair [54].

Evaluation and documentation of testicular smoothness and contour are important in patients presenting for infertility. Infertile men have an increased incidence of testis cancer and testis cancers are known to affect fertility [81]. There should be low threshold to supplement the exam with ultrasound if there is any doubt or difficulty in evaluation.

Presence of the genitourinary ductal system should be evaluated. In the testicles, presence and fullness of the epididymis should be palpated. Then entire epididymis should be palpated, as due to embryologic differences, the head is rarely missing but a missing midbody or tail of the epididymis can indicate a Wolffian duct abnormality or CBAVD related to cystic fibrosis. Unilateral absence of the vas and epididymis suggests Wolffian duct abnormalities that may have involved the ureteric bud and should be evaluated for solitary kidney with ultrasound [82].

Spermatic vein exam is performed to determine the presence and severity of varicocele. Varicoceles are graded from I to III based on exam with grade I felt only with valsalva, grade II palpable without valsalva, and grade III visible through scrotal skin [83]. Smaller varicoceles can be difficult to palpate. Any varicocele should be checked for reduction when laying supine, especially for large or right-sided varicoceles, as these can rarely be associated with retroperitoneal malignant processes and are less likely to reduce due to the nature of venous obstruction [84]. Such findings may help predict issues with cryopreservation and subsequently direct patients to the necessary treatment.

Prostate

In evaluation for fertility, exam of the prostate is reserved for patients with oligospermia, pyospermia, low-volume ejaculate, or any voiding complaints. If they are done, attention should be paid to any signs of obstruction or infection. Tenderness of the prostate may represent an inflammatory process. Midline cysts or dilated seminal vesicles or painful ejaculation suggest obstructive processes in the prostate such as ejaculatory duct cyst or Mullerian duct remnant. I rarely perform rectal exam on young patients without other complaints prior to cryopreservation unless a semen analysis has already discovered an abnormality.

Semen Analysis

Semen analysis is the most commonly used tool to evaluate a man's fertility. It is especially important in analyzing cryopreservation specimens as these values help predict the adequacy of a given specimen, as well as directing the need for further specimens.

When interpreting a semen analysis it should be remembered that it does not account for many aspects of sperm function relating to fertility. Due to variation over time, more than a single semen analysis should be examined. In patients who had 12 monthly semen analyses performed, there were significant differences in all the variables except the percentage of sperm with normal morphology [85]. With cryopreservation, this means that patients should be encouraged to preserve multiple specimens.

The modern semen analysis parameters have been codified by the World Health Organization based on multi-institutional study of men from 14 countries, with the most recent update of normal values in 2010. The men in the study population had

proven fertility with previous children with a time-to-pregnancy period less than 12 months. One-sided lower limits were established as defined by the fifth percentile of these fertile men [86]. These standards remain controversial because there were no comparisons to men who were infertile and use the fifth percentile cutoff which has no known correlation with fecundity, which could be a critical omission for a standard being used to define pathologic states [87]. The updated reference ranges have been significant because up to 15.1% of men who were previously categorized as infertile are now categorized as normal, with new implications for patient access to care [88, 89].

When evaluating infertile men, the entire presentation must be accounted for, rather than relying on the semen analysis alone. In one study of the standard criteria for semen analysis, time to pregnancy was only significantly associated with sperm concentration and total motile count, though with odds ratios scarcely above one [90]. Thus, while semen parameters can assist in predicting fertility and time to pregnancy, they are not definitive.

Collection

Proper collection is necessary for the most accurate semen analysis and for the best cryopreservation specimen. Specimens must be transported to the laboratory at body temperature and processed promptly, at least within 1 h of production. As with normal intercourse, lubricants should be avoided.

Specimen production must be performed with patients abstaining from ejaculation for between 2 and 7 days [91, 92]. Total antioxidant capacity (TAC) was greater in specimens produced with a shorter ejaculatory abstinence interval of 1 vs. 4 days [93]. Specimens produced less than 2 days after previous ejaculation may have sperm count, concentration, and volume adversely affected. Each additional day of abstinence can increase volume by 0.62 mL and sperm concentration by 17.6 M/mL [94]. Long periods of abstinence can be detrimental, with greater than 5 days being associated with decreased sperm motility and periods of 6–7 days being associated with defects of the tail [95, 96].

Volume

Ejaculate volume, along with sperm concentration, determines the absolute number of sperm delivered, and in this case preserved. Normal semen volume is currently defined as >1.5 m [86]. Low volume is most commonly associated with poor collection with partial loss of specimen or inadequate abstinence period, both of which should be documented.

Conditions causing low seminal volume can be divided into poor production of seminal fluid, obstruction, and ejaculatory dysfunction. Poor production of fluid can indicate severe primary or secondary hypogonadism, such as that seen in Klinefelter's syndrome [97]. Treatment of this hypogonadism focuses on increasing gonadotropins, typically with clomiphene citrate, HCG with HMG, or rarely GnRH infusion, depending on the nature of disruption [98, 99]. Seminal fluid production decreases with age, but can rarely be severe with reductions of 22% [100].

Decreased volume related to anejaculation or retrograde ejaculation is differentiated based on post-ejaculate urine analysis. Some semen in post-ejaculate urine is normal, but infertile men can have a greater proportion of their total sperm in their urine [101]. Retrograde ejaculation can be related to conditions disrupting innervation of the genitourinary system, such as diabetes mellitus, or medications such as alpha-blockers [11, 72]. Antegrade ejaculation can be restored with cessation of inciting medications or on-demand pseudoephedrine or imipramine in diabetics prior to cryopreservation [102].

Concentration

Normal sperm concentration is currently defined by the 2010 WHO guidelines as greater than 15 million sperm/mL [86]. Being based on fertile males, this threshold is less helpful in evaluated infertile men or those using assisted reproduction. Due to significant overlap in these populations, studies with reasonable specificity and sensitivity have been unable to determine a functional concentration threshold. A study comparing fertile and infertile men found that subfertile concentrations were less than 13.5 M/mL, with the caveat that the majority of men presenting with infertility had concentrations greater than 25 M/mL [103]. A second study demonstrated a sperm concentration of less than 20 M/mL having a relative risk ratio of 1.51 for not having a pregnancy. Median concentrations were 65 M/mL for those who achieved pregnancy compared to 25 M/mL for those who did not [104].

The most helpful aspect of sperm concentration is differentiation of oligospermia and azoospermia. This distinction is critical for the patient considering male fertility preservation, as it determines which techniques may be required for the patient to have a child. With the use of IVF and ICSI, only a single sperm is required for fertilization of an egg. Similarly, cryopreservation techniques are now able to preserve few or even solitary sperm [105], making true azoospermia a critical distinction.

Motility

After concentration, motility is the most useful characteristic for predicting fertility. While 2010 WHO guidelines set the normal level of motility at greater than 40%, other studies have associated subfertility with motility of less than 32% with the majority of infertile men having sperm motility of greater than 42% [103]. In men studied after varicocele ligation, only improvement to motility was predictive of pregnancy [106]. Total motile count (TMC) can be determined using sperm motility along with concentration and volume. Only TMC was associated with spontaneous pregnancy in a study of patients presenting for infertility without known female factor. Patients with TMC less than five million had a significantly lower spontaneous pregnancy rate than those with TMC of five to ten million [107]. Even with the use of intrauterine insemination (IUI), TMC of less than ten million are associated with significantly lower rates of pregnancy [108].

Morphology

Morphology definitions and the tools used to assess it have changed over time. The current 2010 WHO guidelines use the "strict" Kruger criteria for morphology. These parameters are more stringent and have a much lower cutoff for normal than previous criteria. The Kruger criteria were developed to predict IVF success in semen analyses that were otherwise normal. Since the initial development and validation, the value considered normal has been lowered to greater than 4% normal forms, as this level is predictive of fertility with ART such as IUI which most closely resembles natural conception [109]. A meta-analysis of IUI success predictors found that 11 of 16 papers used greater than 4% as the cutoff for normal morphology, which had low sensitivity for predicting which patients would conceive, but a high specificity for predicting failure of IUI [110].

Seminal pH

Seminal pH is primarily used for cases of low-volume azoospermia. Fluids from the glands contributing to semen volume have different pH values, with prostatic secretions being acidic and seminal vesical secretions being alkaline [111, 112]. If seminal pH is acidic, typically less than 6.8, there may be obstruction of seminal vesicle or ejaculatory duct, or failure of development such as in CBAVD.

Sexually Transmitted Infections

Sexually transmitted infections (STI) are detectable in semen and can cause changes to semen, testicles, epididymis, prostate, urethra, and external genitals with implications for male fertility [113]. Viruses detectable in semen include herpes simplex (HSV), human papillomavirus (HPV), human immunodeficiency virus (HIV), and Zika virus (ZV) [114–116]. Other infections with implications on male fertility found in semen include the bacteria *Chlamydia trachomatis, Neisseria gonorrhoeae*, and even protozoa such as *Trichomonas vaginalis* [117, 118]. Presence of these infections presents a risk of transmission and diminishes male fertility through multiple mechanisms including disruption of spermatogenesis, changes to sperm parameters, or obstruction of the seminal tract [119]. Prior to use of semen for ART, current guidelines from the American Society for Reproductive Medicine (ASRM) recommend screening for HIV, syphilis, hepatitis B and C, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis* [120]. However, routine testing of semen for STI can have a low yield with one study population of almost 9000 tests having only 0.223% tests positive for *Chlamydia trachomatis* or *Neisseria gonorrhoeae* [121]. Once any patient found to be positive are treated for their infections, controversy exists regarding the need for repeated testing [122]. We do not routinely test for other infections of the GU tract unless patients have significant symptoms or leukocytospermia.

Hormone Analysis

Tests of serum are a useful adjunct to semen analysis in evaluating the reproductive system. When discussing cryopreservation with patients, any hormonal abnormalities should be corrected prior to preservation to optimize the cryopreserved specimen. Thoughtful use of a few laboratory tests allows for assessment of the well-defined feedback systems controlling testosterone production and spermatogenesis. In conjunction with semen analysis and physical exam, clinicians can determine both the cause of issues and potential treatments. Initial serum testing includes total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol. If these are abnormal, testing of prolactin and thyroid-stimulating hormone (TSH) can be considered. Patients with severe oligospermia should receive genetic analysis for karyotype and Y chromosome microdeletions.

Testosterone is the most important lab in assessing the infertile male. Serum for testosterone analysis should be drawn early in the day, as there is a circadian driven diurnal variation in testosterone levels with greatest values in the morning, especially in men under 40 years [123, 124]. Normal serum testosterone is validated against morning values, so afternoon draws may give a falsely low value. Testosterone is critical for spermatogenesis, as sperm express receptors to testosterone throughout their maturation [125]. Spermatogenesis requires locally elevated intratesticular levels of testosterone that are much greater than serum, making endogenous production of testosterone a requirement [61].

LH is important for interpreting whether low testosterone is due to disorder of the testicle or hypothalamic-pituitary axis, and must therefore be ordered at the same time as testosterone. Patients with low testosterone as well as low or low normal LH should have evaluation of the pituitary with other laboratory tests (discussed later) and possibly imaging of the pituitary.

FSH can give hormonal insight into the state of spermatogenesis, just as testicular volume was important for gross estimation of spermatogenic activity. Sperm express FSH receptors throughout their production and negative feedback for FSH comes from inhibin B which is released late in spermatogenesis, providing effective regulation [126]. Therefore, if sperm do not progress to the late stages of development, inhibin B is not released and FSH rises without suppression [127]. In azoospermic patients, this allows for differentiation of obstructive azoospermia in which FSH is normal and non-obstructive azoospermia in which FSH is elevated [127, 128]. The vast majority of patients with obstructive azoospermia can be clinically differentiated from those with non-obstructive azoospermia without the use of diagnostic biopsy because 96% of men with obstructive azoospermia will have an FSH of less than 7.6 mIU/mL or testicular length greater than 4.6 cm [129]. This differentiation is important because sperm retrieval procedures are more complicated and have lower success rates in non-obstructive azoospermia vs. obstructive azoospermia. Patients hoping to cryopreserve specimens in this way need to be counseled about this.

Estradiol is produced in the adipose tissue through aromatization of testosterone, allowing it to be elevated in obese patients [130, 131]. As estradiol increases relative

to testosterone, spermatogenesis can be disrupted and produce abnormal semen analyses [132, 133]. Aromatase inhibitors can be used to correct this ratio and improve semen parameters, especially concentration [134, 135].

Disorders of the pituitary can disrupt spermatogenesis through dysregulated LH and FSH. Prolactin is the pituitary hormone most frequently implicated in male infertility as elevated prolactin levels will suppress GnRH, LH, and FSH release and subsequently decrease the secretion of testosterone. Elevated serum prolactin may be caused by prolactin-secreting tumors of the pituitary as well as a variety of medications [136]. Gonadotropin release can also be disrupted directly by a mass lesion. It should be noted that an individual patient's prolactin level can vary considerably and abnormal levels should therefore be confirmed with repeat testing.

Other Laboratory Evaluations

Patients with more significant seminal abnormalities require more intensive testing beyond hormonal analysis. This testing includes genetic testing for abnormal karyotype or AZF microdeletions, and cystic fibrosis testing for those with absence of the vas deferens. This genetic testing seeks conditions that may affect the patient's general medical care (Klinefelter's syndrome or cystic fibrosis), are prognostic for fertility outcomes (AZF microdeletions), or could be inherited by the offspring. This is very important when considering using a preserved specimen for reproduction. Genetic abnormalities are rare, occurring in about 7% of all patients presenting for infertility, but are clustered in those with azoospermia or semen concentrations less than one million sperm/mL. Only 4% of these genetic abnormalities occur in patients with >1 million sperm/mL, and only 1% occur in those with >5 million/mL. Thus, genetic testing is rarely indicated for those without severe oligospermia [137, 138]. In patients with known history of these disorders it should be expected that they may not have sperm to cryopreserve, and the inheritability of these disorders should be discussed prior to storage and usage.

Imaging

Imaging in the evaluation of the infertile male is limited to specific clinical scenarios. The most common imaging modalities used are ultrasound (US), magnetic resonance imaging (MRI), and computed tomography (CT).

US is the gold standard for evaluating the testicle. Testicular US is used to accurately measure size, evaluate vasculature, and screen any masses of the testicle. The resolution of the testicular parenchyma with US makes it the optimal modality for evaluating for testis masses. Any concerning testicular physical exam finding suggestive of a mass should prompt US evaluation.

Clinical sizing of the testicle can be performed on physical exam assisted by calipers or an orchidometer, or with an US. US measurements have the best correlation

with actual testicular water displacement, though Prader orchidometers give a fairly accurate volume [139]. Measurements by Prader orchidometer and US are strongly correlated, though orchidometry can overestimate testicle size by as much as 5.5 cm^3. Normal values used should be specific to the method of testicular measurement [140].

The role of transrectal ultrasound in the infertile male is limited to imaging the seminal vesicles and prostate to evaluate for ejaculatory duct obstruction. Enlarged seminal vesicles with a large prostatic cyst suggest obstruction at the level of the prostate, while small or absent seminal vesicles are pathognomonic of CBAVD [141, 142].

Similarly, CT and MRI are rarely used for fertility evaluation in the male. MRI can be useful in evaluating for possible anatomic or developmental abnormality. This is especially true for conditions like Mullerian duct remnants, prostatic obstructions, non-palpable testis, or CBAVD, when US is non-diagnostic. MRI is also the gold standard for evaluating for masses of the pituitary after suggested by hormonal evaluation [143, 144].

Conclusion

The fertility evaluation is a critical component of preparation for sperm banking. Patients in the United States are delaying childbearing, with the birth rate declining for women under 30, and rising for those aged 30–44 [145]. As this delay increases, the risk of infertility and the cumulative risk of conditions or therapies that may harm fertility may also be expected to increase. The percentage of women aged 22–44 years who have ever used ART increased from 1.0% in 1995 to 5.2% in 2006–2010 [146]. Therefore, the value of fertility preservation techniques can be expected to grow. This makes familiarity with the evaluation and workup of male fertility essential, as this determines whether the patient will require reproductive assistance beyond preservation of ejaculated semen samples (Tables 12.1, 12.2, 12.3, and 12.4).

Table 12.1 Medications associated with male infertility

Medications causing infertility	
Mechanism of infertility	Medication
Cause erectile dysfunction (ED)	Tobacco, alcohol, cocaine, beta-blockers, tricyclic antidepressants, antipsychotics, spironolactone
Decrease libido	SSRI antidepressants, alcohol, beta-blockers, lithium, opiates
Disrupt hormonal balance	Exogenous testosterone, exogenous steroids, antidepressants, antipsychotics
Changes to semen parameters	Tobacco, alcohol, marijuana, exogenous testosterone, alkylating drugs, opioids, antiandrogens, ketoconazole, cimetidine, calcium channel blockers, alpha-blockers, antiepileptics (valproate, phenytoin, carbamazepine), highly active antiretroviral therapy (HAART)

Table 12.2 Pertinent physical exam findings in male fertility evaluation

Relevant physical exam findings in male infertility	
Area	Relevant abnormalities
Overall appearance	Poor virilization, scrotal/inguinal or laparoscopic scars
Penis	Size, meatus stenotic or non-orthotopic, phimosis, curvature
Testes	Small, soft, dense, irregular
Cord	Tender, masses, absent cremaster reflex, varicocele
Epididymis	Tender, masses, unilateral or bilateral absence of epididymal segments
Vas deferens	Diminutive, gaps, sperm granuloma
Prostate	Tenderness, midline cyst, dilated seminal vesicles

Table 12.3 Sexually transmitted pathogens detectable in semen which may affect the health of the male, female, or offspring

Sexually transmitted pathogens detectable in semen	
Family	Pathogen
Viruses	Human immunodeficiency virus (HIV), herpes simplex, Zika virus, human papillomavirus (HPV), hepatitis B, hepatitis C
Bacteria	*Chlamydia trachomatis, Neisseria gonorrhoeae*, Ureaplasma spp., mycoplasma spp., *Treponema pallidum*
Protozoa	*Trichomonas vaginalis*

Table 12.4 Common diagnoses and their associated hormone findings

Hormone analysis				
Condition	LH	FSH	Testosterone	Estradiol
Klinefelter's syndrome (hypergonadotropic hypogonadism)	High	Very high	Low	High
Hypogonadotropic hypogonadism (Kallmann syndrome, acquired hypopituitarism)	Very low	Very low	Very low	Very low
Primary testicular failure	High	High	Low	Low
Exogenous testosterone	Very low or undetectable	Very low or undetectable	High or normal	High or normal
Late-onset hypogonadism	Low or normal	Low or normal	Low	Normal or relatively high
Obesity (aromatization)	Normal	Normal	Low or normal	High

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Chapter 13
Developing a Sperm Banking Consent Process

Lisa Campo-Engelstein and Nanette Elster

Introduction

In the early 1970s commercial sperm banks began to emerge, and men were able to preserve their sperm for future use [1]. As sperm banking continued to evolve so too did the reasons to why a man would choose to store or cryopreserve his sperm. Some reasons, however, might include men undergoing cancer treatment whose fertility may be impacted, men who are going off to war, individuals who are born male but are transgender and undergoing gender affirming surgery, and even men who have concerns about the impact of aging on their future children. To add to the complexity, cryopreservation might also be an option for children and adolescents who are undergoing cancer treatment that might impact future fertility. Because of the varied reasons for preserving sperm, developing an informed consent process that meets the diverse needs and circumstances of those making the decision to cryopreserve sperm is necessary.

In this chapter, we focus exclusively on informed consent in sperm banking only with respect to sperm freezing for autologous use. We consider the necessary legal and ethical elements and individuals to be included in this process. We begin by considering the legal and ethical underpinnings of informed consent. We then explore the elements of consent that are necessary for adults preserving sperm followed by a discussion of the unique components of informed consent when a minor's sperm is being cryopreserved. The chapter concludes with a list of recommended elements to consider in developing an informed consent process for sperm banking for autologous use.

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Background

Informed consent is commonly defined as "an individual's autonomous authorization of a medical intervention or of participation in research" [2]. The Joint Commission provides an even more detailed definition: "Agreement or permission accompanied by full notice about the … service that is the subject of the consent. A patient must be apprised of the nature, risks, and alternatives of a medical procedure or treatment before the physician or other health care professional begins any such course. After receiving this information, the patient then either consents to or refuses such a procedure or treatment" [3].

The process of informed consent has ethical and legal considerations. In both law and ethics, informed consent is much more than a document that must be signed, but rather it is a process. The President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research defines the process "rooted in the fundamental recognition—reflected in the legal presumption of competency—that adults are entitled to, accept or reject health care interventions on the basis of their own personal values and in furtherance of their own personal goals" [4]. In fact, the concept of informed consent has a lengthy history, a discussion of which goes well beyond the scope of this chapter.

Dating back to the time of Hippocrates, however, informed consent was not the cornerstone of medicine and healthcare that it is today. "Classic documents in the history of medicine such as the Hippocratic writings (fifth to fourth century B.C.) and Thomas Percival's Medical Ethics (1803) present an extremely disappointing history from the perspective of the right to give informed consent. The central concern in these writings was how to avoid making disclosures that might harm or upset patients. Physician ethics was traditionally a nondisclosure ethics with virtually no appreciation of a patient's right to consent" [2].

The legal precedent can be traced back to the 1914 case, *Schloendorff v. Society of New York Hospital* [5]. In that case, the court held that "Every human being of adult years and sound mind has a right to determine what shall be done with his own body" [3]. Case law and statutory law continued to enforce the need to provide patients with information prior to biomedical intervention. To date, every state has some statutory law and/or case law regarding informed consent. Such laws may define the age of consent [6]; who may consent for whom [7]; penalties for failure to obtain informed consent [8]; and more. The variability between and among states is tremendous and thus necessitates a consideration of jurisdictional issues when developing an informed consent process. In addition to considering state-specific laws and regulations, consideration of any relevant professional society guidelines is also necessary.

The legal mandates regarding informed consent have distilled the process down to "a legally or institutionally effective approval given by a patient," [2] or essentially a signed form. The form, however, should only serve to memorialize a more interactive and ongoing process. This chapter focuses on the concept of informed consent as an ongoing process rather than the signing of a generic form.

The ethics of informed consent help to flesh out the elements that are necessary for the process to accomplish the goal “to protect and enable meaningful choice” [6]. The key underlying ethical principle at play in informed consent is respect for persons. According to the Belmont Report, respect for persons consists of two components: “first, that individuals should be treated as autonomous agents, and second, that persons with diminished autonomy are entitled to protection” [9]. The principle of respect for persons distinguishes between individuals who have decision-making capacity and those who do not. Individuals with decision-making capacity have the right to autonomy, which includes the right to make their own medical decisions. However, in order to be able to make good and informed decisions, patients need the relevant information. Especially because most patients are not healthcare professionals, it is important for healthcare professionals to provide the relevant medical information in a way that is easy for patients to understand. Furthermore, as previously mentioned, providing this information is not a one-time event. Rather, it is a continuous conversation as time goes by and as new circumstances arise. It also should be thought of as a dialogue with a back-and-forth exchange of questions and answers and an attempt to understand the goals and values of the patient.

Individuals without decision-making capacity (e.g., children, adults with developmental disabilities, individuals who may have a temporary loss of capacity due to illness or injury, and individuals who are not conscious) do not have autonomy rights because they do not have the cognitive ability to make reasoned and rational medical decisions for themselves. In order to respect them as persons, they need protection, which basically translates to having a proxy make decisions for them that uphold their best interests; such a proxy is often referred to as a surrogate decision maker. This will be discussed further below.

Cryopreservation of sperm raises some unique challenges especially with respect to disposition or future use of the previously collected reproductive material. The issues will certainly vary with regard to the age of the participant. In any event, certain factors will need to be discussed and understood including the cost, who may or may not have access to the sperm, and how the sperm can or cannot be used and by whom in the event of the death or future incapacity of the participant. In the following sections, we discuss each of these topics with respect to both adults and minors. The chapter concludes with recommendations of the elements that are necessary for a robust, ethical informed consent process.

Information

One of the essential components of informed consent is the provision of a clear and understandable description of risks, benefits, and alternatives. In the context of consent for sperm banking, certain basic information must be provided to all clients: the actual process, including detailed information about the solution used in preserving the sperm and the mechanism for storage; the cost, including annual storage fees; and duration—the period of time for which sperm will be stored, what happens if

the sperm bank goes out of business, is sold to another entity, suffers a power outage, and/or sperm is lost or inadvertently destroyed. In addition to such general information, the informed consent process may also need to be tailored to the age, marital status, and impetus for cryopreservation.

The Process

Discussion of the process of sperm freezing will involve discussion of such basic information as the culture medium used; the type of equipment used; the temperature at which the sperm is stored; risks, if any, to the quality of sperm as a result of the freezing; and what sort of emergency backup if any exists in the event of an equipment failure, power outage, etc. This may be very important given that in 2013, one storage facility faced 40 lawsuits when a cryopreservation tank malfunctioned resulting in the damage or destruction of sperm [10].

The Cost

Cost is another factor that is integral to the informed consent process. For many, insurance will not be available to cover the cost of storage. The consent process should detail whether fees are paid all at once, annually, monthly, etc. as this may be important information to be factored in by those considering banking their sperm. If the sperm bank has options for pre-payment for extended periods of storage, this too should be detailed in the consent process.

The Duration

Those opting for sperm banking will also need to have information about how long their sperm can be maintained. For example, patients may want to know if any data exists regarding the length of time sperm can be frozen before it is no longer viable and whether the facility in which they are storing sperm has a limit on the amount of time the sperm may be frozen. In some countries outside the USA, for example, legislation may exist limiting the period of time that gametes may be stored [11]. Currently no such law exists in the USA; however, this may be a consideration for both the men preserving his sperm and the cryobank.

Included in the information provided about duration would also be identification of any policies that exist that could result in the inability or unwillingness of the storage facility to maintain the sample. For example, identifying those circumstances under which storage may be discontinued by the facility should be communicated to the man. An explanation of how, when, and if notice will be given prior to transfer or destruction of sperm samples is also necessary.

Special Considerations

In addition to general information about the procedural aspects of sperm donation, the informed consent process must also consider the individual who is storing the sperm. The information will differ if an adult is making the decision for himself or if a parent[1] is deciding for a child.

Considerations for Adults

Reason for Accessing

In developing the informed consent process for cryopreservation of sperm, understanding the patient's reasons for pursuing fertility preservation can improve the informed consent process. Is the man undergoing cancer treatment that may impact future fertility? Is he going to be engaged in combat and concerned that he may be injured? Is the individual transgender and undergoing gender-affirming surgery but wants to preserve the ability to have a genetic child?[2] Each of these reasons may require provision of different information to the patient. Understanding a patient's motivation is necessary to attempt to inform him in a way that respects his goals and values. Respect for the individual's goals and values is a cornerstone of any informed consent process.

Marital Status

Marital status can sometimes factor into individual seeking sperm banking because some healthcare providers refuse in/fertility treatment for unmarried individuals [12]. Additionally, marital status may be a factor in considering disposition of sperm in the future if the patient either loses capacity or dies. For example, if the married patient dies without having documented his wishes for disposition of any remaining sperm as will be discussed below, his spouse may thus have a claim to his sperm.

On the policy side, many insurance companies and state laws regarding insurance coverage of infertility treatment exclude single individuals from coverage for infertility services [13]. Yet, limiting fertility preservation services, such as sperm banking, to married couples ignores the reasons people choose to undergo fertility preservation. Men undergo sperm banking because they want to keep open the pos-

[1] The term "parent" will be used throughout the chapter but may also include a legal guardian.

[2] We use male pronouns in this chapter since most people freezing sperm identify as men. However, as we note here, we recognize that some transgender women may be interested in sperm banking, especially prior to certain gender-affirming surgeries.

sibility of biological children in the future. As Campo-Engelstein argues, "Denying patients fertility preservation because they are unmarried at the time they seek treatment fails to recognize the future-oriented nature of fertility preservation treatment and that patients' marital status may change by the time they decide to use their reproductive material" [14]. As one's goals toward marriage do not necessarily equate with one's goals for parenting, marital status should not be a barrier to cryopreservation. This is especially true given that "the option to use fertility preservation therapies can have positive psychologic effects on cancer survivors" [15]. And, those undergoing fertility preservation in advance of gender-affirming surgery may, in fact, be preserving sperm with a future goal toward marriage and family building, but not yet be married. "This banked spermatozoa can then possibly be used later to inseminate a female partner if the quality is good, or else be used to perform IVF" [16].

Disposition

If a man dies or loses capacity, there are a variety of ways to handle his sperm: it can be destroyed, donated for scientific research, donated for use by an infertile individual or couple, or used to create a child that the man's family or loved ones will raise. The best way to avoid any conflicts regarding disposition of frozen sperm if the sperm provider dies or becomes incapacitated is for the individual freezing sperm to explicitly document his wishes and update them if his circumstances change. In fact, having men annually update their dispositional choices is a helpful way to avoid future conflict. By clearly documenting what he wants and does not want, there is less possibility for disagreement and conflict should he become unable to make his wishes known due to incapacity or death. Most fertility clinics require individuals creating embryos via IVF to complete a document outlining how to handle extra embryos if the couple who created the embryos separate or one individual dies. According to a survey published in 2003, "In 338 of the 340 responding practices, the patient or couple must sign a consent form before their embryos are frozen" [17]. Additionally, the American Association of Tissue Banks also addresses this in its *Standards for Tissue Banking* updated in 2011: "In the case of a Client Depositor the Informed Consent Record shall also include details about costs of tissue cryopreservation, storage, *distribution and disposition options*" [18] (emphasis added) indicating that the same practice should be employed for individuals freezing gametes. This practice should in fact be easier for individuals freezing gametes because the gametes only contain one person's reproductive material whereas embryos contain reproductive material of two people and therefore two people need to agree on all the stipulations of the directive at all times. Additionally, embryos have a different status in the law than gametes, having a heightened "special status," somewhere between person and property [19].

Only the individual freezing his sperm should determine how he would like his sperm to be treated if he is no longer able to make decisions or if he dies. Even if he is married or partnered, his spouse or partner should not be the one making decisions

since the sperm contains only his genetic material. By insuring that all individuals who freeze gametes document their wishes, uncertainties and conflicts regarding disposition could be completely avoided. Additionally, recommending that dispositional options be mirrored in a will or other estate planning document is further protection against future conflicts. In one of the earliest cases addressing the posthumous disposition of cryopreserved sperm, *Hecht v. Superior Court,* the California court resolved a dispute between the decedent's adult children and the decedent's girlfriend over previously frozen sperm. Mr. Kane executed a will wherein he specifically bequeathed his frozen sperm to his girlfriend. After his suicide, his adult child objected to the girlfriend, Ms. Hecht, having access to the sperm and sought to have the sperm destroyed; however, given that Mr. Kane's intent was clearly articulated, the girlfriend was given access to the sperm [20].

Unfortunately, some individuals do not document their wishes regarding their frozen sperm, either in forms provided by the sperm bank or fertility center or in an estate planning document which can lead to confusion and conflict. Family members may be unsure of how to handle his sperm. Furthermore, there can be disagreements about how to handle the sperm and this can be especially problematic if certain individuals want to use the sperm for procreative purposes and others do not. Some believe, however, that the proxy should be the one to decide how sperm should be handled if an individual has not made his wishes clear beforehand. If the individual is married or partnered, then the spouse or partner typically serves as the proxy and if the individual is not married or partnered, then the individual's parents often serve as the proxy. State law, however, may indicate who makes decisions for a decedent and often this will not include a partner.

Ideally, the proxy is someone who knows the individual well and can make decisions based on the substituted judgment standard, which means using the values of the individual to make decisions. There may be a real or perceived conflict of interest for the proxy if that individual wants to use the frozen sperm to create a child and the proxy either does not know or does not think the man would not have condoned posthumous fatherhood. The American Society for Reproduction in a 2013 Ethics Opinion indicated that "After death, where there is evidence that the deceased would still have wanted reproduction to occur, or at least would not have objected, it seems reasonable to allow the survivor to proceed" [21]. If the survivor is not a spouse or partner the decision may be more complex and thus clear directives on who may have access to the sperm, under what circumstances, and for what purposes should ideally be documented at the time the sperm is frozen.

Even if a proxy consents to the use of frozen sperm, some bioethicists object it if there is no documentation that the man agreed to its use. The main reason for this opposition is that it violates the man's autonomy. Even if he is dead or incapacitated, we still have an ethical obligation to uphold his wishes and values. That he froze his sperm shows that he was considering biologically reproducing in the future. However, freezing sperm is generally done so that he can use them himself in the future. The mere existence of frozen sperm does not indicate that he would have agreed to posthumous reproduction. Some individuals who would be thrilled to use

their frozen sperm themselves may vehemently reject posthumous reproduction for personal, religious, or cultural reasons.

Another reason some bioethicists oppose using frozen sperm without a man's previous consent is it seems to use him as a means to an end. In other words, using a man's sperm to create a child without his explicit consent can be seen not only as violating his autonomy and dignity, but also as reducing him to an instrument to achieve another's goal. The man, whether he is dead or incapacitated, does not directly benefit from the use of his sperm. In contrast, the individual using his sperm to create a child directly benefits and that individual's desire for a child may be seen as superseding the man's interests.

Donation to research is another dispositional option for a man if no longer desires or is no longer able to utilize his sperm. In this instance, as with posthumous reproduction, the man's wishes should be made clear at the time that he is freezing his sperm. For many of the reasons discussed above, providing remaining sperm for research raises ethical concerns. This is yet another dispositional option to which the sperm provider should explicitly consent at the time of storage. Becoming aware of any federal, state, or local law or regulation that may require specific consent for research is integral to a sound informed consent process.

Considerations for Minors

Decision Maker

As previously discussed, minors are generally seen as lacking decision-making capacity and therefore require a proxy decision maker. For adults who once had capacity and have temporarily or permanently lost it, proxy decision makers use the substituted judgment standard, which means making a decision based upon the incapacitated adult's values. The best means to assess those values is through explicit documentation. Minors usually have not had the time to develop a robust value system, so proxy decision makers cannot typically rely upon the substituted judgment standard for them. Instead, proxy decision makers for minors are supposed to rely on the best interest standard, which means making a decision for the minor based on what is perceived to be in the best interests of the minor rather than based on the proxy's or someone else's values. The best interest standard, however, is not without its problems, including that reasonable people can disagree about what is in the best interest of the minor [22]. Some have raised concerns that fertility preservation for minors may not be motivated by their best interest, but by the interests of their proxy (e.g., the parents' desire for genetic grandchildren) [23]. Even when fertility preservation is offered for the best interest of the minor, the minor may feel pressure from parents and healthcare providers to undergo treatment [24]. Because a minor may still be able to express his own view, however, seeking his input through assent is an important consideration. Additionally, because parents

themselves may not be in agreement, involvement of a neutral third party such as an ethicist or a mental health professional may be necessary to ensure the voluntariness and understanding of all participants.

Although young children may not be able to consent to treatment they can still assent. Assent is similar to consent in that it is an interactive and ongoing process that assesses a child's willingness and preferences for treatment [25]. Assent differs from informed consent by requiring a lower level of decision-making capacity instead of the deeper level of understanding and reasoning needed to meet the informed consent standard [26]. The American Academy of Pediatrics asserts that children and adolescents should be involved in a process of assent to treatment "to the extent of their capacity" [25]. Furthermore, there is consensus in the medical decisional capacity literature that all minors should be involved in conversations and decisions regarding their healthcare at a level appropriate to their understanding and maturity [27]. Moreover, as treatments move from more objective (e.g., appendectomy for an appendicitis) to more subjective (e.g., sperm banking before cancer treatment), there should be greater involvement of minors because the decision to pursue more subjective care rests more heavily on personal values [28].

Although mature minors who have reached puberty are producing mature sperm, some social barriers to securing a semen sample for preservation may be present. Adolescent boys may not feel comfortable discussing sexuality and masturbation with their parents and healthcare providers. Furthermore, it may be physically or psychologically challenging for them to provide a semen sample via masturbation due to limited sexual activity and knowledge [29]. Parents may also experience discomfort talking about sexuality and some parents may object to their sons masturbating for cultural or religious reasons, especially if pornographic material is used. For those unable or unwilling to produce a semen sample via masturbation, alternatives do exist: electroejaculation, testicular sperm extraction, or microscopic epididymal sperm aspiration [30]. These alternatives, however, are more invasive and carry greater medical risk than masturbation. Additionally, they can be more difficult psychologically, especially for adolescent boys.

The physical and psychological challenges that may arise in providing a semen sample may lead adolescent boys to initially refuse fertility preservation. Furthermore, their limited decision-making capacity and emotional immaturity may prevent them from recognizing the long-term benefits of preserving their fertility. Some clinicians immediately cease discussing sperm banking when an adolescent patient initially declines. Yet, Shnorhavorian et al. argue that "the fertility preservation conversation be a priority at the time of diagnosis, when feasible, for males aged 12 or older, and that there are ethically important reasons that a 'No' should be the beginning of the conversation" [31]. Because respect for persons requires protecting individuals without full capacity, it is important for parents and clinicians take both a short-term and long-term view of a given situation, especially since it is often harder for minors to recognize the long-term consequences of their decisions. Rather than acquiescing to a minor's initial refusal of fertility preservation, parents and clinicians should seek to understand why the minor is refusing and to highlight the long-term benefits of fertility preservation. This may be particularly important

in light of recent research on adult survivors of cancer which found: "While the importance of fertility preservation was generally not instinctively recognized by survivors or parents at the time of cancer diagnosis, retrospective consideration revealed that the issue of fertility frequently emerged as a significant concern later in life" [32].

Disposition

There is a consensus in the literature that reproductive material of minors should be destroyed or donated for scientific research if the minors pass away. According to Fallat and Hutter, citing to the European Society for Human Reproduction and Embryology (ESHR), "If the child dies, the parents should not have discretion over the biological material, and it should be destroyed" [33]. Additionally, Rosoff and Kastur recommend that "children and their parents should sign a consent form declaring that they will destroy the materials if the child dies before reaching his or her majority" [34]. Given our understanding of sperm as "belonging" to the individual whose genetic material it contains, frozen sperm of minors technically belongs to them. However, because they lack decision-making capacity and other legal rights due to their age, their parents or guardians are responsible for securing their future right to their sperm. By protecting the sperm, the parents are ensuring that the child can autonomously make his own decisions when he reaches adulthood. That is, he will have an open future thanks to his ability to use his frozen sperm. If the parents were to use or destroy the minor's frozen sperm while he was alive, that would be a clear violation of his autonomy. Once the minor reaches legal adulthood, his parents should relinquish all rights to his sperm. If the minor dies before reaching adulthood, the parent should destroy or donate his sperm to scientific research [14].

As discussed above, however, parents may be conflicted over what is deemed in the best interest of the child and their own interest in possibly having a legacy/genetic link to their child by using his sperm to create a grandchild. If a real or perceived conflict arises, then involving an ethics consultant or mental health professional may be necessary as discussed above. In any event, as part of the consent process, parent should be advised and informed of any policies regarding future use of a minor's sperm, including whether the sperm will be destroyed if the minor dies prior to reaching adulthood.

Conclusion

Given the complexity and range of issues raised by cryopreservation of sperm, having one blanket consent process would not be appropriate. The process will differ for adults and minors and it may differ based on the circumstances which have

necessitated sperm storage as well as based upon who is making decisions at the time of storage as well as at the time of disposition.

Informed consent for sperm banking should be an ongoing and evolving process involving dialogue and discussion that goes beyond a signature on a formulaic document. To memorialize this process, however, a form is necessary. While documentation of consent may differ depending upon federal, state, local, and institutional requirements, the following items are among the many elements to consider for inclusion:

- The process for cryopreservation
  - How?
  - Where?
  - Emergency backup?
- The cost
  - When are fees due?
  - How frequently is payment made?
  - Do any long-term pay in full options exist?
- Duration
  - How long is sperm viable?
  - For how long will it be stored?
  - Circumstances under which agreement may be terminated and by whom
  - Procedure if sperm bank is sold or ceases to operate
- Reason for freezing
  - Undergoing medical treatment
  - Going into combat
  - Gender affirming surgery
  - Other
- Status of the participant
  - Marital status
  - Age
  - Capacity to consent and appointment of proxy if capacity is diminished
- Dispositional options (may vary depending upon status)
  - Disposal
  - Donation to others for reproductive purposes
  - Donation to research
  - Use by spouse or partner
  - Do any estate planning documents exist mirroring these options?
- Reconsent
  - Annually?

– At the time of reaching majority if sperm belongs to a minor?
– Prior to a final disposition?
– If new dispositional options become available?

The list of elements to consider is in no way inclusive; however, it addresses some of the most salient considerations in preparing a document to memorialize the consent process for sperm banking. "A number of myths about what the law requires impede the practice of obtaining informed consent. If informed consent is viewed as a process of shared decision making, some of the seeming absurdities and excesses that can be associated with it disappear" [35]. Therefore, considering informed consent as an evolving process memorialized by a document is necessary. As technology changes, fertility options expand, medicine advances, and laws change so too will the consent process.

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Chapter 14
The Process of Sperm Cryopreservation, Thawing and Washing Techniques

Sajal Gupta, Rakesh Sharma, and Ashok Agarwal

Introduction

Reproduction is a key need which is central to human experience and drives quality of life. There is a growing list of conditions wherein there is a detrimental impact on fertility as a result of the disease itself or the therapeutic interventions directed towards the treatment of the disease conditions such as cancer or other extreme medical conditions. Sperm cryopreservation is used for these men, as they may desire to have children in the future, but current circumstances prevent the certainty that they will be able to conceive through traditional methods. This can be because of dangerous military deployment overseas, cancer or other toxic treatments which cause infertility, and many other situations.

Sperm cryopreservation procedure involves the collection of the sample, freezing and long-term change storing to storage of the sample in extremely low temperature under liquid nitrogen at −196° C for possible use in the future to help achieve parenthood. All men who undergo treatments detrimental to reproductive function or face the challenge of their fertility being impacted by chemotherapy or radiation therapy should exercise the option of semen collection for cryopreservation [1]. Cryopreservation of human semen is an important procedure for preservation of gametes in patients undergoing gonadotoxic treatment. Even some fertile couples can experience difficulties in conceiving due to geographical or professional challenges and a general deterioration of male reproductive health over time is also an important indication for sperm banking. In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have radically advanced the treatment of male-factor infertility. As a result sperm cryopreservation has become a successful treatment option [2]. ICSI requires a few

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viable sperm for successful fertilization of the oocytes selected based upon their competence [3].

Indications for Sperm Cryopreservation

There are two groups of users for the sperm banking services: donor and directed client depositors [1]. Donors provide samples which can be used by other couples wherein the male partner has refractory male infertility and also could be utilized for same-sex lesbian partners. Directed client depositors freeze their samples exclusively for future use by their sexually intimate partner.

Cancer Patients

Every year more than 1.3 million patients are diagnosed with cancer in the United States. Over 44% of patients referred for sperm banking are cancer patients [1]. Patients receiving gonadotoxic treatment are at high risk for developing infertility [4]. Men with systemic (nonreproductive) cancers have relatively normal semen parameters whereas men with testicular cancer [5–7] and lymphoma [8–10] have poor sperm parameters. Potential chromosomal aberrations in sperm exposed to chemotherapy are compelling reasons for sperm banking before treatment [11]. Early sperm banking is recommended as 77.8% of patients are reported to be azoospermic after receiving cancer treatment [12]. Majority of the patients diagnosed with testicular cancer are in the reproductive age who can benefit from sperm banking [13, 14].

Travelling Husbands

Geographical challenges where one of the partners is absent due to travel is an indication for sperm banking as timed intercourse with ovulation is not possible. Cryopreservation of sperm allows the female partner to utilize cryopreserved sperm when she is ovulating [1].

Pre-vasectomy

Men utilizing bilateral vasectomy as a contraceptive measures can elect to store sperm prior to the procedure. This helps keep their options open in the future if they go through some life-changing circumstances or demographic changes.

Prior to ART Procedure

About 12% of couples are infertile and 30–40% of these couples cannot conceive because the male partner has infertility issues or is azoospermic [15]. In such severe cases the couples may decide to use cryopreserved donor sperm.

Extreme Medical Conditions

Management of medical conditions such as autoimmune disorders, kidney disorders, ulcerative colitis or heart transplant and diabetes involves use of cytotoxic and immunosuppressive drugs. These drugs have the potential to suppress spermatogenesis [16].

Adolescents

One in 1300 US males is a childhood cancer survivor and these adolescent cancer patients need to be offered fertility preservation services. The adolescent can collect a sample if puberty has set in. However, in adolescents who have not achieved puberty, there are limited experimental strategies such as spermatogonial stem cells autotransplantation [17]. The American Society for Clinical Oncologists also recommends discussing fertility preservation options with adolescents.

Emergency Fertility Preservation

Emergency fertility preservation is defined as urgent need for sperm cryopreservation in emergency situations such as scrotal trauma, either unilateral or bilateral, and testicular torsion. Scrotal exploration and emergency TESE is an option for these cases [18].

Posthumous Sperm Retrieval and Freezing

Viable sperm can be obtained through posthumous sperm retrieval and freezing. There are reports of viable cadaver sperm obtained up to 36 h post-mortem. Fertilization rates ranging from 40 to 100% and singleton pregnancies have been reported with the use of posthumous sperm [19]. However use of posthumous sperm remains controversial due to ethical and legal concerns [20, 21].

Techniques of Sperm Cryopreservation

Different cryoprotectants and cryopreservation methods have been used to preserve the viability of the sperm [22]. It is important to use a suitable cryoprotective agent to protect sperm cells during the freezing process and obtain good-quality sperm after thaw. All cryopreservation techniques have some detrimental effects on the overall sperm quality [23].

Slow Freezing

Cryoprotectants function by permeating cells and lowering the concentration of electrolytes. Sperm cells are exposed to less osmotic strain with slow cryopreservation. Although glycerol and egg yolk have been used, egg yolk provides superior post-thaw sperm quality compared with glycerol alone [24]. TES [N-Tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid, pK = 7.5] and Tris [(hydroxymethyl) amino methane] combined with fresh egg yolk, dextrose, and penicillin-streptomycin comprise the cryobuffer known as TEST-yolk buffer (TYB). It is the preferred cryoprotectant with higher longevity [23, 25]. TYB stabilizes sperm by altering phospholipid:cholesterol ratio and reducing damage by reactive oxygen species resulting in improved recovery of higher percentage of motile sperm, increased capacitation and sperm penetration [25].

The manual slow freezing method is used at our institution. It takes about 2–4 h to complete. It involves the gradual cooling of sample from room temperature to –20 °C and the temperature is further lowered to –80 °C at the rate of 1–10 °C/min before plunging into liquid nitrogen at –196 °C. The patient/client depositor collects semen sample in a sterile collection cup by masturbation. The container is labelled with two identifiers. The sample is incubated at 37 °C for 20–30 min (Fig. 14.1). After liquefaction, the volume of the sample is measured and recorded. A note is made if any unusual viscosity, white blood cells or sperm agglutination is observed.

Within 1 h of specimen collection, an aliquot of freezing medium equal to 25% of the original specimen volume is added to the centrifuge tube with a sterile pipette. The specimen with the freezing media is gently mixed on a test tube rocker for 5 min (Fig. 14.2). The steps of addition of the freezing media and mixing of the specimen are repeated three times or until the volume of freezing media added is equal to the original specimen volume (Fig. 14.3). After addition of TYB, sperm motility is examined in the cryodiluted sample using a fixed cell chamber and phase microscope.

The percent motility is recorded as "pre-cryo motility %." Using a sterile serological pipette the well-mixed, cryodiluted semen is equally aliquoted into prelabelled vials (Fig. 14.4). Exposure to freezing conditions should occur within 1.5 h of specimen collection. Two cryovials are loaded into the cryocanes and placed

Fig. 14.1 Preparation of the sample for cryopreservation. The sample is placed in an incubator at 37 °C for complete liquefaction before conducting semen analysis [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

Fig. 14.2 Mixing of the specimen with the freezing media for 5 min on a test tube rocker [liquefy, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

upright in –20° C for 8 min. Labelled portion of cryocanes should be facing the ground while placing the cryovials upright, with the orange top facing (Fig. 14.5) before submersion in the vapour phase for 2 h (Fig. 14.6). Finally, the cryocanes are flipped and immersed in liquid nitrogen for short-term storage. Once the client has completed sperm banking the samples are transferred from short-term storage to

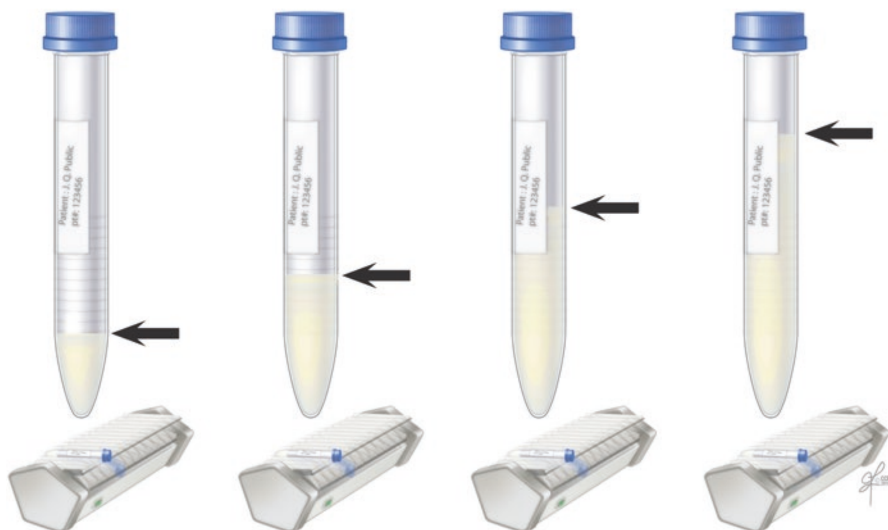

Fig. 14.3 Stepwise addition of Test-Yolk buffer to patient sample. Volume of Test-Yolk buffer equal to ¼ volume of patient sample—added four times, or until total volume in test tube has doubled [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

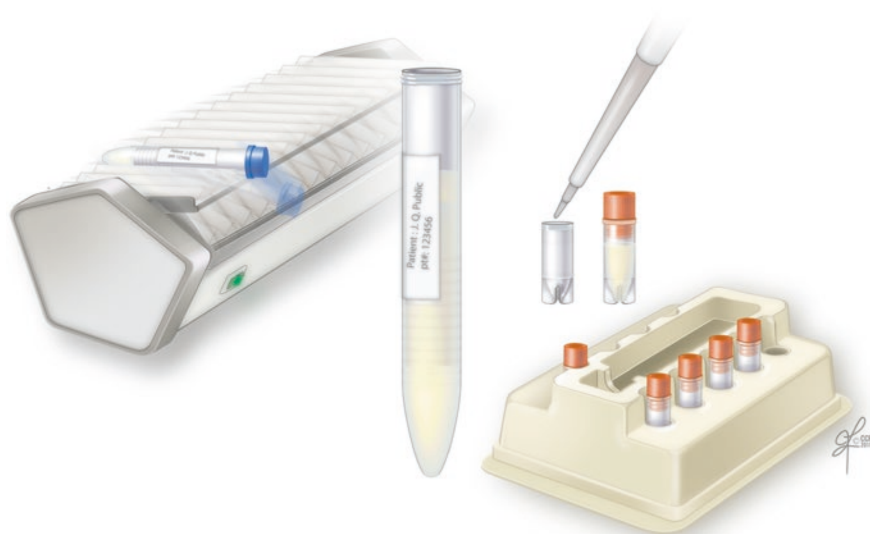

Fig. 14.4 Even distribution of cryodiluted patient sample into cryovials using a sterile serological pipette [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

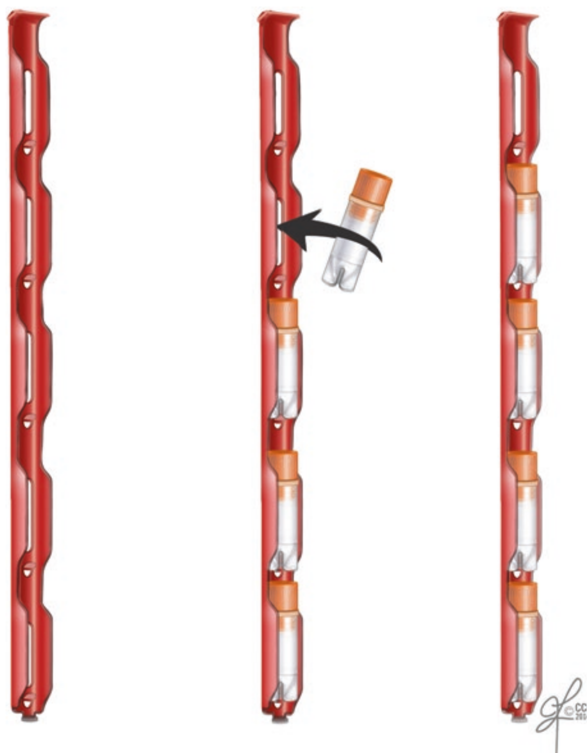

Fig. 14.5 Proper placement of cryovials into cryocanes before immersion in LN_2 vapours [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

long-term storage (Fig. 14.7). The major drawback of this technique is the formation of ice crystals if the cooling is too fast and cell shrinkage if the cooling is too slow.

Rapid Freezing

The aim of utilizing the rapid freezing technique is to minimize the toxicity caused by the cryoprotectant and to lessen the osmotic membrane damage by inhibiting the ice crystal formation [23]. This is achieved by bringing the samples in direct contact with nitrogen vapours at –80 °C after addition of the cryoprotectant. The entire volume of cryoprotectant is added drop by drop at the same time to result in a 1:1 freezing ratio. The mixture is then transferred to a cryovial with a maximum of 1.0 mL volume per vial. Static vapour exposure is performed with the vials placed 3 cm above the liquid nitrogen surface for a 30 min before being submerged in liquid nitrogen [26]. Controlled freezing cannot be achieved in either slow or rapid freezing. This can be overcome by using a programmable freezer where the desired temperature is achieved by a preloaded temperature set-up.

Fig. 14.6 Lowering of canes containing cryovials into LN_2 tank in vapour phase [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

Fig. 14.7 Transfer of samples from short-term to long-term storage [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

Sperm Vitrification

Sperm vitrification was described earlier in 1949 by Polge in Nature [27]. However, the vitrification protocols still require a lot of work and standardization. Several reports provide different approaches for standardization of the sperm vitrification procedure. The investigators have tried different devices (open or closed), different media concentrations media or exposure time [28]. The relative permeability of cryoprotectants determines the level of osmotic stress to which the spermatozoa are exposed. This is an important factor in sperm cryodamage.

Sperm are cooled at a very fast rate of –1000 °C per minute. The basic principle underlying the vitrification technique is to create high viscosity in the solution and produce a glass-like solidification without the formation of ice crystals. The technique has not been standardized as it is difficult to perform the cooling at such high rates and standardization of the high concentration of cryoprotectants has not been achieved. Cryoprotectant-free vitrification using sucrose is associated with better outcomes of post-thaw sperm motility, plasma membrane integrity and acrosome integrity [29].

The principle for vitrification is that small volumes of the cryopreserved specimen mixture are dropped directly into liquid nitrogen to achieve high rate of cooling [30]. Non-permeable cryoprotectants are used for vitrification. Small volume of sperm suspension is mixed with sperm wash media supplemented with 5% HSA and sucrose. The vial is placed at the bottom of a metal strainer and immersed into the liquid nitrogen. The sperm suspension is then dropped with a micropipette directly into liquid nitrogen. The vials with solid spheres in them are packed and placed in liquid nitrogen [15].

Home Sperm Banking

Home sperm banking is a novel option for men who choose to freeze their specimens but prefer to provide semen samples from the comfort of their home. Banking from home helps avoid emotional stress as well as other privacy-related issues. Patients who may be interested in this service include men who have cancer and are going through gonadotoxic treatments, military personnel before deployment and men undergoing infertility treatment. Semen specimens remain viable through a short transport cycle utilizing the specialized sperm collection and transport system called NextGen® [31, 32]. After receipt of the NextGen kit with the sample, cryopreservation is done as per the in-house protocol based on slow cryopreservation technique [31]. The kit retains adequate sperm viability during transit prior to long-term freezing in the tissue bank. Semen samples from infertile men with and without cancer were collected onsite and offsite and shipped using NextGen. Total motile sperm and motility were comparable and no significant differences were observed in the cryosurvival rates of semen samples collected offsite and transported via the NextGen [31].

Sperm Preparation for Cryopreservation

Many times the sample has to be processed before cryopreservation. This is necessary to eliminate the contaminating round cells, leukocytes, dead cells and debris and seminal plasma to obtain a good-quality sperm before freezing. Both abnormal sperm and leukocytes produce reactive oxygen species causing sperm damage and DNA fragmentation. In abnormal semen samples, preparation of sperm has been used to increase the percentage of motile, morphologically normal sperm. This resulted in increased success rates in reproductive treatment outcome in either IUI or ART [33].

Freezing significantly increases abnormally dead, immotile spermatozoa with low DNA integrity after freeze-thawing [34–39]. Preparation of post-thawed spermatozoa is performed to discard the cryoprotectant and select the best quality spermatozoa before using for ART. Sperm preparation before [38, 40–42] and after cryopreservation has been studied [43–45]. Significant improvement in the number of motile spermatozoa with reduced incidence of apoptosis was reported in sperm prepared before freezing [43–46] compared to after freezing [43–45]. Two common methods used to obtain a highly motile sperm fraction are the separation of sperm by a double-density gradient and swim-up technique. Each method has its own advantages and disadvantages [47].

Sperm Preparation by Double-Density Gradient

In this technique, sperm are separated based on their density. After centrifugation, the morphologically normal, highly motile sperm are collected from the pellet at the bottom of the tube. A colloidal suspension of silica particles stabilized with a covalently bonded hydrophilic silane supplied in HEPES medium is used to prepare the double-density gradient. To avoid the detrimental effects of reactive oxygen species produced by high centrifugation speeds, centrifugation speeds are kept no higher than 300 × g to which are detrimental to the sperm. In brief, after the sample has completely liquefied, a manual semen analysis is done for volume, concentration and motility. Lower phase or high density (90%) gradient and the upper phase or the low density (45%) gradient are used. Both gradients and sperm wash medium (modified HTF with 5.0 mg/mL human albumin) are brought to room temperature before loading the sample.

Using a 15 mL conical centrifuge tube, 2 mL of the lower phase is placed at the bottom and carefully layered with 2 mL of the upper phase. A well-mixed semen sample is placed on the upper phase and the sample is centrifuged for 20 min at 300 × g (Fig. 14.8). Seminal plasma, contaminating debris and leukocytes and the morphologically abnormal sperm, is carefully aspirated down to the pellet and discarded. The pellet along with some of the gradient is resuspended in 2 mL of

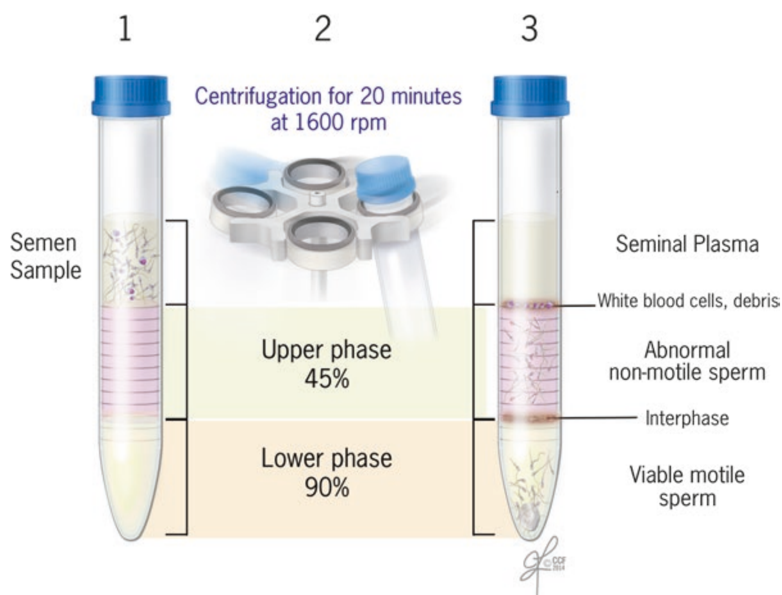

Fig. 14.8 Double-density gradient wash procedure; separation of seminal plasma, abnormal non-motile sperm and viable motile sperm [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

sperm washing medium and centrifuged for 7 min at 300 × g (Fig. 14.9). Following centrifugation, the pellet is finally resuspended in 0.5 mL of sperm wash medium and examined for concentration and motility before cryopreservation.

Sperm Preparation by the Swim-Up Method

This is another common technique for sperm preparation. Swim-up can be performed using either a pre-washed soft-spun pellet or liquefied semen sample. It is placed in an overlaying culture medium (sperm wash medium). Highly motile sperm swim to the top. In brief, the semen specimen is mixed with sperm wash medium (1:4 vol./vol.) and centrifuged for 10 min at 300 × g. After carefully aspirating the supernatant, the pellet is resuspended in 3 mL of the sperm wash medium. The sperm suspension is transferred into two sterile round-bottom tubes. After centrifuging the tubes for 5 min at 300 × g and kept at an angle of 45° for 1 h at 37 °C (Fig. 14.10). The angle and the use of round-bottom tube are important to increase the surface area and allow more motile sperm to swim to the surface. After incubation, the entire supernatant is carefully aspirated from the two tubes and pooled in a 15 mL conical centrifuge tube. Following another centrifugation, the clear supernatant is aspirated and the pellet resuspended in 0.5 mL of the sperm

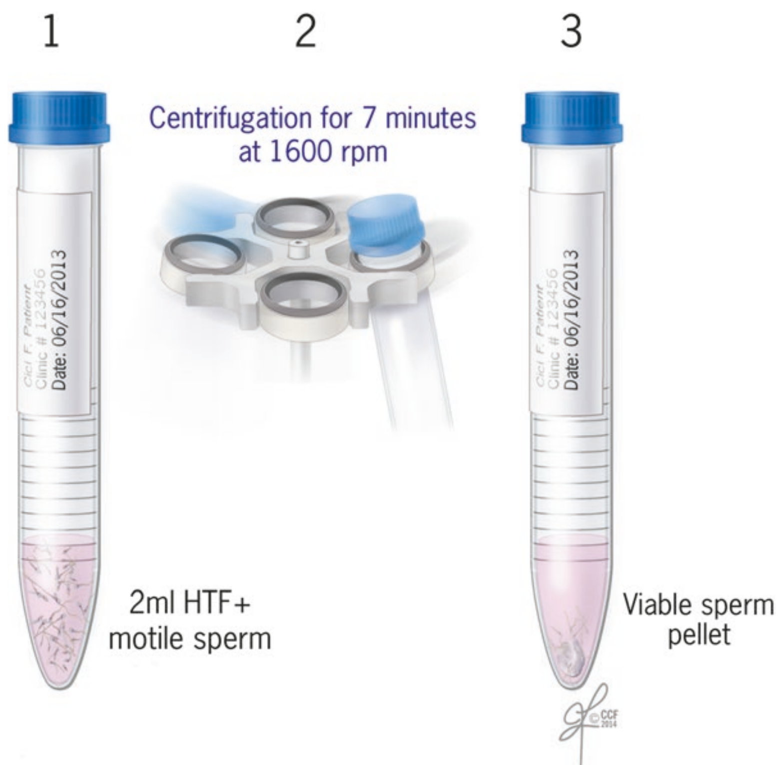

Fig. 14.9 HTF resuspended sample centrifuged to produce viable sperm pellet [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

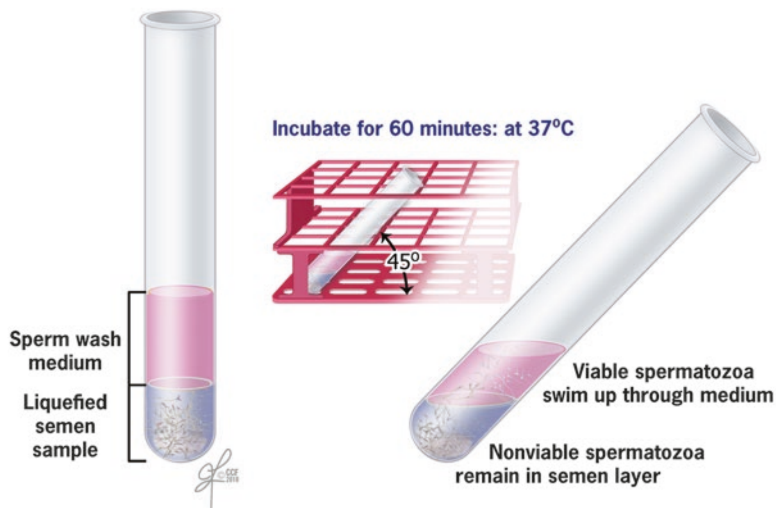

Fig. 14.10 Swim-up procedure showing the separation of highly motile spermatozoa [reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2017. All Rights Reserved]

wash medium. After the initial sperm concentration and motility the sample can be cryopreserved.

Effect of Cryopreservation on Sperm Characteristics

Cryopreservation damage or cryoinjury to the cells can be due to a combination of four factors: osmotic stress/dehydration, intracellular ice formation, cryoprotectant toxicity and oxidative stress [48, 49]. Glycerol in the cryoprotectant can result in cell toxicity. At very low temperatures, such toxic effects can be masked or diminished by slow metabolism of the cells. However at higher temperature such as at thawing, substantial sperm toxicity effects are evident when very high concentration is used. Some of the negative effects of sperm freezing and thawing are osmotic stress, extracellular and intracellular ice crystals resulting in cell injury, production of ROS which in turn has a negative effect on sperm motility, alteration in lipid phase, membrane integrity, mitochondrial function, DNA integrity, cell signalling and metabolism. In addition, sperm freezing can also result in apoptosis and necrotic cell death [50, 51]. Most of these negative effects occur during thawing stage. After thawing, these factors are responsible for a 25–75% loss of sperm motility, decrease in sperm cryosurvival and sperm DNA fragmentation [48, 49].

Motility is the single most affected parameter after cryopreservation [52, 53].The decline in motility is significant especially in patients who have poor sperm quality to begin with and is attributed largely to mitochondrial membrane damage resulting in a decrease in ATP production and poor sperm tail movement [43, 54, 55]. The differences in sperm membrane phospholipid, glycolipid and sterol content contribute to the poor motility [56]. Both osmotic and oxidative stresses have been shown to induce ROS in sperm after freezing and contribute to lethal or sublethal cellular damage [53, 57–59]. In addition, cryopreservation also results in DNA damage, especially DNA fragmentation that varies with the sample and is a result of oxidative stress-induced DNA damage [60–62]. Proteins play key roles in sperm metabolism, membrane permeability, flagella structure and motility, apoptosis, intracellular signalling, capacitation and fertilization that are altered after cryopreservation [63]. In addition, sperm proteome changes have been reported at every stage of the cryopreservation process. This ultimately impairs the fertilizing ability of the sperm, especially at the time of removing the sperm from storage and thawing at 23 °C [64].

Cancer Patients

Teratozoospermia is the most common (93.2%) abnormality among pre-treatment cancer patients [65]. An increase in chromosomal aneuploidy rate is seen in germ cells in men with testicular cancer and Hodgkin's disease even before cancer treatment [66]. Both in healthy individuals and testicular cancer patients, viability and

motility show a similar decrease after cryopreservation. A survival rate of only 44.8% with lowest odds of having total motile sperm count (TMC) above 5×10^6 was reported in men with testicular germ cell tumour (GCT) compared with controls and men with other cancers [67]. The lowest odds of successful intrauterine insemination was also seen in these patients. Non-seminoma germ cell tumour (NSGCT) is associated with higher post-thaw TMC than seminoma patients [68, 69]. Post-thaw TMC and cryosurvival have important clinical implications for couples who desire a pregnancy using ART. Sperm concentration of $>5–10 \times 10^6$ sperm is predictive of successful IUI [70, 71]. Patients with TGCT are recommended to freeze a minimum of 15 vials (about 1×10^6/vial) before beginning their oncological treatment. This ensures availability of adequate sperm for two IUI attempts and sufficient sperm for use with IVF if both attempts fail [67].

Although semen quality is comparable in repeated ejaculates from cancer patients [72]; conflicting results have been reported between cancer stage and semen parameters [67, 73, 74]. Optimizing the post-thaw TMC and cryosurvival is therefore important [75]. Sperm of poor quality can be used for intracytoplasmic sperm injection (ICSI) where the availability of a single motile sperm is the only male factor determining successful fertilization. Success rates of IVF and ICSI treatment using cryopreserved sperm are almost as high as fresh semen. In cancer patients, using cryothawed sperm, the pregnancy rate per cycle and per couple in the IUI group was 11 and 32% while it was 37 and 68% in the ICSI group. ICSI is the preferred method of treatment for achieving pregnancies using cryopreserved sperm in cancer patients [76–81].

Freezing Testicular and Epididymal Aspirates

Patients with non-obstructive and obstructive azoospermia can also freeze their specimen obtained from the testis or the epididymis. For non-obstructive azoospermia, micro-testicular sperm extraction or micro-TESE is used to locate and excise the tubules that show spermatogenesis. Fine-needle aspiration or testicular sperm aspiration (TESA) is used to aspirate multiple testicular sites in case of obstructive azoospermia. Adequate number of sperm are required for IVF using TESA. Micro-TESE has the highest sperm retrieval rate compared to multiple fine-needle aspirate or TESE and the success rates are in the range of 50–60%. After mixing the aspirate with HTF, the mixture is centrifuged, and the pellets are resuspended with HTF. After mixing the suspension with an equal volume of cryoprotectant (e.g. Test Yolk Buffer), the sample aliquots can be loaded in the cryovials.

The procedure is somewhat different for sperm retrieval by testicular sperm extraction (TESE) from that used for testicular aspiration. In this case, tissue biopsies are obtained and shredded into small pieces using a sterile 25-gauge needle or fine scissor, mincer. Enzymatic digestion using type IV collagenase, trypsin, and trypsin inhibitor can also be used to allow the permeating cryoprotectant such as glycerol to fully penetrate testicular homogenate. Each tissue sample is carefully

examined for the presence of motile spermatozoa using an inverted microscope. If no motile sperm are present in the testicular biopsy, further biopsies may be taken from the ipsilateral and contralateral testes until mature sperm are found. Conventional freezing of testicular sperm results in a sperm recovery rate of only 1% [82].

Microsurgical Epididymal Sperm Aspiration or (MESA) or Percutaneous Epididymal Sperm Aspiration or (PESA) is used to harvest epididymal sperm. PESA is effective in cases where the obstruction is at a site distal to epididymis. Compared to PESA, sperm retrieval is better with MESA; both show comparable fertilization and clinical pregnancy rates [83].

Sperm recovery using ICSI with larger cryoprotectant volume can be difficult with the conventional freezing techniques [84]. Different biological or non-biological carriers have been used to freeze microquantities of spermatozoa. The biological carriers can be empty zona [85–88] and Volvox *globator* algae [15, 89] and non-biological carriers include use of straws, mini straws [90, 91], high-security straws [92], cryoloop [91], ICSI pipette microdroplets [15], agarose gel microspheres [15, 93] and sleeper cells [94].

Fresh vs. Frozen Ejaculated Sperm and ART Outcomes

It is recommended that all men who are young adolescents should be offered semen cryopreservation [95, 96]. Several studies have found that the fertilizing capability of frozen sperm is lower than that of freshly ejaculated sperm [97, 98]. This is related with cryopreservation-related sperm dysfunction. However, identical pregnancy rates were reported between frozen and fresh ejaculated sperm using artificial insemination when sufficient number of progressively motile sperm were available in the post-thaw specimen and severe teratozoospermia and asthenozoospermia was not present [99].

Data is conflicting for conventional IVF; while some investigators failed to show any difference in the fertility outcomes between cryopreserved ejaculated sperm and fresh sperm [100, 101], others have identified significant differences [102–104]. Advent and widespread use of ICSI has resulted in a tremendous modification in sperm banking practices as this procedure can overcome many limitations associated with sperm cryopreservation such as poor post-thaw sperm quality and sperm dysfunction. Kuczyński et al. conducted well-designed controlled trials and found no difference in fertilization rates between cryopreserved and freshly ejaculated sperm [105]. Meanwhile, the authors reported higher ongoing pregnancy rates per cycle with fresh sperm (23.7%) vs. frozen (35.2%) [105].

Fresh vs. Frozen Epididymal Sperm

In cases with obstructive azoospermia, both fertilization and clinical pregnancy rates are similar when either fresh or frozen testicular or epididymal sperm were used [106–109]. More than the source of sperm or use of a particular method of sperm

cryopreservation, fertilization rate depends on the ART method, i.e. use of a single viable spermatozoa in case of ICSI. It is influenced by the cause of azoospermia, i.e. obstructive and non-obstructive azoospermia [82, 110, 111]. Use of cryopreserved sperm avoids the need for repeated surgeries in cases of failed fertilization. This minimizes testicular devascularization, fibrosis, and inflammation of the testis [112]. It is also cost effective as it eliminates the need for the couple to undergo surgical procedure on the same day [113]. In general, ICSI outcome is similar in terms of fertilization rates, clinical pregnancy rates or ongoing pregnancy rates between fresh and frozen motile testicular sperm [108, 114, 115]. However, implantation rates are slightly better with the use of fresh motile testicular sperm [108]. Other investigators have concluded that such findings are more evident in men with OA than with NOA [111]. Comparative studies between frozen and fresh epididymal sperm have not detected major differences in ICSI outcome with regard to fertilization rates and ongoing pregnancy rates, particularly when motile sperm were used [116].

Challenges in Sperm Cryopreservation

Cryopreservation of semen samples using conventional freezing techniques shows significant decline in motility, viability, sperm DNA fragmentation and mitochondrial membrane potential. Most of the sperm damage occurs at the time of post-thaw when the temperature is higher after the sample is removed from long-term storage. These methods have been used for a long time; however they are cumbersome, time consuming and expensive. Furthermore, storage in liquid nitrogen creates a safety hazard. Therefore there is a strong need for novel ways to overcome the current challenges. Vitrification is a promising method as the vitrified-warmed spermatozoa have semen parameters and live birth rates similar to conventional freezing. Furthermore, vitrification using sleeper cells is a closed system. Storing sperm in high-security straws is a closed system and another promising alternative to cryoloops. Nevertheless there is a need for improving methods to optimize techniques especially to preserve fewer sperm.

Conclusion

Cryopreservation using the conventional slow freezing continues to be the method of choice for preserving fertility, although newer protocols are being investigated and optimized. Over the past several decades, technologies related to ART and IVF have significantly expanded ways to accomplish fertility preservation. This is likely to continue to increase over the next several decades. Thus more men can now have a biological child as long as they have a single viable spermatozoa utilizing ART technique such as intracytoplasmic sperm injection. Many men in various stages of life could benefit from utilizing sperm cryopreservation. It is advisable for the

physician to engage in a discussion on cryobanking with any patient who may be exposed to gonadotoxic factors at work, is oligo- or azoospermic, has cancer (regardless of age) or another disease that can directly or indirectly affect fertility or is undergoing hormonal therapy that may adversely impact semen quality. It is important to offer the patient the option of fertility preservation rather than precluding his chance of fathering a biological child.

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Chapter 15
Sperm Freezing Injuries

Pankaj Talwar and Pranay Ghosh

Introduction

Cryopreservation refers to the maintenance of cellular life in the form of cells or tissues at subzero temperatures for an extended period of time [1]. Cryopreservation of human spermatozoa is an integral part of assisted reproduction technologies (ART), and has helped overcome various spatiotemporal limitations with regard to sperm availability. As early as in the eighteenth century, Lazaro Spallanzani, an Italian biologist credited for performing the first artificial insemination in dogs, observed the effects of freezing on human sperm and subsequent recovery of motility on warming [2–4]. Montegazza in 1866 proposed the idea of semen banking for soldiers going off to battle to ensure their continued lineage [5].

It was not until 1949 following the accidental discovery by Polge that glycerol could act as a cryoprotectant that boosted the use of cryopreservation in both human and animal models [6]. In 1953, Bunge and Sherman reported three pregnancies following the use of sperm that had been treated with glycerol and frozen in dry ice [7]. Later, Sherman demonstrated that sperm could be stored for longer duration if kept at −196 °C in liquid nitrogen (LN_2), and this conserved flagellar movement post-thawing. Nowadays, semen preservation is regularly used in autologous and donor insemination, and is an especially useful option for preservation of gametes for patients undergoing chemotherapy and/or radiotherapy for malignancy. The longest duration of cryopreservation having resulted in a live birth in humans is around 15½ years, though in bovine model blastocysts have been produced using cryopreserved semen after 37 years [8].

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Sperm Cryobiology

Prolonged storage of sperm and other reproductive cells and tissue is possible only by retarding the cellular reactions by lowering the temperature until the cellular activity ceases. LN_2 (−196 °C) storage has become the standard for sperm freezing since early days of sperm banking. Prolonged storage at −196 °C does not affect cryosurvival since at this temperature there is virtually no movement of atoms or molecules. The atoms and molecules have a tendency to move above −130 °C, while temperatures above −90 °C permit ice crystal growth following even brief exposure. The only potential damage to the cells maintained at −196 °C is degradation of deoxyribonucleic acid (DNA) caused by background radiation. It is estimated that the spermatozoa can maintain their genetic integrity for over 200 years when stored at −196 °C (based on normal background radiation of 0.1 rad/year) [9].

The main objective of any cryopreservation protocol is to prevent intracellular ice crystal formation, regulate cell volume during the procedure and decrease the membrane damage following exposure to subzero temperatures. Solutes in the medium in which the cells are suspended lower the freezing point to −10 to −15 °C below that of water (0 °C). At these low temperatures the water in the extracellular environment freezes, thereby increasing the solute concentration which generates an oncotic pressure, resulting in a solvent flow across the membrane from inside to outside the cell. This causes a reduction in cellular volume and then dehydration. However, the extent of dehydration depends chiefly on the cooling rate. Rapid cooling of cells may lead to incomplete dehydration and intracellular ice crystal formation. On the other hand, if the cells are cooled slowly, this may result in excessive dehydration. Intense dehydration may lead to the cell being rendered unsalvageable (at approximately 40% of the original cell volume), and lead to permanent cellular damage [10].

During thawing, the water is drawn back into the cells (reverse osmosis), thereby restoring the intracellular volume. At this point, there is a risk of recrystallisation injury due to intracellular ice crystal formation. Hence, thawing should be fast in order to avoid the formation of ice crystals. Cellular survival following freeze/thaw depends on a fine equilibrium between an intermediate cooling rate that is fast enough not to cause excessive dehydration but slow enough to circumvent the problem of intracellular crystallisation and a rapid thawing rate [10].

Hence, the outcome of a freeze/thaw cycle depends upon:

1. The cryoprotectant in which the cells are suspended
2. Cooling rate
3. The temperature at which the sample is plunged into liquid nitrogen
4. The temperature at which the sample is stored
5. Warming rate
6. Cryoprotectant removal after thawing

At least 50% of the motile sperm sustain cryoinjury when subjected to the freeze/thaw cycle. Human spermatozoa have a high surface area/volume ratio and have high permeability to water. This results in rapid osmotic equilibrium in the presence of cryoprotectant agent. Moreover, unlike embryos, the genetic material of the sperm is highly

condensed, making it less prone to cryoinjury. Their low water content (50%) also makes them more resistant to cryoinjuries than other cells. Human sperm can withstand a range of temperature variations and are relatively resistant to damage caused by rapid cooling due to the presence of unsaturated fatty acids in the lipid bilayer [11].

Biological Behaviour of Sperm Membrane

Human sperm plasma membrane, like all other mammalian species, is composed of a phospholipid bilayer and associated proteins. The lipid bilayer is a thin polar membrane made of a polar head group with hydrocarbon tails [12]. The sperm plasma membrane plays a very active role in sperm fertilisation capacity and in spermatozoon–oocyte crosstalk. The two leaflets in the membrane of the cap region, overlying the acrosomal vesicle, constitute the area sensitive to the capacitation stimuli. When various steps of capacitation have induced an increased membrane fluidity in this region, a fusogenic process starts between this membrane and that of the outer acrosomal vesicle. The final event is the formation of pores that allow a dispersion of the acrosomal enzymes. During cryopreservation, the sperm plasma membrane undergoes lipid-phase separation, solute effects and osmotic stresses associated with ice crystallisation [13]. Upon cooling, there occurs a reordering of the membrane components, thereby increasing sperm membrane viscosity and decreased fluidity. Though high concentrations of cholesterol and polyunsaturated fatty acids (PUFA) provide more fluidity to the sperm plasma membrane at lower temperatures, the cooling at subzero temperature causes a phase transition of membrane lipids, resulting in a more rigid membrane structure [14–16]. These untoward effects associated with cryopreservation can be prevented by controlling the rate of cooling and by addition of cryoprotective agents.

Cryoprotective Agents (CPA)

A cryoprotectant is a substance used to protect biological tissue from freezing damage (i.e. that due to ice formation). Though they may have varying chemical compositions, all CPAs are highly water soluble, but possess a concentration-dependent toxicity. They cause the lowering of the freezing point of the solution, displace the water from intracellular to extracellular environment and hence alter the solute concentration in the liquid phase [10]. There are two classes of CPAs:

1. Permeating CPA: These agents penetrate the cell membrane, and have low molecular weights (<400 g/mol). They cross the membrane easily, thereby creating an osmotic gradient, leading to shift of water from intracellular to extracellular compartment, and hence further lowering the freezing point. This class includes glycerol, dimethyl sulphoxide (DMSO), ethylene glycol (EG) and 1,2 propanediol (PROH).

2. Non-permeating CPA: These agents do not cross the cell membrane, and have large molecular weights (>1000 g/mol). They increase the concentration of extracellular solutes, thereby creating an osmotic gradient and hence causing cellular dehydration. This class includes sugars like sucrose, fructose, dextrose, trehalose and raffinose.

The most common CPA used for human sperm is glycerol [17]. Other CPAs have been tried subsequently with little success, because of potentially deleterious effects on human sperm. A final concentration of 6.0–7.5% (v/v) seems to be optimum [8]. Glycerol mediates its protective effects by virtue of its colligative properties, freezing point depression and alteration of cell membrane properties by inducing changes in lipid packing structure, and the consequent lowering of electrolyte concentration in the unfrozen fraction. In order to optimise the cryosurvival rates, more complex diluents containing other non-permeable CPAs such as glycine, zwitterions, citrate and egg yolk have been introduced. Almost all CPAs for human sperm cryopreservation contain glycerol (to protect against thermal shock); sugars (which provide the sperm with energy and optimise osmolarity and pH); egg yolk (which improves the fluidity of the plasma membrane, provides structural and functional protection and safeguards sperm integrity); and antibiotics (to protect against microorganisms). Glycerol egg yolk citrate (GEYC) was amongst the earliest and best known extenders. Another commonly used cryoprotective buffer is a zwitterion buffer system containing *N*-tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (TES) and tris-(hydroxymethyl)-aminomethane (TRIS) [18]. This TES-TRIS combination (abbreviated as TEST) is most often used along with egg yolk, citrate and glycerol as the permeating CPA.

Indications of Semen Cryopreservation

Semen can be cryopreserved either by an individual for autologous use in the future or by fertile donors following screening for heterologous use. Due attention should be paid during donor semen banking with regard to the phenotype/blood group matching besides matching physical characteristics, race, hair colour and eye colour [19].

Indications for Autologous Semen Cryopreservation

- Prior to initiation of cancer therapy (chemotherapy/radiotherapy or surgery)
- Non-malignant systemic diseases necessitating cytotoxic therapy (e.g. autoimmune diseases, kidney diseases, inflammatory bowel diseases, transplantation procedures)
- Surgical procedures involving male genitalia (e.g. varicocele ligation, vasovasostomy, vasoepididymostomy)

- Following surgical sperm retrieval (e.g. PESA/TESA/TESE) in cases of azoospermia
- In cases of anticipated absence of male partner on the day of ART procedure (IUI or OPU)
- In case of anticipated performance anxiety on the day of IUI/IVF
- In cases of ejaculatory dysfunction following electroejaculation or penile vibratory stimulation
- Storing and subsequently pooling ejaculates from oligozoospermic patients for their later combined use (although this is of limited value due to the poor thaw survival of sperms from such patients)

Indications for Donor Semen Banking

- Azoospermia
- Severe oligoasthenoteratozoospermia
- Failed surgical sperm retrieval (esp. in cases of NOA)
- Patient suffering from retrograde ejaculation
- Male partner is a carrier of a genetic defect
- Rh incompatibility with isoimmunisation
- Single woman donor insemination
- Recurrent implantation failures/miscarriages

Techniques for Sperm Cryopreservation

The two main conventional freezing techniques used in sperm cryopreservation are slow freezing and rapid freezing, apart from the recent emergence of cryoprotectant-free vitrification [20].

Slow Freezing

This technique was proposed by Behrman and Sawada, and involves progressive sperm cooling over a 2–3-h period in various steps, either manually or automatically using a semiprogrammable freezer [21]. The manual method involves stepwise addition of CPA while simultaneously decreasing temperature and finally plunging the samples in LN_2 [22]. Since reproducibility of this technique was an issue, programmable freezers were investigated. These use software data logging to obtain cooling from 20 °C to −80 °C at a rate of 1.5 °C/min and then at 6 °C/min. At the completion of freezing, the straws are plunged into LN_2 at −196 °C [23]. However, it has been argued that conventional slow freezing, either manual or automated, leads to considerable damage to the sperm due to ice crystal formation (Appendix 1).

Rapid Freezing

This technique was proposed by Sherman and requires direct contact between the straws and liquid nitrogen vapours (LNV) for 8–10 mins, followed by plunging the straws in LN_2 at −196 °C [24]. Initially, the semen sample is mixed with equal volume of CPA in a dropwise manner, and the mixture is then loaded in either straws or cryovials and left to incubate at 4 °C for 10 min. The straws/vials are then placed 15–20 cm above the level of LN_2 for 15 mins, and finally immersed in LN_2.

Vitrification

The cooling and warming processes during slow and rapid freezing associated with the intermediate zone of temperature (−10 to −60 °C), which the cells must traverse twice (once during cooling and then during warming), can be lethal to the sperm. Vitrification method does not require the use of either specially devised cooling programs or CPAs, and is much faster, simpler and cheaper. The method is based on cooling of sperms by direct immersion into LN_2, thereby avoiding intracellular ice crystal formation. Optimal cooling rates are obtained with the following specifically designed packaging systems: open pulled straws [25], the Flexipet denuding pipette [26], micro-drops [27], electron microscope copper grids [28], the Hemi-straw system [29], cryotop [30], cryoleaf [31], cryotip [32] and other carrier devices. Another modification of vitrification is direct dropping of spermatozoon suspension in LN_2 [33].

Cryopreservation of Small Numbers of Spermatozoa

Conventional methods of sperm cryopreservation are not suitable for preserving very small numbers of spermatozoa, such as epididymal or testicular spermatozoa obtained after surgical sperm retrieval. Hence, various novel methods have been devised to store limited numbers of such spermatozoa in a small volume [20]. Though both biological and non-biological carriers have been used for this purpose, no prospective randomised trials have been conducted to show the superiority of one technique over the other. Hence, novel techniques for storing small number of spermatozoa need to be further explored.

Spermatozoa have been successfully cryopreserved using empty zona pellucida by various groups including Borini et al. [34], Cohen et al. [35], Montag et al. [36], Hsieh et al. [37], Liu et al. [38], Cessana et al. [39] and Hassa et al. [40]. This has the advantage of reducing the time in screening to locate motile sperm but carries a potential risk of biological contamination. Similarly, others have used microdroplets for freezing spermatozoa (Gil-Salom et al. [41], Sereni et al. [42], Quintans

et al. [43], Bouamama et al. [44]). This method avoids sperm loss due to adherence to the vessel, but potentially carries the risk of cross contamination. Other methods used for storing limited number of spermatozoa include ICSI pipette [45, 46]; Volvox globator spheres [47]; alginate beads [48]; cryoloop [49–52]; agarose microspheres [53]; and straws [54–56].

Sperm Cryopreservation and Cryoinjuries

Even though spermatozoa seem to be less sensitive to cryodamage as compared to other cell types because of their membrane fluidity and low water content, cryopreservation does render significant structural and functional damage to the sperm. A combination of thermal shock, osmotic shock, cellular dehydration and intracellular ice crystal formation is responsible for the cryoinjuries sustained during freeze/thaw process. As previously discussed, the cryopreservation procedure has a detrimental effect by changing the carbohydrate composition of the glycocalyx, and hence it impairs the function of membrane proteins responsible for ion transport and metabolism, thereby impairing the fertilising ability [57]. Cryopreservation significantly affects post-thaw sperm motility due to membrane swelling and acrosome degeneration [58]. Fatty acids present in the sperm plasma membrane are vulnerable to lipid peroxidation resulting in loss of intracellular enzymes and inhibition of oxidative phosphorylation. The mitochondrial membrane is susceptible to damage at low temperatures, and an alteration in membrane fluidity can cause the release of reactive oxygen species (ROS) [22]. The ROS damage may involve single- or double-stranded DNA breakage. Though the detrimental effects of cryopreservation on the fertilisation capacity, motility, morphology and vitality are well established, there is little consensus on whether cryopreservation induces DNA damage or not. Donnelly et al. studied the pre-freezing and post-freezing sperm DNA integrity in both semen and prepared samples, and found that sperm frozen along with seminal plasma had improved post-thaw DNA integrity, probably due to the presence of abundant antioxidants in seminal plasma [59] (Appendix 2).

Sperm Freezing and DNA Damage

There are three schools of thought as far as the effect of sperm freezing on DNA damage is concerned [20]. Several authors conclusively believe that overall sperm quality deteriorates after freeze-thawing, including sperm DNA damage, as assessed by sperm chromatin structure assay (SCSA) or terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) [60]. Thomson et al. proposed that the cryopreservation-induced sperm DNA damage is mediated predominantly by oxidative stress rather than apoptosis [61].

Few others believe that freeze-thawing does induce sperm DNA damage, but only in infertile men that have a greater incidence of irregular chromatin organisation and show significantly decreased resistance to thermal denaturation as compared to spermatozoa from fertile men. Kalthur et al. evaluated sperm morphology and DNA damage before and after cryopreservation, and reported that the susceptibility of morphologically abnormal sperm to DNA damage during cryopreservation is significantly higher as compared to sperm with normal morphology [62].

In contrast to the above two, the third line of thought believes that freeze-thawing is not associated with a detrimental effect to sperm DNA integrity. Duru et al. noted in their study that though cryopreservation altered the plasma membrane symmetry and was associated with translocation of phosphatidylserine, the DNA integrity remained intact [63]. Similarly, Isachenko et al. concluded that the integrity of DNA is unaffected by cryopreservation while comparing the effects of slow freezing and vitrification on sperm DNA integrity [51].

Apoptosis and Sperm Cryoinjury

Apoptosis, or programmed cell death, has been proposed to play a role in causing cryoinjury to sperm DNA by increased activation of various aspartic acid-directed cysteine proteases, called caspases. Activation of caspases leads to morphological changes and characteristics of apoptotic cells. Initiator caspases include caspases-2, -8, -9 and -10 that activate effector caspases (3, 6 and 7). This results in cleavage of several substrates and culminates in apoptosis. The activation of caspase 3 heralds the "point of no return" in apoptosis; hence caspase-3 is considered the most important effector caspase [22]. Besides the presence of caspases in spermatozoa as a marker of apoptosis, the externalisation of phosphatidylserine on the sperm membrane is considered as a relatively early apoptotic marker. This exposed phosphatidylserine is then amenable to phagocyte-mediated lysis. Apart from this, a family of ligands known as Fas-FasL also plays a significant role in activation of effector caspases. Fas receptor is present only in <10% of healthy ejaculated spermatozoa, as compared to >50% of ejaculated spermatozoa in oligozoospermic men [64].

DNA fragmentation is considered a late-stage marker for apoptosis in spermatozoa, and can partially be caused by activation of caspase-3. The triggers for activation of apoptosis result in the permeabilisation of the outer mitochondrial membrane, first by activation of BAX and BAK proteins followed by release of cytochrome-c. This results in caspase-9 activation along with APAF-1, thereby forming an apoptosome, finally initiating the apoptosis cascade [65] (Fig. 15.1). Chlamydia and mycoplasma infections, and various other microbiological toxins, have been shown to increase apoptosis in human spermatozoa; the resultant high DNA fragmentation levels are amenable to treatment using specific antibiotic therapy [66]. The other triggers for induction of apoptosis may be neoplasia (esp. patients with Hodgkin's disease and testicular cancer), and environmental toxins (e.g. pesticides containing dibromochloropropane) [67].

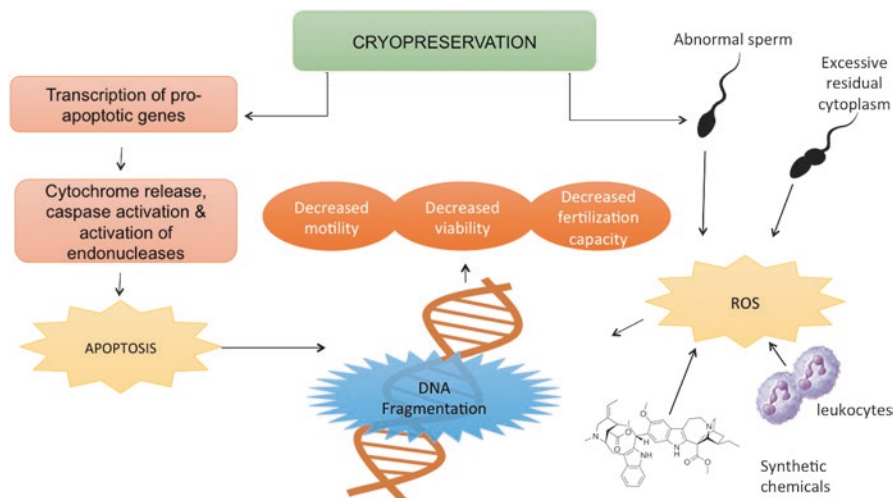

Fig. 15.1 Mechanism of DNA injury during cryopreservation

Apoptosis markers (caspase activation, DNA damage and phosphatidylserine externalisation) have been linked to male infertility in numerous studies. Spermatozoa positive for active caspase-3 demonstrate phosphatidylserine externalisation and DNA fragmentation more frequently than normal controls. Moreover, low-motility spermatozoa demonstrate a higher level of apoptosis markers as compared to high-motility spermatozoa [68]. Various studies have shown that these apoptosis markers tend to increase in spermatozoa following the freeze/thaw process. It has been proven that normozoospermic semen samples are more resistant to the damage induced by freezing and thawing as compared to oligozoospermic samples. Verza et al. reported that motile sperm could be recovered even after five freeze/thaw cycles in normozoospermic men, while motility could be salvaged after only two freeze/thaw cycles in oligozoospermic samples [69]. The extent of damage is correlated to the degree of oligoasthenoteratozoospermia. Moreover, cryopreserved spermatozoa from cancer patients were also found to be having a higher level of DNA fragmentation as compared to healthy controls.

Though literature suggests that a correlation exists between the presence of activated caspases and sperm DNA fragmentation, there are reports which have found no conclusive evidence for the same, and attribute the sperm DNA damage induced during cryopreservation to oxidative stress rather than apoptosis.

Cryopreservation and Mitochondrial Damage

Transmission electron microscopy (TEM) studies have shown an alteration in the ultrastructure of the mitochondria and plasma membranes, and have confirmed that mitochondrial destruction is secondary to widespread cellular destruction. Changes

in mitochondrial membrane potential (M ΔΨ) are assessed using a fluorescent cationic dye, 5,5′, 6,6′ –tetrachloro-1-1′, 3, 3′-tetraethylbenzamidazolocarbocyanin iodide (commonly known as JC-1). Uncoupled mitochondria are suggestive of unhealthy spermatozoa, and hence determination of mitochondrial membrane potential is useful to assess post-thaw sperm survival [70]. In intact mitochondria, M ΔΨ is unaltered and the JC-1 dye aggregates inside the non-damaged mitochondria and fluoresces red. In damaged mitochondria, the M ΔΨ is broken down and the JC-1 dye disperses through the entire cell and fluoresces green.

Cryopreservation and Sperm Motility, Vitality and Morphology

Following freeze/thaw cycles, significant decrease in post-thaw motility and an increase in immotile sperm fraction have been documented. There is a significant decrease in the rapidly progressive fraction (29% pre-freeze vs. 12% post-thaw; $p < 0.05$) and an increase in immotile fraction (24% pre-freeze vs. 64% post-thaw; $p < 0.01$). There is a strong correlation between the deterioration in motility post-thaw and vitality ($r = -0.848$, $p < 0.01$) [70].

Light microscopic examination has revealed an increase in the percentage of spermatozoa with midpiece detachment and coiled tails ($p < 0.05$). TEM evaluation following post-thaw showed various ultrastructural abnormalities including decomposition of the plasmalemma, outer acrosomal membrane, acrosomal content, early acrosomal reaction, chromatin condensation anomalies, and diadem defects. There is a significant increase in acrosomal change defect and subacrosomal swelling. Acrosomal change defect is marked by unaltered equatorial segment but an affected apical acrosomal region. Various apical head alterations occurring in acrosomal change defect include lack of continuity, loss of acrosomal content and appearance of vesicles. Subacrosomal swelling, characterised by detachment of inner acrosomal membrane from the nuclear envelope, is another significant observation [70].

Sperm Cryopreservation and Fertility Outcome

Owing to the plethora of injuries incurred to the spermatozoa, the fertilisation potential of sperm is reduced and hence the pregnancy rates following intrauterine insemination and conventional in vitro fertilisation (IVF) are lower with cryopreserved sperm as compared to fresh sperm [71]. Hence, cryopreservation of sperm before intrauterine insemination or conventional IVF is not recommended. However, the results with testicular frozen spermatozoa when used for ICSI are comparable to fresh spermatozoa from the same subject [72]. No differences in fertilisation rate, cleavage rate, embryo quality, clinical pregnancy rate and ongoing pregnancy rates are noted with the use of cryopreserved testicular spermatozoa as compared to fresh sperm [73]. Even with the use of ejaculated spermatozoa, the fertilisation rates are comparable between fresh and frozen groups [74].

Conclusions

Optimisation of freeze/thaw protocols, cryoprotectant concentrations used and semen preparation techniques is warranted to ensure successful application of cryopreservation for semen preservation [75]. Since the spermatozoa are exposed to a variety of ultrastructural cryoinjuries that may not be discernible by light microscopy examination, different and novel cryopreservation methods (e.g. cryoprotectant-free vitrification) and sperm separation techniques (e.g. MACS—magnetic activated cell sorting) should be explored for different patient populations (normal donors and normozoospermic patients, infertile and oligozoospermic men and patients undergoing treatment for malignancy), since post-thaw survival of each specific group is different with the conventional protocols used [76]. Specific measures should be adopted to minimise the perturbations to the spermatozoa during freeze-thawing because of apoptosis and DNA damage.

Appendix 1: Ideal Sperm Freezing Protocol to Avoid Sperm Freezing Injuries

Freezing Steps

1. Pre-warm the Sperm Freezing Medium for a minimum of 2 h at room temperature.
2. After liquefaction, measure the total volume of the ejaculate and carry out semen analysis as required.
3. Ensure that both semen sample and Sperm Freezing Medium are at room temperature and dilute the semen 1:1 (v/v) with the Sperm Freezing Medium. The medium should be added drop by drop onto the semen and the solution carefully mixed after each addition.
4. The mixture is left at room temperature for a minimum of 10 min.
5. Load the diluted semen into straws or cryo-tubes and seal.
6. Suspend the straws horizontally for 30 min, just above the surface of the liquid nitrogen. Cryo-tubes should be attached to a cane and then suspended above the surface of the liquid nitrogen for the same period of time.
7. Finally, transfer the straws or cryo-tubes into the liquid nitrogen and store at −196 °C.

Thawing

1. Warm straws at room temperature for 5 min.
2. Open the straws or cryo-tubes according to the manufacturer's instructions and remove the thawed semen.
3. Immediately prepare sperm by the density gradient or the swim-up procedure.

[Adapted from ORIGIO Protocol]

Appendix 2: How to Avoid Sperm Freezing Injuries

Serial No	Critical steps to avoid sperm injury	Causes and types of injury	Remarks
1.	Bring the semen sample—raw or prepared to the same temperature as the sperm freezing media (Figs. 15.2, 15.3, and 15.4)	SFM is egg yolk-free and contains • Glycerol and sucrose as the cryoprotective agents • Glycine which improves post-thaw sperm motility, membrane and acrosome integrity • SSR with insulin shown to be a pro-surviving factor Thermal shock to the sperms may occur if both SFM and semen sample are at different temperatures at the time of procedure Sperms from infertile men have more incidence of disordered chromatin organisation and depict reduced resistance to thermal injury related denaturation as compared with spermatozoa from fertile individuals Freezing unprepared semen in seminal fluid seems to be more resistant to freezing injuries as compared to the frozen prepared sperm sample	Endeavour should be keeping them at room temperature before starting the procedure
2.	Using equal volumes of the cryoprotectant media and semen sample (Fig. 15.4)	Osmotic stress may occur to the semen sample during this procedure The phenomenon is marked by the increased coiling of the sperm tail and leading to loss of progressive motility	It is advised to mix them in equal volumes Follow the manufacturer's guidelines
3.	Adding the cryoprotectant to the semen sample (Fig. 15.5)	Osmotic stress if the cryoprotectant is added too rapidly to the semen sample. It is therefore important to allow for gradual osmotic adjustment by slowly mixing the Sperm Freezing medium with your sperm sample	Always add the cryoprotectant media drop by drop over a period of 10 minutes to the equal volume of the semen sample

4.	Gradual cooling of the semen sample and vapour phase cooling (Figs. 15.6, 15.7, and 15.8)	Osmotic stress may occur if we don't cool the sample gradually The intracellular damage that spermatozoa may encounter at rapid rates of cooling may be due to the formation of intracellular ice An osmotic imbalance occurs during rapid cooling rates due to a diffusion limited ice crystallisation in the extracellular fluid The amount of ice forming in the extracellular space in the suspension during the cooling is less than expected leading to cellular injuries during thawing	From room temperature move the sample to 4 °C over a period of 20–30 min so that there is no shock to the spermatazoa Then place the mixed sample in the vapour phase for final step of cooling for a minimum 20 min Ice crystals formation breaches the sperm membranes and affects the organelle function. This leads to impaired cell survival Very slow cooling rate regulates the efflux of water from the internal to the external milieu, thus increasing the concentration of solutes and the osmotic pressure inside the cell Such prolonged and gradual cooling leads to cellular volume changes related with the movement of water outside the cell, dehydration, and toxicity damage due to high solute concentration intracellularly
5.	Storage in the cryo can	Thermal shock to the semen sample may occur to sperms if the suspension is not stored properly at adequate depth in liquid nitrogen or vapour phase	Ensure that the semen sample is always placed at −196 °C for storage for prolonged periods
6.	Thawing protocol (Fig. 15.9)	Thermal and osmotic shock Spermatozoa that have been cooled at high rates are subjected to an osmotic shock at low temperatures during thawing, leading to the observed cellular damage The phenomenon of recrystallization of both intracellular and extracellular ice, in frozen thawed semen sample, occurs as formation of small ice crystals with a rate of recrystallization that increases with increasing temperature during thawing or storage Such chilling injuries can alter the structure and integrity of plasma membranes composed of phospholipids and cholesterol. These can also alter mitochondrial membrane fluidity and lead to alteration in mitochondrial membrane potential and release of Reactive Oxygen Species	Always thaw quickly to avoid recrystallization of ice and subjecting sperms to stress and fracture injuries Warm straws/vials at room temperature for 5 minutes and prepare the sample

(continued)

Appendix 2 (continued)

Serial No	Critical steps to avoid sperm injury	Causes and types of injury	Remarks
7.	Addition of the sperm washing media for the removal of the cryoprotectant (Figs. 15.10 and 15.11)	Osmotic stress injuries	Gradually add the sperm preparation media to the thawed suspension to avoid sudden osmotic stress injuries
8.	Speed of the centrifugation of the semen sample	Sperms are now just recovering from freeze-thaw cycle and are under stress Any unwanted centrifugational stress may lead to membrane injuries	Single wash for less duration at minimal speed
9.	Timing of use of post-thaw semen sample	Frozen-thawed sperms exhibit different dynamics of DNA fragmentation compared with fresh samples with a more rapid increase in the percentage of DNA damaged spermatozoa	If cryopreservation is required, it is recommended that sperm be used in treatment as soon as possible after thawing

Fig. 15.2 SFM is egg yolk-free media and contains glycerol and sucrose as the cryoprotective agents

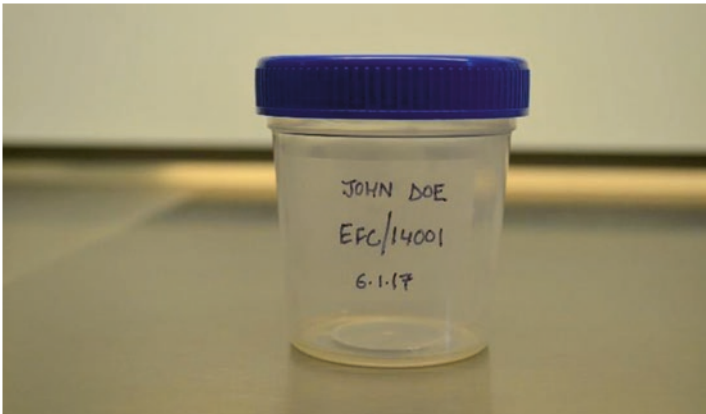

Fig. 15.3 Liquefied semen sample kept at room temperature

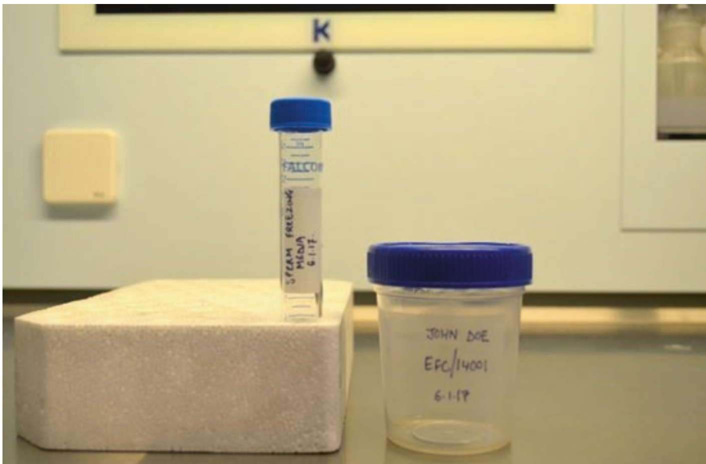

Fig. 15.4 Semen sample and sperm freezing media kept at room temperature in equal volumes

Fig. 15.5 Add the cryoprotectant media drop by drop over a period of 10 minutes to the equal volume of the semen sample raw or unprepared

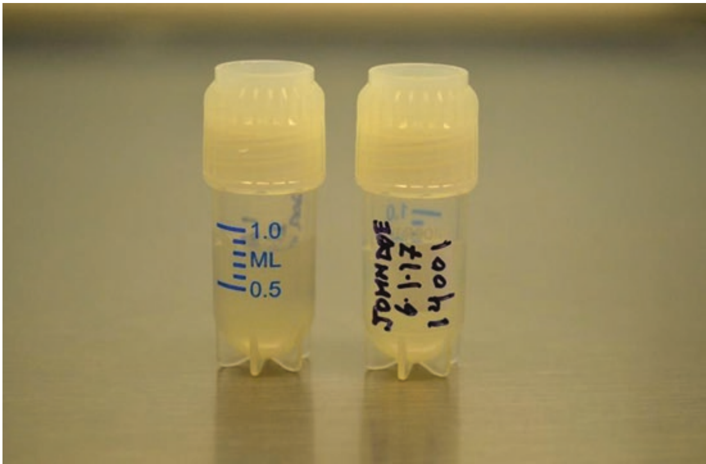

Fig. 15.6 Keeping the vials at room temperature for a period of 10–15 min

Fig. 15.7 Keeping the semen cryovials at the 4 °C temperature for a period of 15–20 min

Fig. 15.8 Placing the sample vial in the vapour phase for final step of cooling for minimum 20 min

Fig. 15.9 Keep vials at room temperature for 5 min for thawing. Prepare the sample after the sweating has stopped and the contents have warmed up

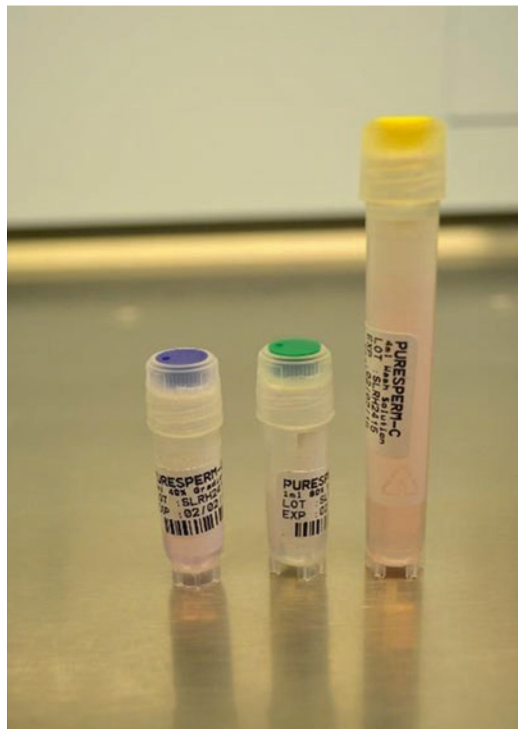

Fig. 15.10 Gradually add the sperm preparation media to the thawed suspension to avoid sudden osmotic stress injuries. Semen sample and sperm preparation media should be at the same temperature to avoid thermal shock

Fig. 15.11 Prepare the semen sample at low centrifugation speed and collect swim-up after 20 min

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Chapter 16
Risk Preparedness in Sperm Banks

Gary N. Clarke

Introduction

Risk preparedness or risk management is a vital element of the operation of any clinical laboratory which aims to provide the highest quality of service to referring doctors and their patients. The aim of this chapter is to provide a reasonably comprehensive description of how this can be achieved, based on the author's more than 40 years of experience in establishing and managing the sperm banking service at the Royal Women's Hospital in Melbourne.

The Risk Analysis Framework

The overall risk analysis framework (Fig. 16.1) separates risk assessment (the analysis of all relevant information relating to a procedure such as sperm banking) from risk management (consideration of the outcome of risk assessments, relevant legislation and regulatory policies and other pertinent information in order to develop, recommend and implement options to mitigate the identified risks). Risk communication should provide for ongoing exchange of information and opinions on elements of the risk assessment and on the proposed and/or implemented risk management steps.

This simplified diagrammatic overview of risk analysis may seem somewhat facile in relation to an apparently simple process such as sperm banking where we may tend to assume that we are aware of all possible risks and have them under control; however it does provide an initial framework from which to embark on an in-depth evaluation of what we do routinely on a daily basis. However, I think it is important

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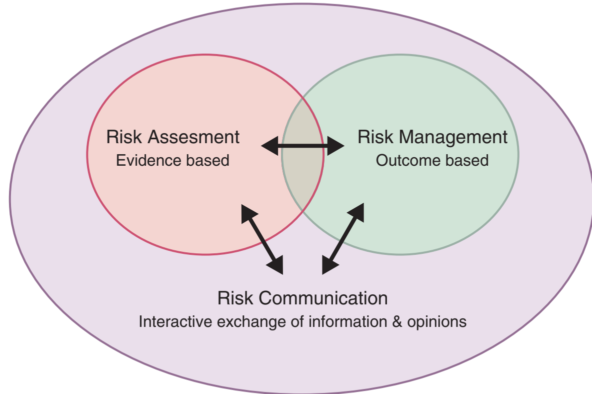

Fig. 16.1 The overall risk analysis/management framework

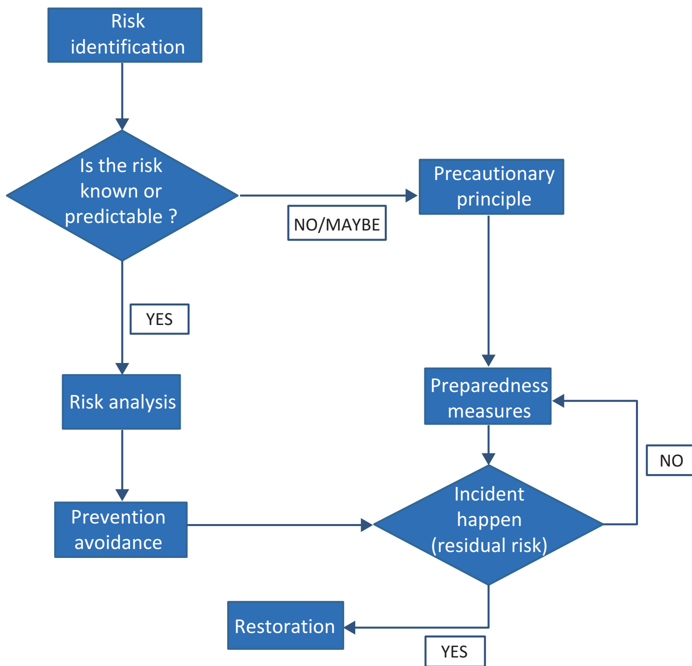

Fig. 16.2 The precautionary principle and risk preparedness as integral components of the risk analysis and management process [adapted from Ganoulis J. Risk Analysis of Water Pollution, Second, Revised and Expanded Edition, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany; 2009. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission] [11]

before considering a more formal risk analysis/management approach, to apply generic laboratory knowledge and experience and the precautionary principle (Fig. 16.2) to delineate the basic requirements for minimising risk during the process of sperm banking.

Institutional and Laboratory Accreditation Requirements

Laboratories performing sperm banking must hold current accreditation as per the regulations in their country of operation, and must be aware of and comply with any relevant laws in their country of operation. For example in the state of Victoria, Australia, this laboratory is inspected and accredited by NATA (National Association of Testing Authorities) to assess adherence of standard operating procedures (SOPs) against the Australian Standard (Medical laboratories-Requirements for quality and competence; AS ISO 15189–2013) and must comply with Victorian legislation (Assisted Reproductive Treatment Act 2008 and Assisted Reproductive Treatment Amendment Act 2013).

Security of Premises

The assessment of the overall security of premises should take into account the local conditions and surrounding environment, not simply guarding against access by unauthorised personnel or intruders. It is essential to perform a realistic evaluation of the potential local risks associated with extreme weather events, earthquakes and major accidents. For example, it would not be wise to establish a storage facility in the basement of a building prone to flooding in the event of extreme weather conditions (for example, the basement of a NY State University building flooded during Katrina—unfortunately the backup generator was in the basement and the resulting malfunction resulted in freezers thawing and loss of hundreds of precious research samples; personal communication). The Christchurch earthquake in 2011 caused major damage to Christchurch Hospital functionality and infrastructure, including basement flooding and loss of power, as detailed in a paper delivered at the 2012 conference of the New Zealand Society for Earthquake Engineering (NZSEE) titled "The Impact of the 22nd February 2011 Earthquake on Christchurch Hospital" [1]: http://www.nzsee.org.nz/db/2012/Paper124.pdf

It is important to factor into the risk assessment that sperm banks often require continuity of service over decades and also that extreme weather events are predicted to increase in frequency over coming decades due to global warming.

Staff Qualifications and Training

In Australia minimum staff qualifications are stipulated in various publications such as the following from NPAAC (National Pathology Accreditation Advisory Council) and RTAC (Reproductive Technology Advisory Council), respectively:

NPAAC Requirements for supervision of pathology laboratories (2007) [2]: http://www.health.gov.au/internet/main/Publishing.nsf/Content

RTAC code of practice for assisted reproductive treatment units (2014) [3]: http://www.fertilitysociety.com.au/wp-content/uploads/RTAC-COP-Final-20141.pdf

Each country or jurisdiction will have its own qualification requirements; however it would generally be accepted that a person working as a scientist performing unsupervised sample processing in a sperm bank facility should have a minimum qualification of a bachelor of science and that the scientist in charge of a facility would have a higher degree such as a master's degree, PhD or equivalent fellowship of a relevant professional body. Supervised technical personnel may have lower qualifications such as relevant diplomas or be studying towards a bachelor's degree or have accepted long-term laboratory experience.

Quality Assurance Participation

In order to monitor the ongoing competency of staff and the suitability of methodology, all laboratories must participate in an external quality assurance (EQA) programme and perform regular internal quality control (IQC) comparisons between staff and also use other means of monitoring the quality of laboratory output (see WHO Manual 5th edition, 2010 [4]). For example, weekly or monthly means charts can be a convenient way to detect drift in laboratory results [5].

Physical Environment

Risk assessment/management for patients/donors should focus on any potential physical hazards and their rectification, for example, provision of wheel-chair access and railings for partially disabled patients and installation of an emergency buzzer in the specimen collection area. It should also be a priority to maintain a pleasant environment including efficient but friendly staff in order to minimise patient/donor anxiety/discomfort during their visit.

Risk Preparedness for Staff

All staff should undergo regular emergency procedure training and competency assessment, chemical spill-kit training and thorough liquid nitrogen handling training and risk awareness. Storage facilities must be adequately ventilated and have oxygen depletion meters installed and two people should work together for safety reasons. The following links provide some good training material to consider:

This training material was prepared by the Division of Facilities Planning and Management of the University of Wisconsin-Madison:

https://www.ehs.wisc.edu/bio/LiquidNitrogenTraining.pdf

The following training video was prepared by the Office of Research Safety of Northwestern University:

http://www.offices.research.northwestern.edu/ors/training/video/cryogen.html

Detailed risk assessments based on the approach outlined in Figs. 16.1 and 16.2 and comprehensively described in Janssens and Cheung (2008) [6] must be performed for all SOPs and it is imperative that properly maintained laminar-flow cabinets should be used for sample processing. Laminar-flow cabinets should be serviced annually by certified technicians and the service records retained by the laboratory for at least 3 years beyond the active life of the cabinet.

Sperm Toxicity Testing of Reagents and Disposable Items Used During Semen Analysis and Cryopreservation

It is important to use reagents and disposable items which have been tested for sperm toxicity, either by the manufacturer or in the laboratory itself. Some manufacturers provide results of testing on each new batch of their product whilst other products require in-house evaluation prior to use. Initially, a new product in the laboratory should be tested against several semen samples, whilst new batches of the same product might be tested against only one or two semen samples to check for a defective batch prior to use.

Security and Risk Minimisation for Cryopreserved Samples

Storage dewars should preferably be kept in a dedicated, locked room fitted with oxygen monitors and appropriate signage about risks associated with liquid nitrogen. All dewars should also be kept locked when not being accessed by authorised personnel.

During initial sample processing for testing and cryopreservation the relevant SOP should stipulate that only one semen sample should be processed per scientist per cabinet to minimise the risk of cross-contamination or sample mix-up. Sterile disposable pipette tips and test tubes should be used when processing storage samples. All sample containers and test tubes must also be labelled on their lids with a sample number which corresponds with the number on the actual container or test tube to guard against swapping of lids resulting in cross-contamination. In addition, double-checking of all paperwork, storage code/ID number assignment and labelling by a second individual prior to freezing and placement of samples in the storage inventory are vital to avoid mistakes with potentially serious consequences for patient welfare.

Holding-time checks must be performed on all liquid nitrogen dewars and dry shippers prior to commissioning for clinical use (Appendix 1). There are multiple aspects to consider when performing a risk analysis/management/mitigation exer-

cise around sample security during liquid nitrogen storage as per the process sketched out in Fig. 16.2. For example, it would not require much reflection to identify dewar failure as a known potential risk to sample security and that the risk analysis/prevention arm should be followed initially. In order to minimise the risk of total sample loss in case of a single dewar failure, particularly in the absence of remote alarms, samples should be split for storage into at least two dewars. However, this would just be the first stage of the process. Some patients have only one vial or straw stored and we ideally don't want to risk loss of any samples, so how can we mitigate this risk? This risk could be essentially eliminated by having all dewars remotely alarmed with SMS/email alerts sent to on-call staff, with an appropriate staff response time to allow a significant chance of recovery of samples prior to actual thawing. Dewar temperature may have risen significantly prior to recovery, but this would be unlikely to significantly affect the sperm if the dewar temperature remained below −130° C. In the case of a dewar reaching higher temperatures, it is worth noting early reports of normal pregnancies and babies born after using sperm stored on dry ice at −79° C for periods up to approximately 1 year [7], despite the fact that some degree of recrystallisation would be occurring at such relatively high temperatures. Initial investigation in my laboratory indicated that sperm stored in liquid nitrogen could be transferred to dry ice (−79° C) for 4–18 h with no change in post-thaw motility or velocity. Sperm transferred from liquid nitrogen to dry ice for 5 h and then into liquid nitrogen vapour for 18 h also showed no difference in post-thaw results compared to sperm thawed directly from liquid nitrogen.

In an alternative scenario, the samples may have thawed, but if they had not been at room temperature for any significant amount of time, they could be refrozen with high likelihood of subsequently being successfully used in IVF/ICSI treatment procedures [8]. In their introduction, the authors of the study just cited state: "It is common practice in ART laboratories to offer at least one repeated freezing cycle to patients to maximize the use of donor or partner sperm for various reasons". This approach has also been used many times at the Royal Women's Hospital and at Melbourne IVF in Melbourne, Australia. However, in the context of inadvertent sperm thawing due to dewar or human failure, this process of recovery would be classed as "restoration" (Fig. 16.2). It is important to note that early studies showed that human sperm can be thawed at widely different rates (1 to 60° C/min) with no apparent difference in post-thaw motility recovery [9, 10], so the trajectory of thawing in the case of either a dewar failure or a human failure to replenish a dewar with liquid nitrogen would be unlikely to significantly affect the success of a refreeze procedure.

It is important to stress that the installation of a liquid nitrogen alarm system definitely does not substitute for regular (at least weekly) manual checks and ongoing records of liquid nitrogen depths (or weight) in all dewars.

Spare dewars containing liquid nitrogen should be available in case of a sudden dewar failure. Minimisation of temperature fluctuations during storage should be a prime consideration, for example during the processes of adding/removing samples from a dewar, routine monitoring and topping up dewars, and sample auditing sessions.

Table 16.1 Risk analysis of cross-infection during sperm cryopreservation, storage and thawing

Risk factor	Risk analysis	Risk management
Cross-infection during sample processing	Higher risk if multiple samples processed in parallel in a single cabinet, or sequentially without swabbing the cabinet	Process one sample per cabinet, then swab the cabinet with 70% ethanol before processing the next sample
Cross-infection during freezing or storage in straws	Higher risk if straw overfilled, resulting in cracking, or if outside of straw contaminated	Leave 15 mm air gap in straw before sealing. Wipe outside of straw with alcohol or hypochlorite before freezing
Cross-infection during freezing or storage in vials	Higher risk if vial overfilled and/or stored under liquid nitrogen	Don't overfill vial, store vials in liquid nitrogen vapour only
Cross-infection during thawing and/or processing for clinical use	Higher risk if straw/vial cracked, or if outside not disinfected prior to cutting straw or opening vial	After thawing, discard any cracked straws. Disinfect outside of straw before cutting to extract sample, use sterile scissors or scalpel blade to cut straw. Disinfect outside of vials before opening

Storage facility staff should follow a strict daily shutdown protocol with appropriate checklist record to avoid potential problems such as leaving samples in the programmed freezing machine, leaving dewar lids open, checking for frosting on the outside of dewars and confirming that the liquid nitrogen alarm system can dial out.

A major consideration pertaining to sample security and integrity during storage concerns the decision as to whether to store the semen samples in straws (pailletes) or cryovials and whether the processed samples should be stored under liquid nitrogen or in the vapour phase [12]. I would recommend storing vials in the vapour phase because of tests conducted in my laboratory which showed that many vials take in liquid nitrogen (and presumably any contaminants in it) during storage under liquid. Additionally, consideration needs to be given to the exact filling and sealing method for straws and whether to opt for high-security straws (e.g. CBS; www.cryobiosystem-imv.com). A risk analysis exercise in this area could address the following question: Is there any significant risk of cross-infection occurring during cryopreservation, storage in liquid nitrogen and subsequent thawing and processing of the sperm for clinical use? Table 16.1 lists the identified risk factors, relevant analysis and possible management steps to mitigate risk.

The decision whether to use quarantine dewars prior to placing samples into the final storage dewar is up to individual facilities. If the precautions described in Table 16.1 are meticulously followed, then the risk of cross-contamination is minimal and quarantine dewars should not be required. However, if a decision is made to use quarantine tanks, then ideally a single quarantine dewar should be used for each sample, and then after use with a sample which is potentially infectious, the dewar should be brought up to room temperature and disinfected prior to being used for another sample. In practice this would require busy laboratories to have many quarantine dewars available, particularly if the laboratory processes a lot of samples from cancer patients which are often on very short notice. The other area of concern

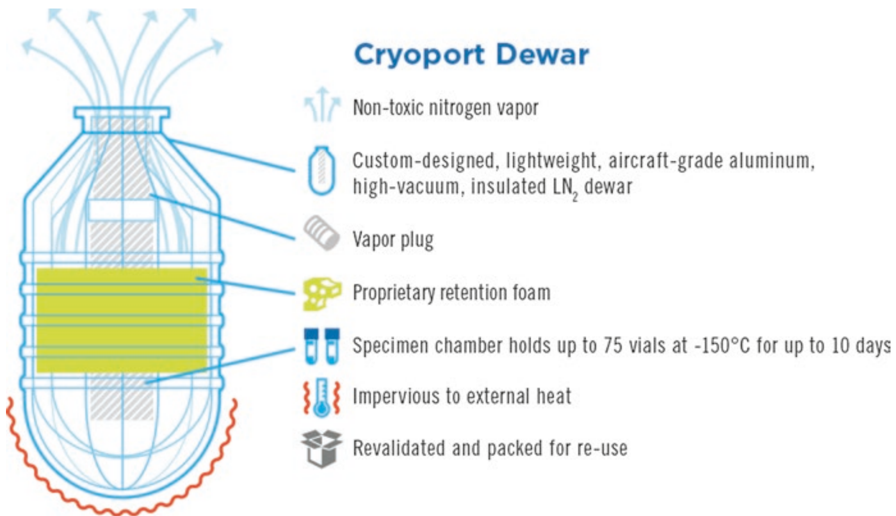

Fig. 16.3 Dry shippers are composed of high-grade aluminium protecting an inner holding chamber. The inner chamber is surrounded by a high-surface-area, low-density open cell foam that allows for holding times of up to 10 days. The dewar design means that the liquid nitrogen remains within the foam compartment and that the specimen chamber remains dry, regardless of the dewar orientation (http://www.cryoport.com) [image used with permission of Cryoport]

with quarantine approach is the question of what policy to follow when a new infectious agent such as Zika virus is identified in semen.

There are a number of important requirements in order to minimise risk during the transport of samples in dry shippers (one type of dry shipper is depicted in Fig. 16.3). Regular holding-time checks should be performed on commissioned dry shippers, correct liquid nitrogen charging procedures must be followed and also check that shippers are at least 90% charged with nitrogen before sending out (Appendix 1). Double-checking of sample labelling by a second individual before send-out is essential. Consideration should be given to temperature logging during transit and the routine use of tracking apps.

My experience indicates that data accuracy is greatly increased by the use of double-checking/validation of data entry for semen testing and storage inventory locations. Adequate labelling of straws/vials using label printers is also beneficial and consideration could be given to barcoding for newly established storage facilities. It is also self-evident but sometimes overlooked that there should be secure and frequent backup of all data pertaining to the samples in storage, including consideration of off-site backup copies. In my laboratory, backups are done daily to two network dives located in a separate building, although we will investigate the addition of a secure "cloud" backup.

The conduct and frequency of audits of storage dewars and the storage database is a matter for individual facilities, based on staffing levels, personnel and sample security considerations and frequency of access of particular dewars.

Business Continuity Planning to Cope with Disaster and Emergency Contingencies

All clinical laboratories must have a written procedure which details how the laboratory staff should proceed in the event of a disaster or an emergency. For example, in case of power outage, does the laboratory have an alternative power supply such as a diesel generator and is the generator function checked (e.g. monthly) and is it serviced regularly (e.g. yearly)? Laboratory managers should be aware of the potential of UPS (uninterruptible power supply) devices and also not to rule out a role for solar power walls as this technology is becoming significantly more efficient.

Principles of Formal Risk Analysis and Risk Management for Sperm Banks

The crucial principle in running any good laboratory is to have rigorous SOPs and general laboratory practices which minimise many overt risks from the outset (prevention/avoidance, Fig. 16.2). Critical SOPs such as semen cryopreservation should incorporate several levels of control aimed at preventing a single error which could result in the wrong sperm being used clinically. Risk can be estimated or at least approximated from the following equation:

Risk score = (Likelihood of error) × (Impact of error)

In the case of an error resulting in the wrong sperm being used, the impact (if the error is detected) is obviously 10/10 for our patients and also has very significant impact on nursing staff, clinicians and laboratory. The likelihood of error is relatively small, but finite if a single technician processes samples on their own, but it can be drastically reduced by a rigorous independent double-checking procedure, as described below:

1. Andrology staff ask the patient to double-check the labelling on their sample and sign as the specimen collector.
2. In the laboratory follow the strict rule: one technician, one workstation, one sample.
3. The technician analyses the sample, adds cryoprotectant and aliquots the sample into straws or vials.
4. The technician checks the storage database to see if the patient has previously been allocated a unique storage code; if so this code is used, and if not a new code is issued.
5. The technician prints labels with the storage code, sample number and date and applies the labels to the straws or vials with all details recorded in a storage workbook with the technician's initials.
6. The technician then calls for a second person to perform a complete double-check which involves checking the doctor's referral, the patient's storage request, the

original sample container, the database, code allocation, labelling of each straw or vial and the information in the workbook before initialling the workbook or pointing out errors to be rectified before initialling.

7. Once the double-check is completed, the technician can proceed to freeze the straws or vials.
8. When the clinic or IVF laboratory orders sperm for a patient treatment, andrology staff cross-check patient name, date of birth and address before issuing the sperm.

Retrospective analysis of our records for 1091 consecutive storage patients showed one major error (0.1%) which was detected during the double-checking process and corrected. This rate of significant human error is in agreement with the NASA analysis of the Kennedy Space Centre Shuttle Switch Throw Database (manual tasks) which estimated an error rate of approximately 0.2% (95% CL 0.05–0.4%) [13]. The authors noted that their estimated error rate concurred with the error probability predictions obtained using the technique for human error rate prediction (THERP) [14]. These analyses indicate that even with highly trained operators and rigorous protocols, human errors are unavoidable. Double-checking by a second person is the most efficient way to detect the majority of errors and rectify them before they cause harm.

Risk Analyses Requiring Potentially Complex Input from Scientific Research

Having introduced the basic precautionary elements described above which are necessary in order to minimise perceived risks in the sperm storage facility, we could then proceed to conduct more rigorous risk analyses for potential risks which are more complex and potentially difficult to evaluate. The following are possible examples:

1. Given geophysical considerations and climate change projections relating to the exact location of the sperm bank, are there real risks of a significant event compromising the integrity of the facility, and if so, how could the risk be at least partially mitigated?
2. Does sperm cryopreservation contribute to epigenetic modification of sperm DNA?
3. Is there any significant risk of chemicals (e.g. ethanol, stains) used during semen analysis affecting sperm samples being processed for cryopreservation in a laminar-flow cabinet?

These risk analyses would require relatively complex evidence gathering based on research into the potential risk factors before a final risk management approach could be developed.

Summary and Conclusions

The key areas for risk minimisation in sperm banks in particular are as follows:

1. Employ scientifically qualified staff who are given specific training in liquid nitrogen handling and ongoing competency assessments for all SOPs. Regular laboratory meetings are essential in order to review laboratory performance and discuss potential improvements.
2. Detailed risk assessments are performed on all SOPs and these are reviewed regularly and updated as procedures change. Any significant changes in the risk assessment status should be communicated to all staff as soon as practically possible.
3. It is imperative that only one semen or sperm sample is processed at a time in each laminar-flow cabinet. Processing should be carried out by one individual with later double-checking of all coding and labelling by a second individual prior to sample freezing, with sign-off by both individuals.
4. All sperm storage facilities should install remote alarming of all liquid nitrogen dewars in addition to regular manual checks and recording of liquid nitrogen depth or weight in each dewar.
5. Thorough evaluation of potential risks in the laboratory and in the surrounding environment is essential. Wherever possible risk mitigation strategies should be implemented and contingency plans prepared.

Appendix 1: Dewar and Dry Shipper Maintenance

All new dewars and dry shippers should have holding-time checks before being used routinely. Regular dewars should be filled with liquid nitrogen to the level that they will be operating at under routine usage conditions and the liquid nitrogen depth and/or weight checked daily for at least one week prior to addition of any storage samples. If the evaporation rate is outside the manufacturer's specifications, the dewar may need to be replaced.

Dry Shipper Procedure

New Dry Shipper

- Determine the "as received" tare weight by weighing the empty shipper with the empty canister and lid in place (=E).
- Record this weight and the shipper serial number on an appropriate worksheet.
- Determine the "complete fill" weight and the "90% charged weight" of the shipper as follows:

1. Fill the dry shipper with liquid to the bottom of the neck tube.
2. Allow the unit to stand undisturbed while the refrigerant is being absorbed.
3. Approximately every 5 min, add liquid to maintain the refrigerant level as the liquid is absorbed by the filler. The procedure can take ten to fifteen cycles.
4. When the liquid level remains at the bottom of the neck tube for at least 30 min, the shipper is full.
5. Pour off any free liquid nitrogen and replace the lid.
   Record the complete fill weight (=F) on the worksheet mentioned above. You now have your baseline "empty" (E) and "full" (F) weights for this shipper. Calculate the 90% charged weight as per the formula below and record it on the worksheet:

$$90\%CW = E + 0.9(F\text{-}E)$$

Holding-Time Check

Check the holding time of the shipper by weighing it daily and checking for liquid nitrogen vapour visually. Record the holding time as the number of days required for the shipper to get down to 10% charged weight with nitrogen vapour still visible.

Acceptable Holding Times

Defined in our laboratory as 3 days less than the manufacturer's static holding time for MVE shippers, or 3 days less than the manufacturer's working time for Taylor-Wharton shippers, however this is up to the laboratory concerned to decide what margin of safety they are comfortable with.

Routine Use of Dry Shippers

- Charge the dry shipper with liquid nitrogen as described above and record the weight on the worksheet used for this particular shipper.
- Do NOT send the dry shipper out unless it is at least 90% charged.
- When the shipper is subsequently returned to the laboratory, re-weigh it (after removing any remaining straws or vials) and record the weight on the worksheet.
- Every 2 years or if the shipper appears to be losing nitrogen more rapidly than on its initial commissioning test run, then allow it to come up to room temperature for re-evaluation of holding time. After the shipper has come up to room temperature, remove the lid and leave the shipper to equilibrate at room temperature for 2–3 days before weighing it again. The shipper is acceptably dry when the tare weight is within 5% of the "as received from the manufacturers" tare weight.

If the shipper does not meet this requirement, then invert it (with lid off) and allow it to stand for 2 weeks before re-weighing it. If necessary, leave it a further 2 weeks. If it is still non-compliant, then it should be de-commissioned and replaced with a new shipper.

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Chapter 17
Licensing and Accreditation of a Sperm Bank for Therapeutic Banking

Michael Jurewicz and Bruce R. Gilbert

Introduction

This chapter provides an overview of the licensing and accreditation procedures for a sperm bank. We discuss the distinction between licensing and accreditation, why each is important, and what is required by each of the associated licensing or accrediting bodies. A comparison of differences between licensing bodies, accrediting bodies, and organizations offering guidance is highlighted in Table 17.1. Requirements on a state-specific basis are described in detail in Table 17.2. Lastly, some basic information about starting a sperm bank is provided.

History

The idea of sperm cryopreservation has been around for a long time. As early as 1776, Spallanzani was noted to report that by cooling sperm they became motionless; however it was not until the 1800s that sperm were actually frozen [1, 2]. As science progressed for the next century scientists refined their technique for freezing sperm. In the 1950s A.S. Parkes and two British scientists developed the method of using glycerol as a sperm cryoprotectant. American Dr. Jerome K. Sherman further refined this method [3–5]. Dr. Sherman developed a protocol for slow cooling of sperm with storage using carbon dioxide. He then demonstrated that thawed sperm were able to fertilize an egg and begin the normal developmental cycle. In 1953 Sherman aided in the first successful human pregnancy with frozen spermatozoa [6, 7]. Due to the difficult social stigma around sperm cryopreservation and assisted

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Table 17.1 Comparison of regulatory body requirements

Blood studies	
FDA	• HIV1, HIV-2, HepB, HepC, syphilis, HTLV1, HTLV-2, NG, CT, CMV
	• Client depositors: No screening required
	• Directed donors do not need to pass donor suitability testing and can be released to parties accepting the risks
AATB	• Anti-HIV-1, anti-HIV-2, HIV-1 NAT, HepBsAg, HepB IgG and IgM, anti-HCV, HCV NAT, anti-HTLV-1, anti-HTLV-2, syphilis, NG, CT, anti-CMV IgG and IgM
	• Client depositors: Anti-HIV-1, anti- HIV-2, HIV-1 NAT, HepBsAg, anti-HCV
	• Donors with positive results are notified
ASRM	• Anti-HIV-1, anti-HIV-2, HIV-1 NAT, HepBsAg, HepB IgG and IgM, anti-HCV, HCV NAT, anti-HTLV-1, anti-HTLV-2, syphilis, NG, CT
	• Additional screening for blood type and Rh
	• Donors with positive results are notified
Medical history	
FDA	• Perform donor screening of relevant medical records for risk factors of or behavior that would be high risk for HIV, HepB, HepC, syphilis, HTLV, NG, CT, Creutzfeldt–Jakob disease, disease associated with xenotransplantation
AATB	• “Donor risk assessment interview” must be completed by the donor or person knowledgeable of donor’s relevant medical history and social behavior that would put them at high risk for disease transmission
ASRM	• A complete detailed personal and sexual history should be obtained using “male donor examination form” from www.Sart.Org to detail any high-risk behavior
Physical exam	
FDA	• None required; however a responsible person must sign off on determination that the donor is free of communicable disease
AATB	• Completed in accordance with AATB’s guidance document no. 1, “tissue donor physical assessment form”
ASRM	• Before acceptance and every 6 months as an active donor, complete physical examination to evaluate for any sign of high-risk behavior should be done using “male donor physical examination form” from www.Sart.Org
Lab requirements/personnel	
FDA	• Must have adequate personnel in terms of education and experience, who are trained to perform their assigned roles
	• Facility should be of adequate size and configuration to prevent cross contamination and transmission of disease
	• Collection and processing should be done in a way to prevent cross contamination
AATB	• Sperm bank should have a director, medical director, and technical staff each with defined qualifications and responsibilities
	• Staff members must demonstrate competency with policies, procedures, and processes and have continuing education as needed. All education should be documented
	• All tissue banks should provide a safe working environment by enforcing safety protocols in accordance with occupational safety and health administration (OSHA) and the CDC
ASRM	• Strict qualifications and responsibilities are defined under CLIA for the lab director, general supervisor, testing personnel, technical supervisors, and clinical consultants
	• Lab must be able to perform semen analysis, sperm penetration testing, sperm cryopreservation, preparation for intrauterine insemination, and computer-assisted semen analysis

(continued)

Table 17.1 (continued)

Quality assurance	
FDA	• Inspection from the FDA is permitted at any "reasonable time in a reasonable manner"
	• Frequency of inspection is up to the FDA
	• A complaint file should be kept and maintained
	• Periodic audits should be performed to ensure adherence to CGTP
AATB	• On-site audits of procedures, policies, and documentation may be performed
	• Twice yearly a bank is responsible for providing documentation that all procedures are done in accordance with AATB standards
	• Routine and focused quality assurance performance audits should be done at least annually to identify trends of recurring problems
ASRM	• All protocols should be validated and reviewed annually by lab director
	• Regular calibration and safety inspections according to National Safety Board Standards
	• Protocol for detecting and reporting errors should be put in place
	• Adverse event file should be kept
Records	
FDA	• Records should be kept of all steps in specimen processing and all personnel involved
	• Records should be kept 10 years after the date of latest disposition of the specimen
AATB	• Records of all steps of processing, storage, and distribution should be documented
	• Records should be kept for 10 years after the tissue is distributed or destroyed
ASRM	• Keep permanent records of each donor's initial selection process and all subsequent evaluations
	• Keep records of outcomes of each insemination cycle and identify heritable diseases

reproduction at the time technology was slow to develop. This finding was finally reported and interest grew in sperm banking after the 11th International Congress of Genetics in 1963 [8]. By the early 1970s this interest developed into the beginnings of commercial sperm banking as we know it today.

Definitions

There are several terms that are commonly used in the standards and licensing documentation that will be defined now as they will be used throughout this chapter.

Donor: A living or deceased individual who is the source of tissue for transplantation in accordance with established medical criteria and procedures.

Directed donor (DD): A reproductive cell or tissue donor who is known to the recipient but is not her sexually intimate partner.

Anonymous donor (AD): A reproductive donor of cells of tissue whose identity is unknown to the recipient.

Client depositor (CD): A person, or persons, who stores reproductive cells of tissues for future use in artificial insemination of assisted reproduction technology procedures for themselves or a sexually intimate partner; not considered a reproductive tissue donor.

Table 17.2 Regulations for reproductive tissue banks by state

State	Requirement	Authority
California	License	CA Health & Safety Code §1635.1—with certain exceptions, all tissue banks operating in California must have a current and valid tissue bank license issued by the Department of Health Services
Delaware	Registration	16 Delaware code § 2801—all sperm banks and tissue banks operating in the state must register with the Department of Health and Social Services by May 1 of each year
District of Columbia	License	DC code 7–1541.03 & CDCR 22–301—all tissue banks operating in the District of Columbia must have a valid license
Florida	Certification	Florida statutes 765.542—an organization may not engage in tissue banking activities in Florida unless it is certified by the Agency for Health Care Administration
Georgia	License	GA comp. Rules & Regs § 290–9–8-04—no clinical laboratory (i.e., tissue bank) may be operated without a license issued by the Department of Human Resources
Illinois	Registration	20 ILCS 2310/2310–330 and 77 Ill. Adm. Code 470.30—all sperm banks and tissue banks operating in the state must register with the Department of Public Health by May 1 of each year
Louisiana	Authorization	32 L.R. § 403 and LAC § 48:I.2901 and 2903—the secretary of the Department of Health and Hospitals will compile and disseminate a list of those nonprofit organ and tissue banks that are authorized to receive donations under this section. The nonprofit tissue banks must submit certain information to the secretary for authorization
Maryland	Permit	MD code Ann. § 17–305—A permit issued by the secretary is necessary to operate a tissue bank in Maryland or to represent or service in Maryland a tissue bank located outside the state
Massachusetts		ALM GL chapter 113, § 7—A tissue bank or storage facility is defined as "a facility licensed, accredited or approved by the Department of Public Health"
Mississippi	Certification	Miss. Code Ann. § 41–39-15—all tissue banks are required to be certified by the Mississippi State Department of Health, which must be renewed annually
New York	License	NY CLS public health § 4364—tissue banks must obtain a license pursuant to this article
Oklahoma	Permit	Oklahoma statutes § 2209.1 and O.A.C. § 310:505–5-1—all tissue banks that procure bone, skin, or connective tissue must obtain a permit issued by the State Department of Health
Oregon	Registration	SB 341 (signed into law 6/11/07)—tissue banks doing business in the state must register with the Department of Health Services

Reproductive tissue (RT): Any cell and/or reproductive tissue from the reproductive tract intended for use in assisted reproductive technology procedures. This might include oocytes, ovarian tissue, embryos, semen, vasal or epididymal fluid, and testicular tissue.

Reproductive tissue bank (RTB): A facility that stores reproductive tissue which might include oocytes, ovarian tissue, embryos, semen, vasal or epididymal fluid, and testicular tissue.

Licensing Versus Accreditation

The main distinction between licensing and accreditation is that regulations and licensing are mandated by a governing body. In the United States this could be the Federal Drug Administration (FDA) which refers to their rules as a "Final Rule" or "Guidance" or states (Table 17.1) which often refer to their rules as a "Regulation." Accreditation is a voluntary process by which the sperm bank agrees to abide by certain standards promulgated from private (nongovernmental) association or governing body. Licensing bodies are generally most concerned with sperm donor eligibility, documentation, and risk of communicable infectious disease. Accreditation delves further into the inner workings of a sperm bank and mandates the bank is managed in a professional manner, with appropriate staff performing tasks in a standard fashion, and has policies and procedures in place to ensure consistent and quality care.

Licensing

In general licensing is needed to provide a minimum set of standards by which a service can be provided, in this case male reproductive tissue banking which we will interchangeably refer to as sperm banking. Licensing is mandatory in the United States by the FDA for all sperm banks and any establishment offering donor sperm services.

Required Reproductive Tissue Bank Regulations

Registration, within 5 days of beginning operation, is required by the FDA (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/EstablishmentRegistration/TissueEstablishmentRegistration/default.htm) for all sperm banks to operate. The reproductive tissue bank must comply with FDA Rules and Guidance and have a valid license if required by the state in which it operates.

What Regulatory Authority Does a Reproductive Tissue Bank Need to Comply with?

There are two regulatory authority mandates that a RTB needs to comply with: the FDA and any state regulations from states that the RTB operates in.

FDA Requirements

The FDA requires registration and compliance with their Rules and Guidance for all RTB that accept, process, or store any human cells or tissue intended for implantation, transplantation, infusion, or transfer into a human recipient. The most recent update from the FDA came in 2007 in a document titled "Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P)." Semen and oocytes are considered HCT/P and thus are regulated as such by the Center for Biologics Evaluation and Research (CBER) under 21 CFR Parts 1270 and 1271. The main tenant of the FDA Rules and Guidance is to establish a system for receiving, processing, storing, and distributing HCT/Ps while minimizing the risk of transmission of communicable disease to HCT/Ps [9].

A summary of the salient portions of 21 CFR Parts 1270 and 1271 as applicable to sperm banks is outlined below. For more detailed information and current guidance as well as registration information please see http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/EstablishmentRegistration/TissueEstablishmentRegistration/default.htm

- General requirements and procedures
  - All tissue banks must be registered with the FDA and list all HCT/Ps with the FDA. The registration must be kept up to date and revised yearly if any changes occur.
  - Each tissue bank registry must include all HCT/Ps recovered, processed, stored, labeled, packaged, and distributed, or on which donor screening or testing was performed.
  - A tissue bank must establish and maintain procedures for all steps of screening, testing, and determining donor eligibility and continually revise and adjust them.
  - All procedures must be reviewed by a responsible person and their guidelines available to those performing the tests.
  - Any deviation from standard procedures must be recorded and justified at the time of occurrence.
  - Inspection from the FDA is permitted with appropriate notification of the RTB. Frequency of inspection is up to the FDA.
- Donor Eligibility (AD, DD)
  - All tissue banks must evaluate donors via screening and testing for transmission of relevant communicable disease agents or diseases (RCDADs). They must be determined to be free from risk factors or clinical evidence of infection from communicable disease. Although not required by the FDA, a sample questionnaire can be found in Appendix 1.
  - For anonymous donors, except for directed donors, a second specimen must be collected and tested 6 months after collection to confirm a communicable disease-free state.

  - RCDADs include human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), human transmissible spongiform encephalopathy, including Creutzfeldt–Jakob disease (CJD), and syphilis (TP).
  - For donors of viable, leukocyte-rich cells and tissues:
  - Human T-lymphotropic virus (HLTV), type I and I, cytomegalovirus (CMV).
  - For donors of reproductive cells or tissues:
  - *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG).
  - Exceptions for screening include:
  - Reproductive cells or tissue donated by a sexually intimate partner of the recipient for reproductive use.
  - A specimen that was originally for use with an intimate partner but is subsequently intended for directed donor use instead (given appropriate measures to screen the donor.)

- Records
  - Records must be maintained that each specimen has an ID number and a statement that the tests for communicable disease were completed together with the results of the screening tests.
  - Records of specimen processing must be kept, including all personnel involved, each step completed, and times and dates of activities.
  - Records should be kept for 10 years from the latest disposition of the specimen.
- Quarantine
  - All specimens are kept in quarantine until donor eligibility testing is completed and required retesting is also completed.
  - Directed donors do not need to pass donor suitability testing and can be released to parties accepting the risks.
- All tissue banks must follow current good tissue practices (CGTP) in order to prevent introduction, transmission, and spread of RCDADs as is outlined in CFR 21 Part 1271 subpart D (AD, DD, CD)
  - Tissue banks should have adequate personnel in terms of education and experience, who are trained and retrained to perform their assigned roles.
  - All facilities must be of suitable size, construction, and location to prevent contamination of HCT/Ps with communicable disease agents and to ensure orderly handling of HCT/Ps without mix-ups.
  - Environmental controls, such as humidity and temperature controls, should be provided in handling and storage areas to prevent contamination or cross-contamination. Inspections of these should be done periodically to ensure their function.
  - All equipment must be installed properly, maintained regularly, and capable of producing valid results.
  - Collection of tissue must be done in a way that prevents contamination or cross-contamination.

  - Processing of HCT/Ps should be done in a way that does not cause contamination or cross-contamination.
  - Labeling and verification must be established and accurate to ensure proper HCT/P identification and to prevent mix-ups.
  - Storage areas must be controlled for temperature when appropriate and corrective action and documentation should be completed when proper storage is not met.
  - Before a sample is distributed its screening and testing must be reviewed to verify that the release criteria are met. A sample that is quarantined, is contaminated, not prepared with the accepted procedure, or is from a donor who has been determined to be ineligible should not be released or must be shipped in quarantine.

- Receipt, Predistribution, and Distribution
  - All HCT/Ps received should be inspected to assure no contamination and decision made to accept, reject, or quarantine the specimen.
  - A specimen should not be released without tracking documentation of its processing, testing, and storage.
  - A specimen should not be released if it is in quarantine, contaminated, or from an ineligible donor unless the recipient has appropriate documentation and accepts these risks.

- Quality Assurance
  - A tissue bank must maintain a quality program that is intended to prevent the spread of communicable disease.
  - It should investigate, evaluate, and document all information regarding core current good tissue practices (CGTP) and possible contamination or deviation from the accept protocol.
  - Corrective actions should be taken and documented for any deficiencies in CGTP.
  - All personnel should be adequately trained and reeducated about current or updated CGTP.
  - Audits should be performed periodically to ensure quality of performance and adherence to standards.
  - Changes in processes should be verified and validated by a responsible person.
  - A complaint file must be created, maintained, and available for the FDA.

State Requirements

Several states require RTB operating in their jurisdiction to be licensed. We have listed those that currently require this in Table 17.2 [10]. However, please check with your state's Department of Health to verify current requirements. Please also be aware that the state in which the RTB facility is located may not be the only state in which licensing is required. If specimens are received or transferred to another state the RTB must also need licensing in that state.

Accreditation

Accreditation is an evaluation process that is voluntary on the part of the RTB, and is used to assess the quality of the services provided by the sperm bank in order for users of the RTB HCT/P to be assured that a certain level of quality will be provided. Accreditation, more so than licensing, is designed to continually refine and improve the quality, efficacy, and effectiveness of the sperm bank's products and services, including its structures, processes, and outcomes.

In general, accreditation uses and adheres to evidence-based standards, which in turn provides the highest quality specimens for storage in a safe and regulated environment to assure the uniform and quality control standards [11, 12]. Participation in the accreditation process is voluntary. It does, however, require that the sperm bank meet and adhere to the standards of the accrediting body and maintain those standards throughout their accreditation.

Accreditation has a variety of benefits to both the consumer and the sperm bank. For the consumer, accreditation provides additional information about the standards and practices of the sperm bank. It provides a level of confidence and reassurance about the quality of care and service they will receive. It may allow them to wade through and choose a bank among a large pool of competitive banks. For a sperm bank, accreditation shows commitment to high-quality care and standards. Although the process can be long and expensive, the knowledge that they are adhering to standards elevates their status among other banks and may draw prospective couples.

Accreditation Organizations

American Association of Tissue Banks

The American Association of Tissue Banks (AATB) was established in 1976 to promote ethics and develop standards of storing human tissue. In 1984 it produced its first manual, which, now in its 14th edition, is regarded as the gold standard for accreditation and licensing of tissue banks in the United States and worldwide. The AATB is the only private tissue banking standard published and is viewed as the most comprehensive and detailed in the world. They serve as an example for federal and state regulations as well as international standards [13]. The AATB membership is composed of individuals with a vested interest and/or expertise in tissue banking. The Standards Committee provides guidelines ("Standards"), which are continuously updated to comply with the most current thinking regarding tissue banking procedures.

A summary of the 2015 AATB requirements for accreditation from "American Association of Tissue Banks: Standards for Tissue Banking" [14] are listed below. For more detailed information and current guidance as well as registration information please see http://www.aatb.org.

- General organization and institutional requirements (AD, DD, CD)
  - The sperm bank should have a governing body (i.e., board of trustees, board of directors).
  - The sperm bank is responsible for making sure that a bank it receives tissue from is in compliance with the AATB standards.
  - Twice yearly the bank is responsible for providing documentation that all procedures are performed in accordance with AATB standards.
  - On-site audits of procedures, policies, and documentation may be performed.
  - Tissue banks must ensure that the laboratory performing infectious disease testing is registered with the FDA and uses FDA-approved donor tests, certified in accordance with CLIA'88 [15], and retain donor infectious disease records for 10 years.
  - Sperm bank should have a director, medical director, and technical staff each with defined qualifications and responsibilities.
  - A quality assurance program should be overseen by the director and annual reviews should be completed. All adverse events should be documented, investigated, and recorded.
  - All banks must develop a donor record system that allows for detailed and reliable documentation:
  - All steps and processing must be documented including consent, donor suitability, tissue recovery, processing, transport, quarantine and infectious disease testing, tissue labeling, storage, release, distribution, and quality control.
  - Records should be kept for at least 10 years beyond the date of distribution.

- General operations (AD, DD, CD)
  - All tissue banks are required to have detailed policies and procedures written in a standard operating procedure manual (SOPM) which includes policies governing every aspect of the bank, from donor suitability to quality assurance and control.
  - Annual review of the SOPM should be completed by the director of all policies and procedures and SOP should be updated to reflect changes in donor suitability and avoid adverse outcomes.
  - The AATB or other regulatory agencies may request SOPM for inspection.
  - All staff members should be trained for competency in policies and procedures from state and federal regulatory agencies in addition to the AATB standards.
  - Staff members must demonstrate competency with policies, procedures, and processes and have continuing education as needed. All education should be documented.
  - All tissue banks should provide a safe working environment by enforcing safety protocols in accordance with occupational safety and health administration (OSHA) and the CDC.
  - The facility should be large enough to meet operational needs. It should have adequate ventilation, lighting, plumbing, etc., as well as be sanitary, orderly, and clean.

 - Equipment should be appropriately selected, calibrated, sterilized, and maintained to service the lab and maintain quality assurances.

- Donor suitability (AD, DD, CD)
  - Must complete "Tissue Donor Physical Assessment Form" (Appendix 2) [14] in compliance with AATB guidelines to identify evidence of high-risk behavior, signs of HIV or hepatitis, other viral or bacterial infections, or trauma.
  - Donor risk assessment should include a past medical history provided by the donor or next of kin if deceased. Should include review of alcohol and drug use and sexually transmitted diseases. Also three-generation family history should be elicited for genetic abnormalities.
  - Disease screening should include HIV-1 and HIV-2 antibodies, HIV-1 NAT, HepBsAg, HepB IgG and IgM, HepC antibodies, HepC NAT, HTLV-1 and HTLV-2 antibodies, syphilis, CMV antibodies.
  - All specimens from donors that are repeatedly reactive on a required screening test should be quarantined.
  - Donors should be notified of results and this should be documented.
  - All directed and anonymous donor semen should be tested, quarantined, and retested 6 months after they are received before release.
  - Semen donors should be younger than 40 years old to minimize genetic anomaly risks.

- Recovery and collection of tissue
  - Informed consent and donor verification are essential and should be documented.
  - All recovery and processing should be done in an environment that limits cross contamination.

- Processing, preservation, and storage
  - Processing should occur in a bacteriologically and climate-controlled environment.
  - Methods for processing should be validated to prevent contamination and cross contamination.
  - The type, amount, concentration, and method of addition of cryoprotectant should be documented and maintained in the standard operating procedure manual (SOPM).
  - Each specimen should be assigned an individual identification number to be used during the collection, processing, storage, and distribution. Second identification numbers will be given to the same donor with subsequent specimens.
  - Pooling of multiple donors should not occur.
  - Reagents and supplies used in processing should remain sterile if indicated, and record kept of lot number, expiration dates, etc.
  - All specimens should be processed and stored in a timely fashion as outlined by the bank SOPM.

  - Records of processing and packaging should be kept and quality control completed by the medical director.
  - Storage areas should be physically separated and clearly marked for quarantined specimens:
  - Tissue from client depositors known to be reactive for infectious diseases such as HIV, hepatitis B, and hepatitis C should be stored separately from seronegative donors.
  - Tissue should be quarantined and stored separately pending completion of processing, preservation, labeling, and quality assurance specifications, or it meets donor suitability criteria.
  - All quarantined tissue records should include reason for quarantine and disposition after release.
  - Tissue should be stored in the liquid or vapor phase of liquid nitrogen.

- Release and transfer of specimens
  - Prior to release each donor should have a release/disposition statement of the person authorizing the release from the processing site stating that all quality control and assurance specifications were completed and reviewed according to the SOPM.
  - Special release circumstances are as follows: In the event that the specimen is reactive for communicable diseases, have not been tested according to current standards, or has not completed the 180-day quarantine or retesting requirements documentation must be provided to include the deviation from the standards and their risks, designation that all has been reviewed by the director, and documentation that this has been reviewed by both parties and all risks were discussed and questions were answered.

- Labeling (AD, DD, CD)
  - Strict labeling protocol should be followed to ensure that errors are minimized and labeling is clear and complete.
  - Cryocontainer labels should include donor or client depositor identification, batch number or code used to determine the date of cryopreservation and stage of development at cryopreservation, and name or code for tissue bank where specimen was processed.
  - Summary of records is required to show that donor was suitable based on screening and testing. A statement that the communicable disease testing was done at an FDA-registered lab which was certified in accordance with CLIA'88.
  - For special release circumstances as noted above, to a sexually intimate partner, the release documentation should include the statements "For use by a sexually intimate partner only," "Not evaluated for infectious substances," and "WARNING: Advise recipient of communicable disease risks." All client depositors with reactive or positive tests should have biohazard label and "WARNING: reactive test results for (test name)."

  - For special release circumstances as noted above a not sexually intimate partner should be labeled accordingly with biohazard label, "WARNING: reactive test results for (test name)," "WARNING: advise recipient of communicable disease risk," or "Not evaluated for infectious substances," accordingly based on the circumstance.
  - For international shipments, requirements from federal government and receiving government should be met.

- Distribution and Dispensing (AD, DD, CD)
  - For a client depositor a sample may only be released with a signed statement that it will be used with a sexually intimate partner.
  - A client depositor who requests release to a nonsexually intimate partner will be treated as a directed donor and be fully tested according to those protocols.
  - Distribution records should be maintained by the distributing tissue bank.
  - Policy should be put in place to receive unused tissue. Given that the shipment container, labeling, and packaging are intact and the specimen is still frozen the tissue may be returned to the bank and not be redistributed.

- Tissue Dispensing (AD, DD, CD)
  - Policies and procedures should be in place to monitor receipt, storage, and disposition of tissue to ensure that all use, transfers, and destruction of samples are safe and traceable.
  - Tissues should only be dispensed with authorization from a physician.
  - When releasing tissue to an intermediary, all labeling materials and other enclosures should be forwarded with the tissue and records maintained of the transfer.
  - Tissue destruction must be authorized by the tissue bank director and the tissue donor.

- Quality assurance (AD, DD, CD)
  - All tissue banks should have quality assurance protocol.
  - Generally quality control, process validation, equipment monitoring, review of donor screening, recovery, and processing should occur with corrective action taken for assessments outside of acceptable limits.
  - Routine and focused quality assurance performance audits should be done at least annually to identify trends of recurring problems.
  - All incidents and adverse events should be documented and resolved.
  - All documentation should be reviewed and archived.
  - Quality control should be in place for work environment, equipment maintenance, process control, reagent and supply monitoring, lab performance monitoring with acceptable variability limits, and corrective actions when necessary.

American Association of Blood Banks

The American Association of Blood Banks (AABB) began developing guidelines for blood banking since the 1950s. In the 1980s the AABB began to release guidance for cellular therapies under which RTB fell. The main goal of the guidelines and accreditation through the AABB is to improve quality and safety of use of HCT/Ps.

A summary of the 2016 AABB requirements for accreditation is listed below [16]. For a more detailed and updated guidance please see http://www.aabb.org/advocacy/regulatorygovernment/ct/hctps/Pages/default.aspx.

- General Organization and Registration
  - Banks must be registered with the FDA using form FDA 3356 and details regarding donor suitability, processing, storage, and distribution for all HCT/Ps must be updated annually.
- Donor Suitability
  - Banks must adhere to the regulations in 21 CFR 1271 Subpart C regarding testing for communicable diseases.
  - While the FDA does not require any specific screening tool for donors, the AABB has developed its own screening tool with which to screen blood bank donors and can be applied to HCT/P (Appendix 3) [17].
  - The required serologic testing is the same as that required from the FDA which includes HIV, HepB, HepC, HTLV 1 and 2, CMV, TP, NG, and CT.
  - Donors should be screened for risk factors for Zika virus and West Nile virus per FDA industry guidance in 2016 [18, 19].
- AABB requires that RTB follow CGTP according to the FDA 21 CFR 1271 Subpart D in regard to facilities, environmental controls, equipment, supplies and reagents, recovery, processing and processing controls, labeling controls, storage, and distribution.
- Reporting
  - Adverse reactions including those that are fatal and life threatening and require medical or surgical treatment must be reported within 15 calendar days.
  - Deviations from the core CGTP in a RTB of contracted facility must be documented and investigated. Information required includes a description of the deviation, information regarding follow-up actions that have or will be taken, and all relevant information related to the HCT/P in question.
- Labeling
  - As per FDA regulations the label should include an identification number, description of specimen, expiration if any, warnings if any, and if feasible.
  - Information that must accompany the HCT/P includes name and address of RTB that provides release of specimen, other warnings, storage temperature, instructions for prevention of transmission, and spread of communicable disease.

Organizations that do Not Accredit or License but Offer Guidelines

American Society of Reproductive Medicine

The American Society for Reproductive Medicine provides guidelines for screening of both sperm and egg donors. They provide the guidelines for gamete and embryo donation as well as guidelines for how to run an andrology lab. Adherence to these guidelines is necessary for their endorsement and is generally accepted by members of the society but observation of the recommendations by a sperm bank is voluntary. They are not involved in performing inspections and no documentation is needed from the ASRM. It is a voluntary set of guidelines that are suggested to ensure quality control of the donor specimen.

A summary of the ASRM guidelines as of 2008 for andrology laboratories and gamete donation is listed below. For detailed information and current guidance as well as registration information please see https://www.asrm.org/Guidelines/.

- General Requirements
  - Must be in compliance with state and federal licensing requirements.
  - Andrology lab is of high complexity and falls under Clinical Laboratory Improvement Act of 1988 (CLIA) regulations.
  - CLIA regulations may change and laboratories must adhere to most current version.
  - Cryopreservation of sperm for therapeutic use is considered transplantation products and as a result must follow the regulations set forth by the FDA for cell/tissue transplantation [20].
  - Personnel requirements: Under CLIA recommendations are made for the responsibilities and qualifications of the lab director, general supervisor, testing personnel, technical supervisors, and clinical consultants. One person may fill multiple roles.
  - ASRM-accredited andrology laboratories should be able to perform semen analysis with sperm viability, sperm membrane integrity tests, and ability of sperm to penetrate human cervical mucus in either a cross-match test or in capillary tubes, sperm antibody testing, sperm penetration or zona-free hamster oocyte test, sperm cryopreservation, preparation of sperm for intrauterine insemination, and computer-assisted semen analysis (CASA) [21–25].
  - Manuals should be produced for the laboratory in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) which should be reviewed annually by the director.
  - Strict logs should be kept of all procedures and personnel completing them, equipment maintenance, and patient specimen log-in.

- Donor suitability [26] (AD, DD).
  - Donor should be of legal age and, ideally, less than 40 years of age.
  - Psychological evaluation and counseling by a qualified mental health professional are strongly recommended.
  - Genetic screening for heritable disease.
  - Donors should be ruled out based on an extensive list of screening questions to elucidate if a donor is at high risk for HIV, STIs, or other transmissible diseases per the "Uniform Donor Application" at www.sart.org (Appendix 4) [27].
  - Donors should undergo complete physical examination with attention to any signs of high-risk behavior that would put them at risk for communicable disease upon donation and every 6 months while an active donor from the "Male donor examination form" at www.sart.org (Appendix 5) [28].
  - Required donor serologic tests: anti-HIV-1 and NAT, anti-HIV-2 or HIV group O antibodies, syphilis, HBVsAg, HBVcAg (IgG and IgM), anti-HCV and NAT, NG, CT, anti-HTLV I and II [29].
  - Donors that test positive must have their samples labeled that way. ASRM requires disclosure and appropriate counseling to the recipient for directed donors while the FDA does not have this provision.
  - Additional screening for blood type and Rh is recommended by ASRM but not FDA.
  - According to the FDA directed donor specimens are exempt from quarantine and only require retesting within 1 week of donation. ASRM states that all donors should be treated similarly and results that exclude or warrant quarantine for anonymous donors should also exclude or warrant quarantine for directed donors.
  - ASRM states that fresh sample use can only be justified with sexually intimate couples due to increased risk of transfer of HIV.
  - The ASRM recommends that all directed donor specimens be frozen and quarantined for a minimum of 180 days, with the donor then retested.
- Records (AD, DD, CD).
  - The ASRM favors much closer record keeping than the FDA. ASRM prefers to have a permanent record of each donor's initial selection process and all subsequent follow-up evaluations. The outcome of each insemination cycle should be recorded and any adverse outcomes should be noted. If a new heritable disease is identified with an offspring both donor and recipient should be tested.
- Laboratory space, design, equipment, and supplies (AD, DD, CD).
  - Must have adequate space and design so that procedures can be performed safely and comfortably.
  - Adequate space available for procedures, record keeping, and administrative work.

- All lab equipment should be kept in good working order and have annual maintenance and should be calibrated regularly.
- Appropriate equipment and supplies should be available and all reagents being used for cryopreservation should be tested for toxicity.
- All supplies used should be labeled with received and opened dates.

• Lab safety and infection control [30] (AD, DD, CD).

- Lab safety manual should be available to all personnel and reviewed annually.
- Policies should be in place to monitor and report adverse incidents and should have corrective action plans.
- Annual safety inspections are mandatory and results kept on file.

• Quality control and assurance [31] (AD, DD, CD)

- All new protocols must be validated.
- Lab director should review and update all procedures yearly.
- All equipment should be calibrated and have maintenance according to the National Safety Board.
- Protocol for detecting and reporting errors should be put in place.
- Adverse incident file should be kept.
- Proficiency testing should be done for all procedures available in the lab.

AUA Statement

No official statement has come out from the AUA on appropriate screening and storage of cryopreserved sperm.

EAU Statement

There is no official statement on accreditation from the EAU but they generally follow the European Directives 2004/23 and 2006/17 and according to the organization "Male Infertility" guideline last updated in 2014 the following suggestions are made [32]:

- Testing should be done to prevent infection and cross contamination.
- Donors should be tested for hepatitis B and C and HIV, *Chlamydia trachomatis* NAT, syphilis, prevalent genetic disorders, and CMV (optional).
- Quarantine should not be used until the results of testing are known.
- Some clinics may store HIV or hepatitis-positive samples separately.
- Facilities that offer long-term storage should have procedures to guard against accidental loss from storage failure.
- Problems may occur ethically with orphan samples.

Specific Guidelines by State

The following is a list of the states with specific requirements for operating a sperm or tissue bank. All states are required to follow the regulations set forth by the FDA and some states have additional requirements below as stated by their bylaws [10]. See Table 17.2:

- California
- Delaware
- DC
- Florida
- Georgia
- Illinois
- Louisiana
- Maryland
- Massachusetts
- Mississippi
- New York
- Oklahoma
- Oregon

International Regulation

The main tenants of the Directive 2004/23/EC of the European Parliament and of the council of 31 March 2004 are the following [33]:

- Quality and safety of the proposed transplanted tissue must be ensured to prevent the transmission of communicable disease.
- Regardless of where the specimen is donated from, one set of high-quality and safety standards should be in place during procurement, processing, testing, and storage to prevent disease.
- Strive for worldwide standards to promote high level of protection for public health.
- Full medical examination is necessary for living donors.
- All human rights and human dignity should be respected in accordance with the Charter of Fundamental Rights of the European Union and protect confidentiality of all health-related information.
- Member states should ensure that all tissue establishments should be accredited authorized, designated, and licensed by a competent authority.

- Member states should ensure that all appropriate measures are in place for quality control and perform inspections at least every 2 years.
- A system for reporting of adverse events should be established by each of the member states.
- Each member state should organize inspection and control measures to ensure that all facilities are complicit in regulations.
- Personnel involved in tissue processing, procurement, preservation, and distribution should be appropriately qualified and trained.
- Labeling systems should be put in place to ensure that all tissue is traceable.
- All establishments should keep a record of their activities and provide authorities annual reports.
- Quality assurance for labeling and safety compliance should be mandatory.
- All establishments should have measures in place to ensure quality control and reliable and accurate documentation.

How to Start the Process of Opening a Reproductive Tissue Bank

The best way to start a RTB is to do your research. This might include reviewing the FDA and state regulations that are readily available online. Review the RTB in your area as well as those located at some distance online. Reach out to those banks and discuss the challenges with the Medical Director. Visit those banks that you would like to emulate. I think you will find that those that are passionate about what they do will be most helpful. The authors of this chapter included.

Conclusion

The ownership and management of reproductive tissue bank is a significant responsibility but is also a source of great satisfaction for those of us with a passion for helping our patients with their reproductive health. Licensing and accreditation are essential to assuring quality for RTB and providing reliable and superior care to couples with their reproductive care. The FDA and state requirements provide a basis for all RTB but are mainly geared toward patient safety through prevention of disease transmission. Accreditation provides additional guidelines and standards for tissue banks to follow in an effort to improve upon what the FDA has set forth and ensure consistent and high-quality HCT/P.

Appendix 1

Patient #: ___________
Date: ______________

Directed Donor Profile

General Information

Donor Name: ______________________

Recipient Name:____________________ Relationship:____________________

QUESTIONS BELOW REFER ONLY TO THE DONOR

Date of Birth: ___________ Place of Birth:___________________

Racial Group/Color Code:
☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_____________ Father:____________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:	
☐ Balding	☐ Curly	☐ Yes	
☐ Thin	☐ Wavy	☐ No	Blood Type:________
☐ Average	☐ Straight		
☐ Thick			

Bone Structure: ☐ Small ☐ Medium ☐ Large ☐ Very Large

Are you predominately: ☐ right-handed ☐ left-handed ☐ ambidextrous

Other distinguishing features (dimples, cleft chin, Roman nose, etc):

____
_

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many
☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

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Educational Background

(circle highest level attained)

High School	1	2	3	4	GPA:_____			
College/University	1	2	3	4	GPA:_____		☐ B.A.	☐ B.S.
Major Area of Study:________________________________								
Post Graduate:	1	2	3	4	5+	GPA:_____	Major:______________	
Degrees Attained:	M.A.	M.S.	Ph.D.	M.D.	J.D.	D.D.S.	Other:______________	
				1				

Personal Characteristics

(Please describe in some detail)

Math
Skills/Ability:____

Mechanical
Skills:____

Athletic
Skills:____

____
_

What is your favorite sport?
____

What languages do you speak?
____

Hobbies / Talents:
____

____
_

Describe your artistic abilities:
____
____
_

What are your favorite foods?
____

____
_

What is your favorite color?
____

Do you like animals? If so, which is your favorite?
____

To where would you like to travel and why?
____

____
_

____
_

How would you describe your personality?
____

____
_

____
_

____

What is your ultimate ambition or goal in life? How do you see yourself in twenty years?

____
_

____
_

____
_

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EMPLOYMENT / OCCUPATIONAL HISTORY

What is your current or most recent occupation?
____

List all jobs you have had in the past five years and any exposure to chemicals and gases. Please consider carefully.

Job/Duties (do not name employer)	Year Employment		Exposure to which chemicals, gases, etc.
	Began	Ended	
1.			
2.			
3.			
4.			
5.			
6.			

FERTILITY HISTORY

Do you have any children? ☐ No ☐ Yes

If yes, how many male children? ____________ female children?______________

For each child, please give age, and list any health problems:

Age Special Health Problems

____

____

____

Have you ever been responsible for any pregnancies other than those listed above? ☐ No ☐ Yes

If yes, what year did itoccur?____

Have you ever been refused as a blood donor? ☐ No ☐ Yes

If yes, explain: ____

Has anyone in your family had difficulty in achieving a pregnancy? ☐ No ☐ Yes

If yes, explain:____

Are there any twins or triplets in your family? ☐ No ☐ Yes

If yes, describe: ____

SOCIAL AND SEXUAL HISTORY

1. Have you had non-therapeutic injected drug use within the past 5 years? ☐ No ☐ Yes
2. Have you had anal or oral intercourse with another man within the past 5 years? ☐ No ☐ Yes
3. Have you been the recipient of factor VIII or factor IX concentrate which was not heat treated or otherwise virally inactivated? ☐ No ☐ Yes
4. Have you ever been treated or given the diagnosis of HIV (AIDS) infection? ☐ No ☐ Yes
5. Have you been an inmate of a correctional facility for 72 consecutive hours or longer with the past 12 months? ☐ No ☐ Yes
6. During the past 12 months are any of your heterosexual partners known to be HIV (AIDS) positive or in any other of the above categories (questions 1 –5)? ☐ No ☐ Yes
7. Have you or any of your partners engaged in prostitution within the past 5 years? ☐ No ☐ Yes
8. How many sexual partners have you had in the past 6 months? _______
9. Is your partner(s)subject to vaginal infections? ☐ No ☐ Yes ☐ Don't know
10. Have your partner ever had any of the following illnesses?

    If so, please note date and treatment:

    Herpes simplex (Type I or II): ☐ No ☐ Yes ☐ Don't know

    Pelvic inflammatory disease (PID): ☐ No ☐ Yes ☐ Don't know

    Venereal disease (VD): ☐ No ☐ Yes ☐ Don't know

    Gonorrhea (GC): ☐ No ☐ Yes ☐ Don't know

    Non-specific urethritis (NSU): ☐ No ☐ Yes ☐ Don't know

    Syphilis: ☐ No ☐ Yes ☐ Don't know

    HIV (AIDS) ☐ No ☐ Yes ☐ Don't know

FAMILY MEDICAL HISTORY

Note: The following questions require knowledge about your family's medical history. You may wish to have your mother or father assist you in obtaining the necessary information.

Has any member of your family, including yourself, had a problem or defect in any of the following body systems?

1. Circulatory System ☐ No ☐ Yes
2. Gastrointestinal System ☐ No ☐ Yes
3. Genital/Urinary System ☐ No ☐ Yes

4. Metabolic (hormones, enzymes, etc.) ☐ No ☐ Yes
5. Nervous System (brain, spinal cord, etc.) ☐ No ☐ Yes
6. Respiratory System ☐ No ☐ Yes
7. Skeletal System (bones, joints, muscles) ☐ No ☐ Yes
8. Organ (heart, lung, kidney, etc.) ☐ No ☐ Yes
9. Other: ______________________ ☐ No ☐ Yes

If yes to any of the above, please list below the specific defect in each case.

Type of birth defect	Affected family member	Age at diagnosis	Relevant Circumstances

Do you have any brothers or sisters who died in infancy or childhood? ☐ No ☐ Yes
If yes, what was the cause?____

____

Are there any diseases or abnormalities that appear to run in your family? ☐ No ☐ Yes
If yes, indicate the disease(s) and the family member(s) affected.___________________________

____

Has anyone in your family, including yourself, experienced recurring and/or chronic symptoms that have not been evaluated by a physician? (Please include those symptoms that you may not consider serious.) ☐ No ☐ Yes
If yes, pleasedescribe:____

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FAMILY MEDICAL HISTORY
(continued)

Relatives	Mother	Father	Siblings		Grandparents				Aunts		Uncles		Maternal Cousins		Paternal Cousins	
			F	M	MGM	MGF	PGM	PGF	Mat	Pat	Mat	Pat	F	M	F	M
Please indicate the # of each in the blank boxes	1	1														

Medical Problem	You	Mother	Father	Siblings		Grandparents				Aunts		Uncles		Maternal Cousins		Paternal Cousins		No One
				F	M	MGM	MGF	PGM	PGF	Mat	Pat	Mat	Pat	F	M	F	M	
1. Cardiovascular																		
a.congenital heart defect																		
b. atherosclerosis																		
c.arteriosclerosis																		
d. heart attack																		
e. high blood pressure																		
f. stroke																		
g. other																		
2. Blood																		
a. anemia																		
b. sickle cell anemia																		
c. hemophilia or other bleeding problem																		
d. leukemia																		
e. immune defiency																		
f. other																		
3. Respiratory (lungs)																		
a. hay fever																		
b. asthma																		
c. emphysema																		
d. tuberculosis																		
e. lung cancer																		
f. pneumonia																		
g. other																		
4.Skin																		
a. acne																		
b. eczema																		
c. melanoma																		
d. skin cancer																		
e. pigmentation disorders																		
f. other																		

6 Patient #: ___________

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Medical Problem	You	Moth er	Fath er	Siblings		Grandparents				Aunts		Uncles		Maternal Cousins		Paternal Cousins		No One
				F	M	MGM	MGF	PGM	PGF	Mat	Pat	Mat	Pat	F	M	F	M	
5. Gastro-Intestinal																		
a. ulcer of stomach or duodenum																		
b. gall stones																		
c. hepatitis A (infectious)																		
d. hepatitis B (serum)																		
e. other liver disease																		
f. colon cancer																		
g. ulcerative colitis																		
h. Crohn's disease																		
i. cystic fibrosis																		
j. intestinal cancer																		
k. other																		
6. Urinary																		
a. kidney disease																		
b. disease of urinary tract (urethra, bladder, ureter)																		
c. other																		
7. Genital/Reproductive System																		
a. undescended testicle																		
b. hypospadias																		
c. prostate cancer																		
d. uterine fibroids																		
e. ovarian cysts																		
f. cancer of cervix or uterus																		
g. breast cancer																		
h. ovarian cancer																		
i. other																		

Comments:____

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Medical Problem	You	Mother	Father	Siblings		Grandparents				Aunts		Uncles		Maternal Cousins		Paternal Cousins		No One
				F	M	MGM	MGF	PGM	PGF	Mat	Pat	Mat	Pat	F	M	F	M	
8. Metabolic/ Endocrine																		
a. diabetes mellitus																		
b. hypoglycemia																		
c. thyroid cancer																		
d. thyroid disease																		
e. goiter																		
f. adrenal dysfunction or disorder																		
g. other																		
9. Neurological																		
a. migraines																		
b. mental retardation																		
c. senility before age 50																		
d. Alzheimer’s disease																		
e. multiple sclerosis																		
f. cerebral palsy																		
g. epilepsy or seizure disorder																		
h. hydrocephalus																		
i. disorders of spinal cord																		
j. Huntington’s disease																		
k. Gaucher’s disease																		
l. Wilson’s disease																		
m. delay in growth and/or development																		
n. learning disorder, learning problem																		
o. Other																		
10. Mental health																		
a. schizophrenia																		
b. manic depressive illness																		
c. other mental health disorders requiring hospitalization																		
d. sever depression with periods of inability to function																		
e. other																		

Comments:____

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Medical Problem	You	Mother	Father	Siblings		Grandparents				Aunts		Uncles		Maternal Cousins		Paternal Cousins		No One
				F	M	MGM	MGF	PGM	PGF	Mat	Pat	Mat	Pat	F	M	F	M	
11. Muscle/Bones/ Joints																		
a. muscular dystrophy																		
b. other chronic muscle disease																		
c. lupus																		
d. deformity of spine																		
e. osteoporosis																		
f. dwarfism																		
g. hereditary low back disease																		
h. arthritis (rheumatoid, osteo-, unknown type)																		
i. gout																		
j. other																		
12. Sight/ sound/ smell																		
a. deafness before age 60																		
b. significant hearing loss																		
c. deformity of the ear																		
d. cataracts before age 50																		
e. blindness																		
f. color blindness																		
g. glaucoma																		
h. deviated septum																		
i. any other sight/ sound/ smell disorder																		
13. Other																		
a. alcoholism																		
b. drug abuse, misuse, addiction																		
c. any other cancer not mentioned above																		
d. any other condition not mentioned above																		

Comments:____

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PERSONAL HEALTH HISTORY

Do you currently have any allergies? ☐ No ☐ Yes
If yes, they are to : ☐Food ☐ Drugs ☐Plants ☐Other

Please list specific substances and reaction(s) produced:

Substance	Reaction

Describe any childhood allergies you had:____

How is your vision (without corrective lenses)? ☐ Excellent ☐ Good ☐ Fair ☐ Poor
Do you were corrective lenses? ☐ No ☐ Yes Your vision is:20/___
Are you: ☐ Nearsighted ☐ Farsighted ☐ Other (specify)_______________

Do you have any hearing impairments? ☐ No ☐ Yes
If yes, please describe: ____

Condition of your teeth (check one): ☐ Good ☐ Fair ☐ Poor

Your diet is: ☐ Good ☐ Fair ☐ Poor
Any dietary restrictions?____

Dietary Supplements (vitamins, etc):____

How often do you exercise? ☐ Regularly ☐ Occasionally ☐ Rarely
Type of exercise:____

Have you ever had surgery? ☐ No ☐ Yes
If yes, please list all surgeries:
1.____ Year _______

2.____ Year _______

3. ____ Year _______

Have you had any hospitalization not mentioned? ☐ No ☐ Yes
If yes, please explain: ____

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PERSONAL HEALTH HISTORY
(continued)

Have you had major x-ray exposure or other radiation exposure? ☐ No ☐Yes
If yes, please explain: ____

Have you or your sexual partners ever had:			Myself	Partner	When
NSU (non- specific urethritis)	☐ No	☐ Yes	________	______	______
Chlamydia	☐ No	☐ Yes	________	______	______
Genital warts	☐ No	☐ Yes	________	______	______
Genital Herpes	☐ No	☐ Yes	________	______	______
Other(s)	☐ No	☐ Yes	________	______	______

Type(s):____

Have you ever been treated for sexually transmitted disease(s)? ☐ No ☐ Yes
If yes, for which disease(s): ____

When?________________ Details?____

When was the last time you were treated?____

Have you ever had any major illness such as amoebic dysentery, hepatitis, pneumonia, mononucleosis, Etc? ☐ No ☐ Yes
If yes, please explain:____

Do you have any chronic medical problems or conditions? ☐ No ☐ Yes
If yes, please explain:____

Have you ever been exposed to herbicides or toxic chemicals? ☐ No ☐ Yes
If yes, please explain: ____

Have you ever served overseas in the military? ☐ No ☐ Yes
If yes, please explain: ____

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PERSONAL HEALTH HISTORY
(continued)

Please list any medications you are currently taking:____________________________________

Please list any prescription, non-prescription or recreational drugs that you have used or are currently using.

Describe any drug use as indicated below.

Name Of Drug	Date Started	Date Ended	Frequency of Use	How Used?
___________	__________	_________	_____________	_________________
___________	__________	_________	_____________	_________________
___________	__________	_________	_____________	_________________
___________	__________	_________	_____________	_________________
___________	__________	_________	_____________	_________________
___________	__________	_________	_____________	_________________

How many alcoholic drinks do you consume during an average week? _____________________

Have you ever had a drinking problem? ☐ No ☐ Yes

If yes, describe: ____

Have you ever been treated for alcohol or drug abuse? ☐ No ☐ Yes

If yes, describe: ____

Do you smoke cigarettes? ☐ No ☐ Yes

If yes, how many packs/day?____

How long have you been smoking regularly? ____

Patient # __________
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FAMILY HISTORY SECTION

Pages 15 through 26 contain detailed information regarding the donor's family members, including his parents, siblings, grandparents, aunts and uncles. One page is used for each family member. Therefore if the donor has more than one sister, you will find more than one page 17. If the donor has no sisters, page 17 will be blank. The same applied to brothers, aunts and uncles.

For a summary of the number of family members, please refer to the top portion of page 6 in this profile.

Family History
Mother of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:
☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_____________ Father:____________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:
☐ Balding
☐ Thin
☐ Average
☐ Thick

Hair Type:
☐ Curly
☐ Wavy
☐ Straight

Corrective Lenses:
☐ Yes
☐ No

Blood Type:________

Other distinguishing features (dimples, cleft chin, Roman nose, etc):__________________
____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many
☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe her health: ☐ Excellent ☐Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:
____

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________ 16
v.051403

Family History
Father of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:
☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_____________ Father:____________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐No
☐ Average	☐ Straight	☐ Thick

Blood Type:________

Other distinguishing features (dimples, cleft chin, Roman nose, etc):
____

Skin Characteristics:
Freckles: ☐ None ☐ Few ☐ Many
☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe his health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:____

What kind of person is/was he?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # _________ 17
v.051403

Family History
Sister of Donor

Year of Birth: 19__ Place of Birth: ___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother: _______________ Father: ________________

Religion Born Into:

Donor: ____________ Mother: _______________ Father:_________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________

☐ Average	☐ Straight	☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):______________________
____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many

☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe her health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:____

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________ 18
v.051403

Family History
Brother of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:
☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_____________ Father:_______________

Religion Born Into:
Donor:____________ Mother:_____________ Father:________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________

☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):
____

Skin Characteristics:
Freckles: ☐ None ☐ Few ☐ Many
☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe his health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:___________________________________

What kind of person is/was he?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________
v.051403

Family History
Maternal Grandmother of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_____________ Father:________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________
☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):
____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many
☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ______________________________________

If living describe her health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:____

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________ 20
v.051403

Family History
Maternal Grandfather of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_____________ Father:________________

Religion Born Into:
Donor:____________ Mother:_____________ Father:_________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________

☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):

____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many

☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe his health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:_______________________________________

What kind of person is/was he?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Family History
Paternal Grandfather of Donor

Year of Birth: 19__ Place of Birth:__________________

Racial Group/Color Code:
☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:______________ Father:_______________

Religion Born Into:
Donor:____________ Mother:_____________ Father:____________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Cobr:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:	
☐ Balding	☐ Curly	☐ Yes	
☐ Thin	☐ Wavy	☐ No	Blood Type:________
☐ Average	☐ Straight		
☐ Thick			

Other distinguishing features (dimples, cleft chin, Roman nose, etc.):
____
_

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many
☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____
_

Education: ____

Special Skills or Characteristics: ____

If living describe his health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:
____

What kind of person is/was he?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________ 26
v.051403

Family History
Paternal Grandmother of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_______________ Father:________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair: ☐ Balding ☐ Thin

Hair Type: ☐ Curly ☐ Wavy

Corrective Lenses: ☐ Yes ☐ No

Blood Type:________

☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):

____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many

☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe her health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:____

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________
v.051403

Family History
Maternal Aunt of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:________________ Father:________________

Religion Born Into:
Donor:_____________ Mother:________________ Father:________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________

☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):

____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many

☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe her health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:____

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________ 23
v.051403

Family History
Maternal Uncle of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_______________ Father:________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________

☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):

____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many

☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe her health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:_______________________________________

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Family History
Paternal Aunt of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_______________ Father:________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________

☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):

____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many

☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe her health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:______________________________________

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

Patient # __________ 25
v.051403

Family History
Paternal Uncle of Donor

Year of Birth: 19__ Place of Birth:___________________

Racial Group/Color Code:

☐ Caucasian/White ☐ Black/Black ☐ Asian/Yellow ☐ Other/Red

Ethnic Origin/ Ancestry: Mother:_______________ Father:________________

Religion Born Into:
Donor:____________ Mother:_______________ Father:________________

If Jewish: ☐ Ashkenazi ☐ Sephardic ☐ Oriental

Height: _____ Weight:_____ Eye Color:_____ Hair Color:_____

Hair:	Hair Type:	Corrective Lenses:
☐ Balding	☐ Curly	☐ Yes
☐ Thin	☐ Wavy	☐ No

Blood Type:________

☐ Average ☐ Straight ☐ Thick

Other distinguishing features (dimples, cleft chin, Roman nose, etc):

____

Skin Characteristics:

Freckles: ☐ None ☐ Few ☐ Many
☐ Very Fair (little to no ability to tan on sun exposure)
☐ Fair (skin will tan lightly on sun exposure)
☐ Medium (light color but will tan moderate to dark)
☐ Olive (pigmentation of unexposed skin) ☐ Light ☐ Moderate ☐ Dark
☐ Dark (unexposed skin) ☐ Light Tan ☐ Dark Tan ☐ Brown ☐ Black

Occupation:____

Education: ____

Special Skills or Characteristics: ____

If living describe her health: ☐ Excellent ☐ Good ☐ Fair ☐ Poor

If deceased, give cause and age at time of death:_______________________________________

What kind of person is/was she?

Optimistic	1	2	3	4	Pessimistic
Assertive	1	2	3	4	Passive
Leader	1	2	3	4	Follower
Easy going	1	2	3	4	Controlling, rigid

NEW YORK CRYO

900 Northern Blvd., Suite 230, Great Neck, New York

Telephone (515) 487-2700 Fax (516) 487-2007

Screening of potential donors of oocytes or sperm

In order to safeguard those who donate and receive tissue, the NY State DOH, FDA and AATB requires testing of potential donors. The questionnaire below seeks your complete honesty and accuracy.
If you have any questions during the screening process, please ask your nurse or physician for assistance.

	Review of risks for Creutzfeldt-Jakob disease	Yes	No	NY Cryo comments
1	Have you or a family member have confirmed (gene sequencing) Creutzfeldt-Jakob ("Mad Cow") disease, Variant Creutzfeldt-Jakob disease, or risk for CJD? • If family members have confirmed diagnoses of CJD or vCJD, how many family members were diagnosed? [________]			
	Did you reside in the UK (includes England, Northern Ireland, Scotland, Wales, Isle of Man, Channel islands) for ≥3 months between 1980 and 1996?			
2	Were you a member of the US military, a civilian military employee or a dependent of a member of the US military who spent a total of 6 months on, or associated with, a military base stationed in northern Europe (Germany, Belgium, Netherlands) between 1980 -1990?			
	Were you a member of the US military, a civilian military employee or a dependent of a member of the US military who spent a total of 6 months on, or associated with, a military base stationed in Spain, Portugal, Turkey, Italy or Greece between 1980 and 1996?			
3	Have you visited or lived in the UK for three or more months between 1980 and 1996?			
4	Have you received blood or blood components in the UK or France since 1980?			
5	Have you traveled or lived a cumulative time of 5 years or more since 1980 to the present in any combination of countries in Europe?			
6	Have you received human pituitary-derived growth hormone (used until 1985) or a non-synthetic dura mater (brain covering) graft?			
7	Have you been treated with bovine (beef) insulin (used to treat diabetes) since 1980?			
8	Do you have a biologic relative who has been diagnosed with CJD? (Biologic relative in this setting means father, sibling, grandparent, aunt, uncle or children).			
9	Have you been diagnosed with dementia or any degenerative or demyelinating disease of the central nervous system?			
	Have you been diagnosed with any other neurological disease of unknown cause?			
	Have you, or your sexual partner, resided in Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Niger or Nigeria after 1977?			
	Review of risks for HIV	Yes	No	NY Cryo comments
10	Have you ever had prior reactive (positive) screening test for HIV?			
11	Have you had sex with some-one who has been diagnosed with HIV?			
12	Have you used needles to take injectable drugs for non-medical use, including steroids, or anything not prescribed by a doctor (including intravenous, intramuscular and subcutaneous injections) within the past 5 years?			
13	Have you engaged in sex in exchange for money or drugs in the past 5 years?			
14	Have you received human-derived clotting factor concentrates for a bleeding disorder such as hemophilia or related blood clotting disorder within the past 5 years?			
15	Have you participated in male-to-male sexual activity, or participated in sexual activity with a man who had male-to-male sexual activity, in the past 5 years?			

16	Were you or your sexual partners born or lived in certain countries in Africa after 1977			
17	Have you received a blood transfusion or any medical treatment that involved blood in Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Niger or Nigeria after 1977?			
18	Have you had sexual contact in the past 12 months with anyone described in questions 12-17?			
	HIV signs and symptoms	Yes	No	C F P comments
19	Do you have unexplained weight loss (10 pounds or more in less than 2 months), night sweats or swollen lymph nodes (lumps in your neck, armpits or groin) for longer than one month?			
20	Have you had an unexplained temperature of >100.5°F for 10 or more days?			
21	Have you had unexplained white spots or unusual blemishes in the mouth?			
22	Do you have blue/purple spots under the skin or mucous membranes?			
23	Do you have unexplained cough, shortness of breath, persistent diarrhea or other infection(s)?			
	Risk of HTLV (Human T-lymphotrophic virus)	Yes	No	NY Cryo comments
24	Have you ever had a prior reactive (positive) screening test for HTLV?			
25	Have you ever tested positive for Adult T-cell leukemia.			
26	Have you ever experienced weakness in your lower extremities (paraparesis)?			
	Risk of Hepatitis infection	Yes	No	NY Cryo comments
27	Have you ever had prior reactive (positive) screening for Hepatitis B or C virus?			
28	Have you had unexplained jaundice (yellow skin, yellowing of the whites of the eyes) or enlarged liver?			
29	Have you been diagnosed with clinical, symptomatic viral hepatitis since age 11? • If 'Yes', at the time of illness, was it documented as being caused by hepatitis A, Epstein-Barr Virus (EBV) or cytomegalovirus (CMV)?			
30	Have you received a tattoo or body piercing within the past 12 months in which sterile procedures were not used?			
31	Have you been exposed in the preceding 12 months to known or suspected HIV, HBV or HCV-infected blood through needle-0stick or through contact with an open wound, non-intact skin or mucous membrane?			
32	Have you had close contact within 12 months with another person having clinically active hepatitis B or hepatitis C infection, i.e. living in the same household, where sharing of kitchen and bathroom facilities occurs regularly?			
33	Have you ever been incarcerated (jail, juvenile detention, lock-up or prison) for more than 72 hours during the past 12 months?			
	Risk factor assessment	Yes	No	NYCryo comments
34	Have you had sex with any person known or suspected to have clinically active hepatitis B infections or hepatitis C infection?			
	Risk of sepsis	Yes	No	NYCryo comments
41	Have you had an unexplained fever (>100.4°F), fast heart rate (>90 beats/minute) and fast respiratory rate (>20 breaths/minute) within the past 7 days?			
42	Have you been diagnosed with or treated for sepsis or have elevated white blood cell count (>12,000/mm^3) or positive blood cultures associated with sepsis within the past 7 days?			
43	Do you currently have severe signs and symptoms of sepsis; unexplained low oxygen in the blood, very low urine output, altered mental functioning or low blood pressure?			

	Risk of Syphilis/STD infection	Yes	No	NYCryo comments
44	Have you been treated for Syphilis in the past 12 months?			
45	Have you been treated for Gonorrhea in the past 12 months?			
46	Have you contracted Chlamydia, venereal warts (HPV) or genital herpes in the past 12 months?			
	Risk of Vaccinia (Smallpox)	Yes	No	NYCryo comments
47	Have you had a Smallpox vaccination in the past 8 weeks? • If 'Yes', did the scab separate spontaneously? • If 'Yes', did you develop any complications (i.e. skin rashes/sores beyond the vaccination site, infection of the cornea, or general illness) related to the vaccination?			
48	Have you developed skin rashes/sores/skin lesions since close contact with someone who received a Small pox vaccination? • If 'Yes', did the scab(s) separate spontaneously?			
	Risks of West Nile virus	Yes	No	NYCryo comments
50	Have you had a medical diagnosis of WNV (including diagnosis based on symptoms and laboratory results or confirmed viremia)? • If 'Yes', was the diagnosis supported by an FDA licensed or investigational WNV NAT?			
51	Within the past 7 days, have you; • Had a fever and headache, body aches or eye pain? • Had swollen lymph glands? • Had a skin rash on the trunk of the body? • Had a severe illness; encephalitis, meningitis, meningoenceph-alitis, paralysis? • Had severe illness with headache, high fever, neck stiffness, disorientation, coma or tremors? • Experienced convulsions, muscle weakness or paralysis?			
	Xeno-transplantation (transplant or other medical procedure that involved being exposed to live cells, tissues or organs from an animal)	Yes	No	NYCryo comments
57	Are you a recipient, or have you had intimate contact with a recipient, of a xeno-transplantation product? (Surgical transfer of live cells, tissues or whole organs from an animal). • If 'yes', or if this person resides in your household, have you been exposed to blood, saliva, or other body fluids from this person?			

I certify that all the information provided above is complete and true to the best of my knowledge.

____________________ ____________________ ___/___/______

Donor name (Print) Donor signature Date

____________________ ____________________ ___/___/______

NYCryo witness name (Print) NYCryo witness signature Date

Appendix 2

Sample Tissue Donor Physical Assessment Form

Identification
Name stated on Consent (Authorization) : ______________________________

Age: _______ days months years Recovery Agency ID#: ______________________

Sex/gender: Male Female Race: ________________ ____________ID#:______________

Weight: _________ lbs. kgs Weight is: estimated/team , reported (source: ___________) , actual

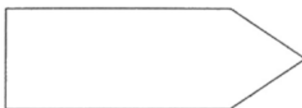
Height: _________ft. in. cm. Height is: estimated/team, reported (source: ___________), actual

Manner identified by: hospital ID band, toe tag, other (describe) ______________________

Identification Band/Tag

ID re-created as closely as possible,

or circle N/A (if not present).



Personnel confirming donor identification: ______________________________Date/time:__________

Evidence of Donation/Autopsy

Eye donation: whole eyes, corneas only, N/A ; Organ donation: Yes No UNOS#: __________

Autopsy: tissue recovery is pre-, or post-autopsy (full, limited); no autopsy planned; or, plan unknown

Recovery Team Assessment:
Is there evidence of:

Jaundice —————————— Yes ——— No

Genital lesions —————————— Yes ——— No

Enlarged lymph nodes —————————— Yes ——— No

Tattoo/piercing —————————— Yes ——— No

White spots in the mouth —————————— Yes ——— No ——— Unable to visualize

Nonmedical injection sites —————————— Yes ——— No

Enlarged liver (hepatomegaly) —————————— Yes ——— No

Insertion trauma/perianal lesions —————————— Yes ——— No

Rash/scab/skin lesion (nongenital) —————————— Yes ——— No

Blue/purple (gray/black) spots/lesions —————————— Yes ——— No

Trauma/infection to potential retrieval sites ——— Yes ——— No

Abnormal ocular finding (e.g., icterus, scarring)—— Yes ——— No ——— Unable to visualize

Notes/Explain if "unable to visualize," or if any answers are "Yes": ______________________

____

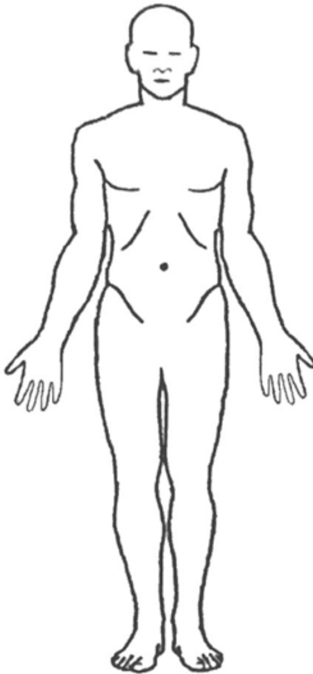
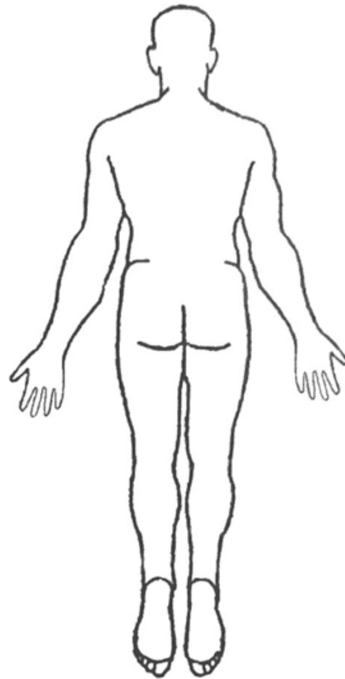
General Appearance
Cleanliness: Good Poor; Describe if "poor" ______________________

Personnel performing physical assessment: ______________________________Date/time:__________

______________________________	______________________________	__________	__________
Name of Person Completing Form (Print)	Signature	Initials	Date

AATB Sample Tissue Donor Physical Assessment Form
Recovery Agency ID#: ______________

Recovery Team Assessment: (continued)

Key to schematics:

(A) Abrasion	(J) Team blood draw site	(T) Tattoo – requires description
(B) Bruise/Contusion	(L) Laceration/Wound	(U) Urethral catheter
(C) Cast/Ortho device	(M) ID band/tag	(V) Skin lesion
(D) Dressing/Bandage	(N) Needle entry site	(W) Scab
(E) ET tube/NG tube	(O) Organ recovery incision	( ) ______________
(F) Fracture/Dislocation	(P) Body Piercing – requires description	( ) ______________
(H) Hematoma	(R) Rash	( ) ______________
(I) IV/Arterial line	(S) Scar (surgical/trauma)	( ) ______________

Summary
A review of available medical records and physical assessment findings were completed and found to be acceptable/not acceptable prior to recovery. ______________________ ____________
(Circle one) (Responsible person) (Date/time)

Page 14 of 16

Appendix 3

Full-Length Donor History Questionnaire (DHQ)

	Yes	No
Are you		
1. Feeling healthy and well today?	❑	❑
2. Currently taking an antibiotic?	❑	❑
3. Currently taking any other medication for an infection?	❑	❑
4. Have you taken any medications on the Medication Deferral List in the time frames indicated? (Review the Medication Deferral List.)	❑	❑
5. Have you read the educational materials today?	❑	❑
In the past **48 hours,**		
6. Have you taken aspirin or anything that has aspirin in it?	❑	❑
In the past **8 weeks,** have you		
7. Donated blood, platelets or plasma?	❑	❑
8. Had any vaccinations or other shots?	❑	❑
9. Had contact with someone who was vaccinated for smallpox in the past 8 weeks?	❑	❑
In the past **16 weeks,**		
10. Have you donated a double unit of red cells using an apheresis machine?	❑	❑
In the past **12 months,** have you		
11. Had a blood transfusion?	❑	❑
12. Had a transplant such as organ, tissue, or bone marrow?	❑	❑
13. Had a graft such as bone or skin?	❑	❑
14. Come into contact with someone else's blood?	❑	❑
15. Had an accidental needle-stick?	❑	❑
16. Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?	❑	❑
17. Had sexual contact with a prostitute or anyone else who takes money or drugs or other payment for sex?	❑	❑
18. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything not prescribed by their doctor?	❑	❑
19. Male donors: Had sexual contact with another male?	❑	❑
20. Female donors: Had sexual contact with a male who had sexual contact with another male in the past 12 months?	❑	❑
21. Had sexual contact with a person who has hepatitis?	❑	❑
22. Lived with a person who has hepatitis?	❑	❑
23. Had a tattoo?	❑	❑
24. Had ear or body piercing?	❑	❑
25. Had or been treated for syphilis or gonorrhea?	❑	❑
26. Been in juvenile detention, lockup, jail, or prison for more than 72 consecutive hours?	❑	❑

In the past **three years,** have you		
27. Been outside the United States or Canada?	❑	❑
From **1980 through 1996,**		
28. Did you spend time that adds up to 3 months or more in the United Kingdom? (Review list of countries in the UK)	❑	❑
29. Were you a member of the U.S. military, a civilian military employee, or a dependent of a member of the U.S. military?	❑	❑
From **1980 to the present**, did you		
30. Spend time that adds up to 5 years or more in Europe? (Review list of countries in Europe.)	❑	❑
31. Receive a blood transfusion in the United Kingdom or France? (Review country lists.)	❑	❑
Have you **EVER**		
32. Female donors: Been pregnant or are you pregnant now?	❑	❑
33. Had a positive test for the HIV/AIDS virus?	❑	❑
34. Used needles to take drugs, steroids, or anything not prescribed by your doctor?	❑	❑
35. Received money, drugs, or other payment for sex?	❑	❑
36. Had malaria?	❑	❑
37. Had Chagas disease?	❑	❑
38. Had babesiosis?	❑	❑
39. Received a dura mater (or brain covering) graft or xenotransplantation product?	❑	❑
40. Had any type of cancer, including leukemia?	❑	❑
41. Had any problems with your heart or lungs?	❑	❑
42. Had a bleeding condition or a blood disease?	❑	❑
43. Have any of your relatives had Creutzfeldt-Jakob disease?	❑	❑

Use this area for additional questions	Yes	No

Appendix 4

Donation Application Form **DONOR NUMBER ________________** Page 1

UNIFORM DONOR APPLICATION FORM

Date filled out: _____/_____/_____ (Month/Day/Year)

To become a sperm or egg donor, we need to learn some information about your personal and medical history. Your responses to these questions will help us to make sure that your health and medical history are compatible with the donation process and in particular for egg donors that it will not involve any increased risks for you. This effort will also help us to match you to an appropriate recipient.

Please provide complete and accurate information to these questions. If you do not know the answer, ask a parent or family member. Any information you provide during the donation process, will remain completely confidential. Some of the information from this questionnaire will be given to the recipient(s) as noted but all identifying information is removed.

A “yes” response will not necessarily eliminate you as a potential donor. Most people will have at least one of these conditions in themselves or a family member. The accuracy of the information you will be giving will provide information to potential families you may help to create.
Instructions:

1. **Please fill in all blanks completely.** Please complete all questions and write “N/A” if not applicable.
2. Please be specific. Avoid expressions such as “natural” or “old age” (for causes of death). List any health problems as specifically as possible. If you do not know the age, put the approximate age or ask a relative to help you. List exact relationships such as “first cousin through my mother’s sister”.
3. Please provide information on all the relatives requested. Do not write their names.
4. If you have any questions, please call your donor coordinator.

Last name: __________________________ First name: ___________________ Middle Initial: _____

Sex: Male ______ Female ______ Age: _______

Date of Birth: ____/____/____ Place of Birth:_________________

Soc. Security #: ______-______-________ Are you a US citizen or permanent resident? ☐ Yes ☐ No

Driver’s License #:______________________ State:__________

Marital Status: ____single ____married ____ divorced ____ widowed ______engaged _____partnered

Length of Current Relationship: _____ years

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
FORM NUMBER:	**EFFECTIVITY DATE:**

Donation Application Form **DONOR NUMBER** ________________ Page 2

DEMOGRAPHICS

MAILING ADDRESS:

Street: ____City: ___________________________

State/Province: ____________ Zip/ Postal code: _______________ Country: ______________

OK to leave message?

Home Phone Number: ( ) _______- ________ ☐ Yes ☐ No

Work Phone Number: ( ) _______- ________ ☐ Yes ☐ No

Cell Phone Number: ( ) _______- ________ ☐ Yes ☐ No

Email Address: ____

Do you have medical insurance? ____Yes ____No

If yes, name of carrier: ____________ ID #:______________ Group #__________________

Employer: ____

DONATION HISTORY:

Have you applied or been screened to be an egg or sperm donor before? ____Yes ____No

If yes, list name and location of donor program (s): ____

____

Have you donated before? ____Yes ____No If yes, how many times did you donate or cycle? ____

Are you currently enrolled as an egg or sperm donor in another program? ____Yes ____No

How did you hear about our program?

☐ Radio (which station)_________________ ☐ Friend (name)______________________
☐ Newspaper (which one) _______________ ☐ Magazine (which one)___________________
☐ Website (which one) ________________ ☐ Other (specify)____________________

Did you consult with your family when completing your family medical history? _____Yes _____No

I hereby attest that all information disclosed in this application is accurate, true, and up-to-date to the best of my knowledge. ____

(Signature of Applicant)

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
FORM NUMBER:	**EFFECTIVITY DATE:**

Donation Application Form **DONOR NUMBER** ________________ Page 3

PERSONAL HEALTH HISTORY

Are you currently under a physicians care for any reason? _____Yes _____No

If yes, please explain: ____

Have you ever had any major illnesses such as amoebic dysentery (infection of the intestine), hypertension, blood clots, pneumonia, mononucleosis, etc.? ____Yes ____No

If yes, when? ____

Have you had any serious illness in the past? _____ Yes _____ No
If yes, please describe: ____

Did you have any complications or concerns with anesthesia? ____

Have you had any hospitalization(s) not mentioned above? ____

____

Please list any surgical procedures:
____
____
____
____

Have you ever had any broken bones? _____Yes _____ No If yes, please list: ______________________________

How many days in the preceding 12 months did you miss work because of illness (colds, flu, accidents, surgery, etc.)?
Please explain:____

Has anyone in your family, including yourself, experienced recurring and/or chronic physical symptoms that have not been evaluated by a physician (Please include those symptoms that you may not consider serious.)? _____Yes _____No
If yes, please describe:____.

Have you ever been seen by psychiatrist, psychologist, social worker, counselor, or any other mental health professional for any reason? ____Yes _____No
If yes, when, for how long and for what reason? ____

Have you ever used medications such as antianxiety or antidepressants to treat an emotional or psychological problem?
____Yes ____No
If yes, list why and date last used ____

Have you been vaccinated in the last 6 months? _____Yes _____No

If yes, what were you vaccinated for? ____

List all medications that you have taken in the preceding 12 months (prescription):

Medication	How Often	Reason
____________________	_________	____
____________________	_________	____
____________________	_________	____
____________________	_________	____

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
FORM NUMBER:	**EFFECTIVITY DATE:**

Donation Application Form **DONOR NUMBER** ________________ Page 4

PERSONAL HEALTH HISTORY (continued)

List all current over-the-counter medications (include hormones, vitamins, aspirin, antacids, laxatives, herbal & sports supplements, performance-enhancing supplements including steroids, etc.)

Medication	How Often	Reason
____________________	_________	____
____________________	_________	____
____________________	_________	____
____________________	_________	____

Have you ever taken anti-malarial drugs or had malaria? _____Yes _____No

Have you had a blood transfusion? _____Yes _____No If yes, when? _______________

Have you ever been refused or denied as a blood donor? _____Yes _____No If yes, why? ____________________

Are you eligible to work in the United States? _____Yes ____No Is your work schedule flexible? ____Yes ____No

List all the jobs you held in the past five years:

Jobs/Duties	Year Began	Year End

Have you had radiation exposure or x-ray exposure? _____Yes _____No
If yes, please explain: ____

Have you ever been exposed to "agent orange" or any other herbicides or chemicals (military, forestry, highway service, or elsewhere)? _____Yes _____No

If yes, which substance(s)? ____

When? __________________________________ Where? ______________________________________

In the preceding six months, were you exposed to the following in your job, living environment or while involved in hobbies? If yes to any of these, give dates and how often you have been exposed. Please consider carefully.

Exposed to:	Response		When?	How Often?
Toxic Chemicals or Substances	Yes	No		
Sprays	Yes	No		
Fumes/Exhaust	Yes	No		
Radiation	Yes	No		
Flea Powder/Sprays	Yes	No		
Lead/Lead products	Yes	No		
Asbestos/Asbestos products	Yes	No		
Pesticides/Herbicides	Yes	No		
Cleaning solutions/solvents	Yes	No		

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
FORM NUMBER:	**EFFECTIVITY DATE:**

Donation Application Form **DONOR NUMBER** ________________ Page 5

PERSONAL HEALTH HISTORY (continued)

Do you take hot baths, saunas, hot tubs, or steam baths? _____Daily _____Weekly _____Occasionally _____Never

Within the past 6 months have you been exposed to UV rays in a tanning booth? _____ Yes _____ No

What is your caffeine usage? Number of cups of coffee: _____ Soda _____ Tea _____ Energy Drinks _____

Do you currently smoke cigarettes? Daily Occasionally Rarely Never If yes, how many per day? _____

Have you ever smoked cigarettes? ____Yes ____No
If yes, how many cigarettes per day? __________
If no, what year/month did you stop? __________
How many years did you smoke? _____

What best describes your alcohol consumption? ____Never drink
____ Rarely drink/Drink in small amounts ____Even amounts through the week ____Drink in concentrated periods

What type of alcohol do you usually consume? _____Beer _____ Wine _____Liquor

If you do drink, how many drinks do you usually consume in a week? ____1-3 ____4-9 ____10-15 ____16 or more

Have you ever used recreational or illicit drugs (cocaine, marijuana, LSD, heroin, barbiturates, narcotics, opiates, amphetamines, hallucinogens, tranquilizers, PCP, steroids, or etc.)? _____ Yes _______ No
If yes, which one (s) and when did you last use them? ____

Do you sleep well? _____Yes _____ No If no, how do you manage this?_________________________________

Have you had acupuncture, ear and/or body piercing or tattooing in which sterile procedures may not have been used?
____Yes _____No

Please list and describe all of your tattoos and body piercings:

Date Received:	Description:	Location on Body:	Sterile Needles Used?

Have you ever had any problems with the law (i.e. DUI, custody issues, lawsuits)? _____Yes _____No

If yes, please explain ____

Please list any arrests, convictions, sentences, etc.: ____

____

Have you ever been incarcerated? If yes, please describe:
____

FORM TITLE: Donor Application Form	**REVISION:** 3/4/09
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Donation Application Form DONOR NUMBER ________________ Page 6

SEXUAL AND CONTRACEPTIVE HISTORY

Sexual Orientation (please circle): Homosexual Heterosexual Bisexual

Number of current sexual partners: ______Number of sexual partners during the last six months: ______

Total number of past sexual partners: ______

In the last 6 months have you had unprotected sex (intercourse without a condom) with a new partner? ___Yes ___No

Have you ever injected drugs or had a sexual partner who did so? ____Yes ____No

CONTRACEPTIVE HISTORY:

Currently use: IUD Type _____ Diaphragm _____ Condom _____ Birth Control Pills _____

Rhythm _____ Spermicide _____ Depo-Provera _____ Tubal Ligation _____ None _____

If Birth Control Pills: __________________________ (name) How long on Birth Control Pills? ___________________

Why did you start taking Birth Control Pills? ___________________________

If Depo-Provera, when was your last injection? _________________________

To your knowledge, have you or any of your sexual partners been in contact with anyone or have you been personally tested or been treated for any of the following:

	Self	Partner	If yes, when:	How many times?	When was the last time?
HIV (AIDS)					
NSU (non specific urethritis)					
Syphilis					
Gonorrhea					
Chlamydia					
Trichomonas					
Venereal Warts					
Herpes, Genital					
Viral Hepatitis B or C					
Genital Sores					
Penis Discharge					
Other sexually transmissible diseases					

FORM TITLE: Donor Application Form	**REVISION:** **3/4/09**
FORM NUMBER:	**EFFECTIVITY DATE:**

Donation Application Form **DONOR NUMBER** _________________ Page 7

MENSTRUAL AND REPRODUCTIVE HISTORY: FOR EGG DONORS

Age at onset of menses: _______ Date of Last Menstrual Period: ____________

Are your menstrual periods regular: _____Yes _____No

How long is your monthly cycle (first day of one period to first day of the next)? ________days

Are you periods regular when you are not on any type of hormonal birth control such as the pill, etc.? ____Yes ____ No

If no, how many times per year do you menstruate? ___________

How many days does your period usually last? ______ days

Do you bleed or spot between periods? _____Yes _____No

Do you get menstrual cramps before, during, or after your period? ____Yes ____No
If yes, are your cramps: mild moderate severe?

If yes, do you use medication alleviate the pain? _____Yes _____No
If yes, what medications do you use? ____

Have you ever had any medical treatment for menstrual problems? ____

Date of last Pap Smear: ________________ Result: ____

Have you ever had an abnormal PAP: __________________ If yes, when and why: ______________________________

Have you ever been told you were infertile: ______________ If yes, when and why:_______________________________

Have you ever had a pelvic infection requiring treatment with antibiotics ____Yes ____No

Do you want children in the future? ____Yes ____No

REPRODUCTIVE HISTORY (or partner for sperm donors)

FERTILITY HISTORY:

Number of pregnancies:___________________

Number of miscarriages: __________________ Date(s) of miscarriages: ___________________________________

Number of ectopic pregnancies: _____________ Date(s) of ectopic pregnancy: _______________________________

Number of abortions: _____________________ Date(s) of abortions___________________________________

Number of stillbirths: _____________________ Date(s) of each stillbirth: ___________________________________

Number of children: ______________________ Are you Currently Breastfeeding? ____Yes ____No

Length of time it took you or your partner to get pregnant. Shortest _____________ Longest ______________

Pregnancy # Boy/Girl	Delivery Date	Type of Delivery (Vaginal or C-Section)	Complications	Weeks pregnant when delivered (prematurity)	Height / Weight
1					
2.					
3.					
4.					

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
FORM NUMBER:	**EFFECTIVITY DATE:**

Please note that the remaining portion of this application will be shared and viewed by recipients.

PHYSICAL CHARACTERISTICS

THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

Are you adopted? ____Yes ____No Blood Type if known: ____________

Height: _______ Weight: ______

Recent weight loss/gain?____Yes ____No If yes _______lbs loss/gain (circle one)

What was your weight at age 21? _______

Please circle responses that best describe you below:

Right Handed Left Handed Ambidextrous

Bone Structure: Small Medium Large Very Large

Complexion: Very Fair Fair Light Medium Olive Light Brown Dark Brown Ebony

Tan ability: None Slight Medium Easy Freckle

Skin Condition: Oily Medium Dry Combination **Dimples?** ____Yes ____No

Eye Color: Blue Brown Lt. Brown Dark Brown Green Hazel

Eye set: Narrow Average Wide **Eye Size:** Small Average Large **Shape:** Round Oval Almond

Natural Hair Color: Black Light Blonde Medium Blonde Dark Blonde Light Brown Medium Brown Dark Brown Red

Hair Type: Curly Wavy Straight **Hair Texture:** Fine Medium Coarse **Fullness:** Thin Medium Thick

Baldness: ____ Yes ____No **Baldness in Family:** ____ Yes ____ No

Premature Graying: ____Yes ____No If yes, at what age____

Body and Facial Features: Small Medium Large

Condition of your teeth: Poor Fair Good Excellent

Have you had any periodontal or orthodontic work? ____Yes ____No If yes, at what age? _____

Hearing (without corrective aids): Poor Fair Good Excellent

Vision (without corrective lenses): Poor Fair Good Excellent Prescription (If known): _________

FORM TITLE: Donor Application Form	**REVISION:** 3/4/09
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Donation Application Form **DONOR NUMBER** _________________ Page 9

PERSONAL HEALTH HISTORY
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

Do you wear glasses or contacts or have you had laser surgery? _____Yes _____No

If yes, are/were you: _____Nearsighted _____Farsighted ____Other (specify):____________

Do you have astigmatism (blurred vision due to an irregularity in the curvature of the cornea.? ____Yes ____No
If yes, age diagnosed ______.

Do you have any Allergies? _____Yes _____No

If yes, are they to: ____Food(s)_____Medication(s) _____Environmental _____Latex

Please list any childhood allergies that you have outgrown: _________________________________

For each medication allergy, describe specific substance and reaction(s) and age first noticed:

Substance: ____________________ Reaction(s):_________________________ Age: _____

Substance: ____________________ Reaction(s):_________________________ Age: _____

Substance: ____________________ Reaction(s):_________________________ Age: _____

SOCIAL HISTORY AND HABITS
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

Religion Born Into: _____________________________ Religion Practiced:_____________________________

Grade Point Average (GPA): ___________ SAT Scores: Verbal _____ Math _____ ACT Score: _____

Education:
_____ Did not Complete High School
_____ Received GED
_____ Completed high school
_____ Currently in college, pursuing degree in ____
_____ Completed college, degree in _________________________________ GPA:______________
_____ Currently pursuing an advanced degree in ____
_____ Completed advanced degree in ____

Did you have any learning disabilities or weaknesses in school? If yes, describe: _________________________________

Academic Strengths (i.e. math, reading):____

How many languages do you speak? _______________Which one (s): ____

Musical Talent or Instrument: ____Years Experience______

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
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SOCIAL HISTORY AND HABITS (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

Artistic Talent: ____

Athletic Skills / Favorite Sports: ____

Other skills/hobbies/talents/interests do you have (i.e. writing, reading, ability to do games or crossword puzzles, handcrafts)? Describe: ____

Current Occupation: _____________________________ How long have you been at your current job? __________

HABITS:

Exercise Habits: _____None _____Occasional _____Regular Type of Exercise: _______________________________

Your diet is: ____Vegetarian ____Non-vegetarian Your diet is: poor average excellent

Do you have any dietary restrictions? ____

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REPRODUCTIVE HISTORY
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

YOUR CHILDREN	**1**	**2**	**3**	**4**
Age				
Sex				
Eye color				
Hair Color				
Frame size				
Grade in school				
Personality				
Artistic ability				
Intelligence				
Distinguishing characteristics				
Wears eye glasses				
Discipline problems				
Any medication				
Dyslexia				
Reading difficulties				
Speech difficulties				
Any special services at school				
Seen by Social worker/ psychiatrist				
Grade functional level: Normal / Above/ Below Average				

FAMILY HEALTH HISTORY
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

How many blood siblings are in your immediate family (including yourself and half siblings)? _________

Number of Brothers __________ Number of Sisters __________

Number of Maternal Aunts ___________ Number of Maternal Uncles ___________

Number of Paternal Aunts ___________ Number of Paternal Uncles ___________

Do you have any brothers or sisters that died in infancy or childhood? _____Yes _____No

If yes, what was the cause? ____

Are there any members of your family with a history of learning disabilities or autism? _____Yes _____No

If yes, please explain ____

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
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Donation Application Form **DONOR NUMBER** ________________ Page12

FAMILY HEALTH HISTORY (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

Describe genetic family members according to the following characteristics. Use natural eye and hair color; fair/dark, etc. complexion. If they are deceased, please list cause of death. Please do not put “natural” as a cause of death. If unknown, write “unknown.”

	Eye Color	Hair Color	Complexion	Height	Weight	Bone Structure	Occupation/ Education	Age if living	Age at time of death	Cause of death
Sister(s)										
Brother(s)										
Mother										
Father										
Maternal Grandmother										
Maternal Grandfather										
Paternal Grandmother										
Paternal Grandfather										

FORM TITLE: Donor Application Form	**REVISION:** 3/4/09
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Donation Application Form **DONOR NUMBER** ________________ Page13

FAMILY HEALTH HISTORY (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

Carefully review the following list of medical problems and identify which ones you or one of your genetic relatives have or had. Please consider each condition carefully for each family member. Explain any conditions you check below, indicating which side of the family (maternal or paternal), the age at the time of onset, and any other pertinent information. If you and none of your indicated family members have a history of the specific medical condition, please indicate none.

***PLEASE REFER TO THE GLOSSARY ON THE LAST PAGES OF THIS FORM FOR DEFINITIONS**

	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
CANCER									
Breast									
Colon or Intestinal									
Lung									
Ovarian or Uterine									
Prostate or Testicular									
Skin									
Stomach									
Thyroid									
Blood (e.g. leukemia)									
Other									
HEART									
Stroke									
Heart Attack									
Congenital Heart Disease									
Heart Disease or Defect									
Hardening of the Arteries									
High Blood Pressure									
High cholesterol level									

FORM TITLE: Donor Application Form	**REVISION: 3/4/09**
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Donation Application Form **DONOR NUMBER** ________________ Page14

FAMILY HEALTH HISTORY (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
BLOOD									
Anemia									
Sickle-Cell Anemia									
Factor V Leiden thrombophilia (blood clots or strokes)									
Hemophilia or other Bleeding/Clotting Disorders such as Von Willebrand's Disease									
Immune Deficiency									
Leukemia									
Lymphoma or Swollen Lymph Nodes									
HIV									
Thalassemia									
Polyarteritis Nodosa									
Other Blood Disorder									
RESPIRATORY									
Asthma									
Hay Fever									
Emphysema									
Tuberculosis									
Pneumonia									
Alpha-1 antitrypsin Disorder									
Blood in Sputum									
Other Lung Disease									

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FAMILY HEALTH HISTORY (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
GASTRO-INTESTINAL									
Appendicitis									
Ulcer of Stomach or Duodenum									
Gallstones									
Hepatitis A,B or C									
Cirrhosis of the Liver									
Other Liver Disease									
Ulcerative Colitis									
Crohns Disease									
Pyloric Stenosis									
Multiple Polyps of the Colon									
Rectal Disorder									
Inflammatory Bowel Disease									
Any other problem of the digestive system									
METABOLIC/ ENDOCRINE									
Diabetes requiring insulin therapy									
Diabetes not requiring insulin therapy									
Childhood Diabetes									
Thyroid disorder									
Goiter									
Hypoglycemia									
Adrenal Dysfunction or Disorder									
Phenyl ketonuria (PKU) or inherited Metabolism Disorder									
Obesity									
Dwarfism									

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FAMILY HEALTH HISTORY (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
URINARY									
Kidney Problems									
Polycystic Kidney Disease									
Other disease/ defect of urinary tract (urethra, bladder, ureter)									
GENITAL/ REPRODUCTIVE									
Hermaphroditism/ Ambiguous Genitals									
Hypospadias or undescended testicle									
Uterine Fibroids									
Ovarian Cysts or Ruptured									
Lumps or Cysts in Breast or Discharge									
Polycystic Ovarian Syndrome (PCOS)									
Pelvic Inflammatory Disease (PID)									
Endometriosis									
REPRODUCTIVE OUTCOMES									
2 or more Miscarriages									
Stillborn									
Premature Menopause									
Death of a newborn infant									
Childhood death									
Birth defects									
Infertility									
Premature Birth									

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FAMILY HEALTH HISTORY (continued)
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	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
NEUROLOGICAL									
Migraines									
Mental retardation									
Senility or Mental Deterioration before age 50									
Multiple Sclerosis									
Cerebral Palsy									
Neurofibromatosis									
Epilepsy / Seizures									
Attention Deficit Disorder/ Hyperactivity									
Autism / Asperger's									
Alzheimer's Disease/Dementia									
Hydrocephalus									
Tuberous Sclerosis									
Parkinson's Disease									
Creutzfeldt-Jakob Disease									
Scoliosis									
Myasthenia Gravis									
Huntington's or Wilson's Disease									
Tourette's syndrome									
Other diseases of the nervous system									
MENTAL HEALTH									
Anxiety / Panic Attacks									
Anorexia / Bulimia/other eating disorders									

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FAMILY HEALTH HISTORY (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
Depression									
Schizophrenia									
Manic Depressive or Bipolar Disorder									
Other mental health disorder requiring hospitalization									
Suicide Attempts									
Other mental health problems that warranted counseling (please list)									
MUSCLE/BONE/ JOINTS									
Muscular Dystrophy									
Achondroplasia – form of dwarfism with abnormal bone growth									
Other Chronic Muscle Disease									
Osteogenesis imperfecta (brittle bone disease)									
Loss of Muscle Coordination									
Osteoporosis									
Marfan Syndrome									
Arthritis									
Rheumatoid or Juvenile Arthritis									
Spinal Muscular Atrophy									
Hereditary Low Back Disorder or Deformity of Spine									
Reiter's Disease									
Myasthenia Gravis									
Gout									

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FAMILY HEALTH HISTORY (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
Metabolic Bone Disease (be more specific)									
Lupus (systemic lupus erythematosus – SLE)									
SIGHT/SOUND/ SMELL									
Deafness before age 60									
Deformity of the ear									
Cataracts before age 50									
Blindness									
Color Blindness									
Severe Myopia									
Glaucoma									
Retinoblastoma									
Retinitis Pigmentosa									
Deviated Septum									
Any other Sensory Disorder									
SKIN									
Acne									
Albinism									
Eczema									
Excessive Facial Hair (Hirsutism)									
Pigmentation Disorders									
Psoriasis									
Neurofibromatosis									

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FAMILY HEALTH HISTORY (continued)
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	None	Self	Mother	Father	Sibling	Grand-parents	Aunt/ Uncle	Cousin	Explanation (which side of family, age of onset, etc.)
Other disorders of the skin									
Infectious Skin Disease									
More than 5 purple- or coffee- colored spots on skin (size of quarter or larger)									
CONGENITAL ABNORMALITIES/ BIRTH DEFECTS									
Cleft Lip / Palate									
Congenital Hip Problems									
Club Feet									
Heart Defect									
Hearing Problems									
Spina Bifida -Neural Tube (open spine)									
Microcephaly									
oloprosencephaly – a single-lobed brain structure and severe skull and facial defects									
Other									
CHROMOSOMAL ABNORMALITIES									
Down Syndrome									
Other (i.e. Turner, Fragile X, Klinefelter's etc.)									
OTHER									
Alcoholism									
Drug abuse, Misuse or Addiction									
Premature degeneration of any organ system									
Any other condition not mentioned above									

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More information about the above medical conditions are located at: http://www.mazornet.com/genetics/index.htm

Explain: ____

____

____

GENETIC HISTORY

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Ethnic origin (e.g., French, Irish)

Mother: ____________________________ Father: ________________________________

Race: Check all that apply for your ancestors:

African American	___Mother___Father___MGM___MGF___PGM___PGF
Eastern European (Ashkenazi) Jewish	___Mother___Father___MGM___MGF___PGM___PGF
Mediterranean (Greek, Italian)	___Mother___Father___MGM___MGF___PGM___PGF
Hispanic	___Mother___Father___MGM___MGF___PGM___PGF
Indian (from India)	___Mother___Father___MGM___MGF___PGM___PGF
Southeast Asian (Laotian, Vietnamese, Cambodian)	___Mother___Father___MGM___MGF___PGM___PGF
French Canadian	___Mother___Father___MGM___MGF___PGM___PGF
Cajun	___Mother___Father___MGM___MGF___PGM___PGF

(**MGM**=Maternal Grandmother, **MGF**=Maternal Grandfather; **PGM**=Paternal Grandmother, **PGF**=Paternal Grandfather)

Have you or anyone in your family ever been tested positive as a carrier or had any of the following diseases?

Blooms Syndrome	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Canavan	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Cystic Fibrosis	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Fabry Disease	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Familial Dysautonomia	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Familial Mediterranean Fever	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Fanconi Anemia Grp. C:	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Gaucher	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Niemann-Pick type A	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Mucolipidosis type IV	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Sickle Cell	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Tay-Sachs	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown
Thalassemia	No	If yes:	_____ disease	_____carrier	_____negative	_____unknown

Is there anything else we should know about your family?

____

____

____

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PERSONAL AND MOTIVATIONAL
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In your own words, describe your personality, temperament, and character:____

____

____

____

____

What physical, artistic, intellectual or social abilities do you feel best about?
____

____

____

____

____

____

What are your present and future career goals?________________________________

____

____

____

What are your present and future personal goals? ________________________________

____

____

____

____

List the 3 achievements you are most proud of:
____

____

____

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PERSONAL AND MOTIVATIONAL (continued)
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What is your favorite movie? ____

What is your favorite book? ____

What is your favorite color? ____

What is your favorite food? ____

What is one of your most memorable moments and why?
____
____
____

If you could change one thing about yourself, what would it be and why?
____
____
____

Is there a person alive or dead whom you admire and why?
____
____
____

What would you do on a "perfect" day if you could do anything you wanted?
____
____
____

Describe your personality and temperament as a child:
____
____
____

What was your favorite thing to do as a child?
____
____

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PERSONAL AND MOTIVATIONAL (continued)
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What did your parents teach you to value?

How were you in comparison to other children?

Describe your personality and temperament as a teenager:

Did you have any problems as a child and/ or as a teenager? Explain:

Who was the most important influence on you and why?

What were your ambitions/ goals as a teenager?

What were your best and worst subjects in school?

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PERSONAL AND MOTIVATIONAL (continued)
THIS PAGE WILL BE SHARED AND VIEWED BY RECIPIENTS

Please provide the following information about your family:

	Intellectual/Academic Achievements	Artistic Achievements
Mother		
Father		
Sisters		
Brothers		

Reasons for wanting to donate eggs or sperm : ____

____

____

____

____

If you could pass on a message to the recipient(s) of your eggs or sperm, what would that message be?

____

____

____

If you could write a message to the child born through your participation as an egg or sperm donor for when he/she turns 18 years old, what would you tell him/her?

____

____

____

____

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Please attach several photographs of yourself
(Ages 1 – 8 years, no adult photos please).
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GLOSSARY- INHERITED DISEASES

DEFINITIONS

Inherited – A disease or characteristic that is transmitted through genes from parents to offspring. Inheritance patterns include the following:

Autosomal Dominant – Disorders caused by one mutated copy of a gene. An affected person usually has one affected parent. Autosomal dominant disorders usually occur in every generation of an affected family. When a person carries an autosomal dominant gene mutation, each of his/her offspring has a 50% chance for inheriting the gene mutation.

Autosomal Recessive – Disorders caused by two mutated copies of a gene. An affected person usually has unaffected parents who each carry one copy of the mutated gene. Autosomal recessive disorders are not usually seen in every generation of a family. Carrier parents have a 25% chance for having an affected child.

X-linked dominant – Disorders caused by mutations in genes located on the X chromosome. Females are more frequently affected than males, and the chance to pass on an X-linked dominant disorder differs between men and women. Fathers cannot pass the X-linked traits or disorders to their sons. Females who have an X-linked dominant gene mutation have a 50% chance to have an affected child.

X-linked recessive – Disorders caused by mutations on genes on the X chromosomes. Males are more often affected than females, and the chance to pass on the disorder differs between men and women. Families with X-linked recessive disorders often have affected males, but rarely affected females, in each generation. Females who carry an X-linked recessive gene mutation have a 50% chance to pass it on to each of her children.

Multifactorial – Disorders caused by a combination of the effects of multiple genes or by interactions between genes and the environment.

Sources and additional information:

Talking Glossary of Genetic Terms http://www.genome.gov/10002096; http://www.genome.gov/glossary.cfm#g

Fact Sheets http://www.genome.gov/10000202

Cancer Dictionary http://www.cancer.gov/dictionary/

Genetics Home Reference National Library of Medicine http://ghr.nlm.nih.gov/

National Institutes of Health Genetic and Rare Diseases Information Center

http://rarediseases.info.nih.gov/GARD/Default.aspx?PageID=4

Gene Tests http://www.genetests.org/

GLOSSARY- INHERITED DISEASES (continued)

Cancer

- **Hereditary Breast/Ovarian Cancer** – Mutations in *BRCA1* or *BRCA2* genes predispose to breast cancer and ovarian cancer as well as prostate cancer *(BRCA1)* and other cancers *(BRCA2)*. Hereditary breast/ovarian cancer is inherited in families in an autosomal dominant pattern. Each child of an individual with a *BRCA1* or *BRCA2* cancer-predisposing mutation has a 50% chance of inheriting the mutation.
- **Hereditary colon cancer**
  - **Hereditary non-polyposis colorectal cancer** - Hereditary non-polyposis colon cancer (HNPCC) is caused by an autosomal dominant inherited gene mutation. HNPCC is characterized by an increased risk of colon cancer and other cancers (e.g., of the endometrium, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, skin). Each child of an individual with a HNPCC cancer-predisposing mutation has a 50% chance of inheriting the mutation.

Heart

- **Congenital heart disease** - Congenital heart disease is a common type of birth defect or malformation in one or more structures of the heart or blood vessels that occurs during pregnancy while the fetus is developing. The cause of congenital heart disease is not known in most affected people. There are some recognized factors that are associated with an increased risk for congenital heart disease including: (1) genetic or chromosomal abnormalities such as Down syndrome; (2) taking certain medications, alcohol or drug abuse during pregnancy; and (3) maternal viral infections such as German measles in the first trimester of pregnancy. The risk of having a child with congenital heart disease is higher if a parent or a sibling has a congenital heart defect.

Blood

- **Sickle cell anemia -** Sickle cell disease is a group of disorders that affects hemoglobin, the molecule in red blood cells that delivers oxygen to cells throughout the body. Individuals who have sickle cell disease have atypical hemoglobin molecules called hemoglobin S, which can distort red blood cells into a sickle, or crescent, shape. Signs and symptoms include a low number of red blood cells (anemia), repeated infections, and periodic episodes of pain. The severity of symptoms varies from person to person. Sickle cell anemia is inherited in an autosomal recessive manner. Each child of carrier parents has a 25% chance to be born with sickle cell anemia.
- **Factor V Leiden thrombophilia -** Factor V Leiden thrombophilia is an inherited disorder of blood clotting. Factor V Leiden is the name of a specific mutation that results in thrombophilia - the increased tendency to form abnormal blood clots in blood vessels. People who have the factor V Leiden mutation are at somewhat higher than average risk for a type of clot that forms in veins, such as the deep veins of the legs (deep venous thrombosis), or a clot that travels through the bloodstream and lodges in the lungs (pulmonary embolism). Factor V Leiden thrombophilia can be inherited in families in an autosomal dominant and autosomal recessive manner.
- **Hemophilia -** Hemophilia is a bleeding disorder that slows the blood clotting process. People who have hemophilia often experience prolonged bleeding or oozing following an injury, surgery, or having a tooth pulled. The major types of this condition are hemophilia A (also known as classic hemophilia) and hemophilia B (also known as Christmas disease). Hemophilia A and hemophilia B are inherited in an X-linked recessive manner. In X-linked recessive inheritance, a female with one altered copy of the gene in each cell is called a carrier. She can pass on the altered gene to her children, but usually does not experience signs and symptoms of the disorder
- **Tay-Sachs -** Tay-Sachs disease is a rare inherited disorder that causes progressive destruction of nerve cells in the central nervous system (the brain and spinal cord). Affected infants progressively lose motor skills such as turning over, sitting, and crawling. Children who have the severe infantile form of Tay-Sachs disease usually survive only into early childhood. Tay-Sachs disease is inherited in an autosomal recessive manner. Carrier parents have a 25% in each pregnancy to have an affected child.

GLOSSARY- INHERITED DISEASES (continued)

- **Thalassemia -** Beta thalassemia is an inherited blood disorder that reduces the production of hemoglobin. Symptoms of beta thalassemia occur when not enough oxygen gets to various parts of the body due to low levels of hemoglobin and a shortage of red blood cells. Beta thalassemia is inherited in an autosomal recessive manner. Carrier parents have a 25% chance in each pregnancy to have an affected child.

Respiratory

- **Alpha-1 antitrypsin disorder -** Alpha-1 antitrypsin deficiency is an inherited condition that can cause lung disease in adults and liver disease in adults and children. This disorder is inherited in an autosomal co-dominant pattern. Co-dominance means that two different versions of the gene may be expressed, and both versions contribute to the genetic trait.

Gastrointestinal

- **Cystic Fibrosis -** Cystic fibrosis is an inherited disorder of the mucus glands that affects many body systems. The most common signs and symptoms of cystic fibrosis include progressive damage to the respiratory system and chronic digestive system problems. Cystic fibrosis is inherited in an autosomal recessive manner. Carrier parents have a 25% chance in each pregnancy for having an affected child.
- **Pyloric stenosis -** Pyloric stenosis (also called infantile pyloric stenosis or gastric outlet obstruction) is a condition that involves a narrowing of the pylorus, the lower part of the stomach through which food and other stomach contents pass to enter the small intestine. When an infant has pyloric stenosis, the muscles in the pylorus become enlarged to the point where food is prevented from emptying out of the stomach. Pyloric stenosis is known to run in families. When a parent has pyloric stenosis, then, their infant has an increased risk of developing the disorder.

Metabolic/Endocrine

- **Phenylketonuria -** Phenylketonuria (also known as PKU) is an inherited disorder that increases the levels of a substance called phenylalanine in the blood. Phenylalanine is a building block of proteins that is obtained through the diet. If PKU is not treated, phenylalanine can build up to harmful levels in the body, causing mental retardation and other serious health problems. PKU is inherited in an autosomal recessive manner. Carrier parents have a 25% chance with each pregnancy to have an affected child.
- **Dwarfism** – There are a number of different types of dwarfism and many are inherited in families. Examples of types of dwarfism include: achondroplasia, thanatophoric dysplasia, and Robinow syndrome.

Urinary

- **Polycystic kidney disease -** Polycystic kidney disease is a disorder that affects the kidneys and other organs. Cysts, develop in the kidneys, causing them to become enlarged and can lead to kidney failure. Cysts may also develop in other organs, particularly the liver. There are two major forms of polycystic kidney disease distinguished by the age of onset and their pattern of inheritance. The autosomal dominant form (sometimes called ADPKD) has signs and symptoms that typically begin in adulthood, although cysts in the kidney are often present from childhood. The autosomal recessive form of polycystic kidney disease (sometimes called ARPKD) is much rarer and is often lethal early in life.

GLOSSARY- INHERITED DISEASES (continued)

Genital/Reproductive

- **Hypospadias –** Hypospadias is a birth defect of the urethra that happens in males. It involves an abnormally placed opening in the penis. Instead of opening at the tip of the penis, a hypospadic urethra opens anywhere along the line running from the tip along the underside of the shaft to the where the penis and scrotum meet. In most males hypospadias is not inherited, nor is their family recurrence. In some cases, hypospadias happens as a result of a chromosomal abnormality called a pericentric inversion of chromosome number 16.

Reproductive Outcomes

- **2 or more miscarriages –** Miscarriage (also called spontaneous abortion) is the term used for a pregnancy that ends on it's own, within the first 20 weeks of gestation. The causes of miscarriages are varied, and most often the cause cannot be identified. During the first trimester, the most common cause of miscarriage is chromosomal abnormality - meaning that something is not correct with the baby's chromosomes. In some cases the chromosome abnormality in the developing fetus is the result of a parent carrying a balanced chromosomal arrangement called a translocation. This can lead to multiple miscarriages.
- **Birth defects –** A birth defect is a problem that happens while the baby is developing in the mother's body. Most birth defects happen during the first 3 months of pregnancy. A birth defect can affect almost any part of the body. Causes of birth defects include a family history of birth defects, maternal age, certain drugs taken during pregnancy, alcohol use and smoking during pregnancy.

Neurological

- **Mental Retardation -** Mental retardation is a term used to describe a person who has certain limitations in mental functioning and difficulties in communicating, taking care of him or herself, and social skills. These limitations will cause a child to learn and develop more slowly than a typical child. Causes of mental retardation include genetic conditions such as Down syndrome, problems during pregnancy, problems at birth and health problems such as malnutrition.
- **Cerebral palsy -** Cerebral palsy is the term for a group of disorders that involve the loss of movement or loss of other nerve function. Cerebral palsy is caused by injuries to the largest part of the brain (cerebrum) which happen as the baby grows in the womb or near the time of birth. There are multiple causes of cerebral palsy including birth defects that affect the brain, spinal cord, head, face, lungs or metabolism, and certain hereditary and genetic conditions.
- **Neurofibromatosis –** There are two types of neurofibromatosis. Neurofibromatosis type 1 is a disorder characterized by changes in skin coloring (pigmentation) and the growth of tumors along nerves in the skin, brain, and other parts of the body. The signs and symptoms of this condition vary widely among affected people. Neurofibromatosis type 1 is considered to have an autosomal dominant pattern of inheritance. Neurofibromatosis type 2 is a disorder characterized by the growth of noncancerous tumors in the nervous system. Neurofibromatosis type 2 is also considered to have an autosomal dominant pattern of inheritance. However, unlike most other autosomal dominant conditions, in which one altered copy of a gene in each cell is sufficient to cause the disorder, two copies of the NF2 gene must be altered to trigger tumor formation in neurofibromatosis type 2. A mutation in the second copy of the NF2 gene happens in other cells in the nervous system during a person's lifetime. Almost everyone who is born with one NF2 mutation acquires a second mutation in many cells and develops the tumors characteristic of neurofibromatosis type 2.

GLOSSARY- INHERITED DISEASES (continued)

- **Autism/Asperger's –**
  - Autism and autism spectrum disorders are complex neurodevelopmental conditions. The genetics of autism are complex and it is thought that there are multiple genes involved.
  - Asperger's syndrome is one of several autism spectrum disorders, with symptoms of difficulty in social interactions and restricted, stereotyped interests and activities. Children who have Aspergers syndrome do not usually have language or cognitive developmental delays. Genes are believed to play a role in Aspergers syndrome, and it seems to run in some families.

- **Hydrocephalus –** Hydrocephalus is a condition in which the primary characteristic is excessive accumulation of fluid in the brain. The excessive accumulation of fluid causes an abnormal widening of spaces in the brain called ventricles. This widening creates potentially harmful pressure on the tissues of the brain. The causes of hydrocephalus are still not well understood. Hydrocephalus may be caused by inherited genetic abnormalities (such as the genetic defect that causes aqueductal stenosis) or developmental disorders (such as those associated with neural tube defects including spina bifida and encephalocele). Other possible causes include complications of premature birth, and diseases such as tumors or hemorrhage which block the fluid.
- **Tuberous sclerosis -** Tuberous sclerosis is a genetic disorder characterized by the growth of numerous noncancerous tumors in many parts of the body. These tumors can occur in the skin, brain, kidneys, and other organs, in some cases leading to significant medical problems. Tuberous sclerosis is inherited in an autosomal dominant manner, which means one copy of the altered gene in each cell is sufficient to cause the disorder. In about one-third of families, an affected person inherits an altered gene from a parent who has the disorder. About two thirds of cases result from new gene mutations. These cases occur in people with no history of tuberous sclerosis in their family.
- **Creutzfeldt-Jakob Disease –** Creutzfeldt-Jakob disease is a prion disease. Prion diseases are group of progressive conditions that affect the nervous system. Prion diseases impair brain function, causing memory changes, personality changes, a decline in intellectual function, and problems with movement that worsen over time. The signs and symptoms of these conditions usually begin in adulthood, and these disorders lead to death within a few months to several years. Only a small percentage of prion disease cases run in families. Most cases occur in people without any known risk factors or gene mutations. Creutzfeldt-Jakob disease is acquired by eating beef products obtained from cattle that have prion disease.
- **Huntington Disease** - Huntington disease is a progressive brain disorder that causes uncontrolled movements, mental and emotional problems, and loss of thinking ability. Adult-onset Huntington disease, is the most common form of this disorder, with onset usually in a person's thirties or forties. An early-onset, less common form of Huntington disease begins in childhood or adolescence. This condition is inherited in an autosomal dominant manner, which means one copy of the altered gene in each cell is sufficient to cause the disorder.
- **Gaucher Disease -** Gaucher disease is an inherited disorder that affects many of the body's organs and tissues. The signs and symptoms of this condition vary widely among affected individuals. There are several types of Gaucher disease based on their particular features. Some types do not affect the brain and spinal cord while others do. Type 1 Gaucher disease, for example, is the most common form of this disorder. Major signs and symptoms of Type 1 Gaucher disease include enlargement of the liver and spleen, a low number of red blood cells, easy bruising caused by a decrease in blood platelets, lung disease, and bone abnormalities such as bone pain, fractures, and arthritis. Types 2 and 3 Gaucher disease, on the other hand, have problems that affect the central nervous system. Gaucher disease is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. The parents each carry one copy of the mutated gene, but they do not show signs or symptoms of the disease.

GLOSSARY- INHERITED DISEASES (continued)

- **Wilson Disease -** Wilson disease is an inherited disorder in which excessive amounts of copper accumulate in the body, particularly in the liver, brain, and eyes. Typically, signs and symptoms of Wilson disease first appear during the teenage years. Wilson's disease is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. The parents each carry one copy of the mutated gene, but they do not show signs or symptoms of the disease.
- **Tourette syndrome -** Tourette syndrome is a complex disorder characterized by repetitive, sudden, and involuntary movements or noises called tics. Tics usually appear in childhood, and their severity varies over time. In most cases, tics become milder and less frequent in late adolescence and adulthood. Individuals who have Tourette syndrome are also at risk for other associated problems including attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), anxiety, depression, and problems with sleep. A variety of genetic and environmental factors appear to play a role in causing Tourette syndrome. Most of these factors are unknown to date. Among family members of an affected person, it is therefore difficult to predict who else may be at risk of developing the condition.

Mental Health

- **Depression –** Clinical depression is an illness that can challenge a person's ability to perform even routine daily activities and in some cases lead a person to contemplate or commit suicide. There are several different types of depression (mood disorders that include depressive symptoms) such as major depression, bipolar disorder, and seasonal depression. The causes of depression are complex. Genetic, biological, and environmental factors can contribute to its development. In some people, depression can be traced to a single cause, while in others, a number of causes are involved. For many, the causes are never known. Certain types of depression seem to run in some families. Research is ongoing as to exactly which genes are involved in depression.

Muscle/Bone Joint

- **Muscular dystrophy -** Muscular dystrophies are a group of genetic conditions characterized by progressive muscle weakness and wasting. The Duchenne and Becker types of muscular dystrophy primarily affect the skeletal muscles, which are used for movement, and the muscles of the heart. These conditions occur much more frequently in males than in females. Both Duchenne and Becker muscular dystrophy are inherited in an X-linked recessive pattern, with the mutated gene that causes the disorder on the X chromosome. Males are affected by X-linked recessive disorders much more frequently than females.
- **Achondroplasia -** Achondroplasia is a disorder of bone growth. particularly in the long bones of the arms and legs. All people with achondroplasia have short stature. Health problems commonly associated with achondroplasia include breathing difficulties (called apnea), obesity, and recurrent ear infections. Achondroplasia is inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder. About 80 percent of individuals with achondroplasia have average-size parents; these cases result from a new gene mutation in that individual. In the remaining cases, people with achondroplasia have inherited a gene from one or two affected parents.
- **Osteogenesis imperfecta -** Osteogenesis imperfecta (OI) is a group of genetic disorders that mainly affect the bones. People who have OI have bones that break easily, often from mild trauma or with no apparent cause. Multiple fractures are common, and in severe cases, fractures can occur even before birth. Milder cases may involve only a few fractures over a person's lifetime. There are at least eight recognized forms of osteogenesis imperfecta, designated type I through type VIII, distinguished by their signs and symptoms. Most types of osteogenesis imperfecta have an autosomal dominant pattern of inheritance, which means one copy of the altered gene in each cell is sufficient to cause the disorder. Many people with type I or type IV osteogenesis imperfecta inherit a mutation from a parent who has the disorder.

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GLOSSARY- INHERITED DISEASES (continued)

- **Marfan syndrome -** Marfan syndrome is a connective tissue disorder. Connective tissue provides strength and flexibility to structures throughout the body such as bones, ligaments, muscles, the walls of blood vessels, and heart valves. Marfan syndrome affects most organs and tissues, especially the skeleton, lungs, eyes, heart, and the large blood vessel that distributes blood from the heart to the rest of the body called the aorta. Individuals who have Marfan syndrome often are tall and slender, have elongated fingers and toes, a long narrow face, highly arched palate, and have an arm span that exceeds their body height. About half of all people with Marfan syndrome have vision problems caused by a dislocated lens (ectopia lentis) Most people with Marfan syndrome have abnormalities of the heart and the aorta. This condition is inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is needed to cause the disorder. At least one quarter of classic Marfan syndrome cases result from a new gene mutation. These individuals have no history of the disorder in their family.
- **Spinal muscular atrophy -** Spinal muscular atrophy is a disorder that affects the control of muscle movement. It is caused by a loss of specialized nerve cells, (motor neurons), in the spinal cord and the part of the brain that is connected to the spinal cord (the brainstem). The loss of motor neurons leads to weakness and shrinkage of muscles used for activities such as crawling, walking, sitting up, and controlling head movement. In severe cases of spinal muscular atrophy, the muscles used for breathing and swallowing are affected. There are a number of different subtypes of spinal muscular atrophy based on the age of onset and symptoms. Most types of spinal muscular atrophy are inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. One type of spinal muscular atrophy is inherited in an autosomal dominant manner, which means one copy of the altered gene in each cell is sufficient to cause the disorder.
- **Reiter's disease -** Reiter's syndrome, also known as reactive arthritis, is a type of arthritis that occurs as a reaction to an infection somewhere in the body. Most infections that cause the disease begin in the bladder, urethra, penis, or vagina and are spread through sexual intercourse, a form of the disease called genitourinary Reiter's syndrome, or urogenital Reiter's syndrome. Other infections that can cause reactive arthritis include gastrointestinal infections due to eating contaminated food or handling contaminated substances, a form of the disease called gastrointestinal Reiter's syndrome, or enteric Reiter's syndrome. Reiter's syndrome affects mostly young men, between the ages of 20 and 40. Although researchers are not sure why some people develop reactive arthritis in response to certain infections, a genetic factor (presence of the HLA-B27 gene) appears to increase the risk.

Sight/Sound/Smell

- **Deafness –** There are several types of deafness including conductive hearing loss, neural hearing loss (nerve deafness), and mixed hearing loss (a combination of conductive and neural hearing loss). Some people are born deaf. Usually the cause is unknown. Although deafness is inherited in some families, deaf parents often have hearing children and hearing parents often have deaf children. Diseases and injuries of the ear can also cause deafness.
- **Blindness –** Blindness is a condition of lacking visual perception that is due to physiological or neurological factors. Blindness has a number of causes including disease and malnutrition. Blindness may have a genetic cause, and may also be a symptom of a particular genetic disorder. Recent advances in mapping of the human genome have identified genetic causes of low vision or blindness, for example the disorder called Bardet-Biedl syndrome.
- **Color blindness –** Color blindness is the inability to perceive differences between some of the colors that other people can distinguish. It is usually genetic in nature, but may also be due to eye, nerve, or brain damage, or to exposure to certain chemicals. Color blindness can be inherited in families. Since the mapping of the human genome there have been many causative gene mutations discovered. Mutations capable of causing color blindness originate from at least 19 different chromosomes and many different genes.

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GLOSSARY- INHERITED DISEASES (continued)

- **Retinoblastoma -** Retinoblastoma is a rare type of eye cancer that develops in the retina, the part of the eye that detects light and color. Although this disorder can occur at any age, it usually develops in young children. Most cases of retinoblastoma occur in only one eye, but both eyes can be affected. Retinoblastoma can be inherited in an autosomal dominant pattern which means that one copy of the altered gene in each cell is sufficient to increase cancer risk. A person with retinoblastoma may inherit an altered copy of the gene from one parent, or the altered gene may be the result of a new mutation. For retinoblastoma to develop, a second mutation in the other copy of the RB1 gene must occur in retinal cells during the person's lifetime. When there is a family history of retinoblastoma or if the person develops tumors in both eyes, the gene mutation is probably in all of the person's cells, and that person is said to have an inherited form of retinoblastoma. A smaller number of individuals have retinoblastoma as a result of missing portions of chromosome 13 that are not inherited.

Skin

- **Albinism –** Albinisim is a condition in which there is a lack of melanin pigment in the eyes, skin, and hair (or more rarely the eyes alone). Albinisim is hereditary and results from inheritance of recessive gene mutations. There are two main categories of Albinism - (1) **oculocutaneous albinism** in which there is a lack of melanin pigment in skin and hair and (2) **ocular albinism**, in which only the eyes lack pigment. People with oculocutaneous albinism can have anywhere from no pigment at all to almost-normal levels. People who have ocular albinism have generally normal skin and hair color, and many even have a normal eye appearance. Albinism may also be a feature of a genetic syndrome such as Hermansky-Pudlak syndrome.

- **Neurofibromatosis –** There are two types of Neurofibromatosis – Type 1 and Type 2. Neurofibromatosis type 1 is a disorder characterized by changes in skin coloring and the growth of tumors along nerves in the skin, brain, and other parts of the body. The signs and symptoms of this condition vary widely among affected people. Neurofibromatosis type 2 is a disorder characterized by the growth of noncancerous tumors in the nervous system. The most common develop along the nerve that carries information from the inner ear to the brain (the auditory nerve). Tumors that occur on nerves in other areas of the brain or spinal cord are also commonly seen with this condition. Both Type 1 and Type 2 Neurofibromatosis are considered to have an autosomal dominant pattern of inheritance. People with Neurofibromatosis Type 1 and Type 2 are born with one mutated copy of either the NF1 or NF2 mutated genes in each cell. In about half of cases, the gene mutation is inherited from an affected parent. The remaining cases result from new mutations in the gene and occur in people with no history of the disorder in their family. Unlike most other autosomal dominant conditions, in which one altered copy of a gene in each cell is sufficient to cause the disorder, two copies of either the NF1 or NF2 gene must be altered to trigger tumor formation in neurofibromatosis. A mutation in the second copy of the NF1 or NF2 gene occurs during a person's lifetime in specialized cells surrounding nerves. Almost everyone who is born with one NF1 or NF2 mutation acquires a second mutation in many cells and develops the tumors characteristic of the disease.

Congenital Abnormalities/Birth Defects

- **Cleft lip/palate –** Cleft lip and palate are common birth defects that affect the upper lip and the roof of the mouth. There are many causes of cleft lip and palate. Gene alterations passed down from one or both parents, drugs used or maternal viruses during pregnancy can cause cleft lip and/or palate. Cleft lip and palate can also be part of a genetic syndrome or occur with other birth defects. Risk factors for cleft lip and palate also include a family history of cleft lip or palate and other birth defects.

- **Congenital hip problems –** Congenital hip problems, also called hip dysplasia, involve problems with formation of the hip joint in children. The location of the hip dysplasia can be either the ball of the hip joint (femoral head), the socket of the hip joint (the acetabulum), or both. Hip dysplasia, called congenital dysplasia of the hip (or CDH) in the past, is now called developmental dysplasia of the hip (DDH). There are a number of factors that contribute to cause DDH. One known risk factor is having a family history of hip dysplasia. Other causes include when the baby is born in breech position or when there is a lack of intrauterine fluid (oligohydramnios) during pregnancy.

GLOSSARY- INHERITED DISEASES (continued)

- **Club feet –** Clubfoot is a condition where the foot turns inward and downward. It is a congenital condition, meaning it is present at birth. Other terms for clubfoot are Talipes equinovarus and Talipes. Clubfoot is the most common congenital disorder involving the legs, and can range from mild and flexible to severe and rigid. Although the exact cause is not known, clubfoot may be passed down in some families. Family history, therefore, is a risk factor for clubfoot, as is being a male.
- **Heart Defect -** A congenital heart defect involves an abnormal structure of the heart that is present at birth. Congenital heart defects are the most common type of major birth defect. There are multiple causes of congenital heart defects including environmental and genetic factors. Genes that can cause congenital heart defects are now being discovered, such as a gene that can cause an atrial septal defect and one that may contribute to hypoplastic left heart syndrome. Congenital heart defects can also be a part of a wider pattern of birth defects and genetic syndromes such as Down syndrome, Turner syndrome and velocardiofacial syndrome.
- **Hearing problems -** There are several types of hearing loss including conductive hearing loss, neural hearing loss (nerve deafness), and mixed hearing loss (a combination of conductive and neural hearing loss). Some people are born with hearing loss. Usually the cause is unknown. Although hearing loss is inherited in some families, deaf parents often have hearing children and hearing parents often have deaf children. Diseases and injuries of the ear can also cause deafness.
- **Spina bifida -** Spina bifida is one of a group of birth defects called neural tube defects. Spina bifida occurs during fetal development when a portion of the neural tube fails to develop or close properly causing defects in the spinal cord and in the bones of the backbone. Spina bifida, like many other birth defects, appears to be caused by a combination of genetic and environmental risk factors, such as a family history of neural tube defects, folic acid deficiency, and medical conditions such as diabetes and obesity.
- **Microcephaly -** Microcephaly is a disorder in which the circumference of the head is smaller than normal because the brain has not developed properly or has stopped growing. Microcephaly can be present at birth or it may develop in the first few years of life. It is most often caused by genetic abnormalities that interfere with the growth of the cerebral cortex during the early months of fetal development. Microcephaly is associated with genetic syndromes such as Down syndrome, chromosomal syndromes, and neurometabolic syndromes. Babies may also be born with microcephaly if their mother abuses drugs or alcohol during pregnancy, or becomes infected with the German measles, chicken pox.
- **Holoprosencephaly -** Holoprosencephaly is a disorder caused by the failure of the embryonic forebrain (*prosencephalon*) to divide properly into the double lobes of the cerebral hemispheres. As a result, the baby has a single-lobed brain structure and severe skull and facial defects. In most cases of holoprosencephaly, the malformations are so severe that babies die before birth. In less severe cases, babies are born with normal or near-normal brain development and facial deformities that may affect the eyes, nose, and upper lip. Often, no specific cause for holoprosencephaly can be identified. There are some specific chromosomal abnormalities that have been identified as the cause of holoprosencephaly in some patients. In some families, holoprosencephaly is inherited in autosomal dominant or X-linked recessive inheritance. Several genes have also been identified that play a role in causing holoprosencephaly.

GLOSSARY- INHERITED DISEASES (continued)

Chromosomal Abnormalities

- **Down syndrome -** Down syndrome is a chromosomal disorder that is associated with mental retardation, a characteristic facial appearance, and poor muscle tone in infancy. Individuals who have Down syndrome may also have heart defects, digestive problems such as gastroesophageal reflux or celiac disease, hearing loss, and cancer of blood-forming tissue (leukemia). Some people with Down syndrome have hypothyroidism. Down syndrome also appears to be associated with an increased risk of Alzheimer disease Down syndrome is usually caused by the presence of an extra chromosome number 21, called trisomy 21, which means each cell in the body has three copies of chromosome 21 instead of the usual two copies. Most cases of Down syndrome are not inherited, but occur as random events during the formation of egg or sperm. One type of Down syndrome, called translocation Down syndrome, can be inherited.
- **Fragile X syndrome -** Fragile X syndrome is a genetic disorder that involves a range of developmental problems including learning disabilities and mental retardation, and behavioral problems such as hyperactive behavior and attention deficit disorder. Males are usually more severely affected by this disorder than females. Many males with fragile X syndrome have characteristic physical features that become more apparent with age such as a long and narrow face, large ears, prominent jaw and forehead, unusually flexible fingers, and enlarged testicles after puberty. Most cases of fragile X syndrome are caused by a mutation in which a DNA segment, known as the CGG triplet repeat, is expanded within the FMR1 gene. Fragile X syndrome is inherited in families in an X-linked dominant pattern.
- **Turner syndrome -** Turner syndrome is a chromosomal disorder that affects development in females. Women with Turner syndrome are often shorter than average and are usually unable to conceive children because they lack ovarian function. Other features of Turner syndrome can include extra skin on the neck, puffiness or swelling of the hands and feet, skeletal abnormalities, heart defects, and kidney problems. Developmental delays, learning disabilities, and behavioral problems may also be present, although these characteristics vary among affected females. In most cases, Turner syndrome is not inherited. Rather, it occurs as random events during the formation of egg or sperm.
- **Klinefelter syndrome -** Klinefelter syndrome is a chromosomal disorder that affects male sexual development. Most males who have Klinefelter syndrome have one extra copy of the X chromosome in each cell. The presence of an extra X chromosome interferes with male sexual development causing their testicles to develop abnormally, and leading to low levels of the hormone testosterone beginning during puberty. A lack of testosterone can lead to breast development, reduced facial and body hair, and an inability to father children. Boys who have Klinefelter syndrome may have learning disabilities and difficulty with speech and language development. Klinefelter syndrome is caused by the presence of one or more extra copies of the X chromosome in a male's cells. Klinefelter syndrome is not inherited, but usually occurs as a random event during the formation of egg or sperm.

Genetic History

- **Bloom syndrome -** Bloom syndrome is an inherited disorder that is characterized by a high frequency of breaks and rearrangements in an affected person's chromosomes. Individuals who have Bloom syndrome are usually much smaller than average and often have a high-pitched voice and characteristic facial features including a long, narrow face; small lower jaw; and prominent nose and ears. They tend to develop pigmentation changes that often appear as a butterfly-shaped patch of reddened skin on the face. Other features of the Bloom syndrome may include learning disabilities, mental retardation, chronic lung problems, diabetes, and immune deficiency that leads to recurrent pneumonia and ear infections. Men with Bloom syndrome are usually not able to father children because they do not produce sperm. Women with the disorder generally experience menopause earlier than usual. Chromosome instability in Bloom syndrome also results in a high risk of cancer in affected individuals. Bloom syndrome is inherited in families in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations.

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GLOSSARY- INHERITED DISEASES (continued)

- **Canavan disease -** Canavan disease is an inherited disorder that causes progressive damage to nerve cells in the brain. The signs and symptoms of Canavan disease usually begin in early infancy; however, the course of the disorder can be quite variable. Infants with Canavan disease usually appear normal for the first few months of life. By age 3 to 5 months, these infants begin to have developmental delays in motor skills, weak muscle tone, large head size, and mental retardation. They may also develop feeding and swallowing difficulties, seizures, and sleep disturbances. Canavan disease is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations.
- **Fabry Disease -** Fabry disease is an inherited disorder that begins in childhood and results from the buildup of a particular type of fat in the body's cells. Characteristic features of Fabry disease include episodes of pain, particularly in the hands and feet; clusters of small, dark red spots on the skin; a decreased ability to sweat; cloudiness of the front part of the eye; and hearing loss. Individuals with Fabry disease are also at risk for potentially life-threatening complications such as progressive kidney damage, heart attack, and stroke. Fabry disease is inherited in an X-linked pattern; however, unlike other X-linked disorders, Fabry disease causes significant medical problems in many females who have one altered copy of the mutated gene. These women may experience many of the classic features of the disorder.
- **Familial Dysautonomia-** Familial dysautonomia is a genetic disorder that affects the development and survival of certain nerve cells. The disorder causes disturbances in autonomic nerve cells, which control involuntary actions such as digestion, breathing, production of tears, and the regulation of blood pressure and body temperature. It also affects activities related to the senses, such as taste and the perception of pain, heat, and cold. Familial dysautonomia is also called hereditary sensory and autonomic neuropathy, type III. Problems related to this disorder first appear during infancy and include poor muscle tone, feeding difficulties, poor growth, lack of tears, frequent lung infections, and difficulty maintaining body temperature. Developmental delays in walking and speech, are usually present, although some affected individuals do not show signs of developmental delay. Familial dysautonomia is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations.
- **Familial Mediterranean Fever -** Familial Mediterranean fever is an inherited disorder that involves recurrent episodes of painful inflammation in the abdomen, chest, or joints. These episodes are often accompanied by fever and sometimes a rash. The first episode usually occurs by the age of 20. For some affected individuals, however, the initial episode occurs much later in life. The episodes usually last 12 to 72 hours and may vary in severity and length of time between attacks. A buildup of protein deposits occurs in some cases of familial Mediterranean fever and this can lead to kidney failure if left untreated. This condition is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have the mutations. Rarely, familial Mediterranean fever may be inherited in an autosomal dominant pattern, which means one copy of an altered gene is sufficient to cause the disorder.
- **Fanconi Anemia -** Fanconi anemia is a rare, inherited blood disorder that causes bone marrow failure. Fanconi anemia causes the bone marrow to stop making enough new blood cells for the body to function normally. Infants born with Fanconi anemia are at higher risk for having birth defects. Fanconi anemia can also cause the bone marrow to make many abnormal blood cells, which can lead to serious health problems such as cancer. This condition is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have the mutations.
- **Niemann-Pick, Type A -** Niemann-Pick disease is an inherited disorder that involves lipid metabolism - the breakdown, transport, and use of fats and cholesterol in the body. In affected individuals the abnormal lipid metabolism causes harmful amounts of lipids to accumulate in the spleen, liver, lungs, bone marrow, and brain. There are four main types of Niemann-Pick disease. Type A presents during infancy and is characterized by an enlarged liver and spleen, failure to thrive, and progressive deterioration of the nervous system. Children born with Niemann-Pick, Type A generally do not survive past early childhood. Niemann-

GLOSSARY- INHERITED DISEASES (continued)

Pick, Type A is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations.

- **Mucolipidosis Type IV -** Mucolipidosis Type IV is a genetic disorder, primarily which is characterized by severe neurological and ophthalmologic abnormalities. Also known as ML4, the disorder usually presents during the first year of life with mental retardation, corneal opacities, and delayed motor milestones. Children with ML4 begin to show signs of developmental delay during their first year of life. They usually attain a maximum developmental age of 15 months in language and motor function, although their receptive abilities are more advanced. They may also experience retinal degeneration that severely limits their vision. ML4 is inherited in an autosomal recessive pattern which means both copies of the gene in each cell have mutations.

Appendix 5

Male Donor Physical Examination

Instructions for use: FDA requires that a physical examination be performed and documented to assess a donor for signs of a relevant communicable disease and for signs suggestive of any risk factor for a relevant communicable disease. FDA has provided specific guidance on what clinical and physical evidence to look for when screening a donor (http://www.fda.gov/cber/gdlns/tissdonor.htm). Utilization of this form will assist you in documenting such clinical and physical evidence. A donor should be determined to be ineligible if they exhibit physical evidence of relevant communicable disease or high-risk behavior associated with these diseases.

Donor Name or Distinct ID Code _____________________________________

Date of Birth____________________

Photo Identification_______________________________________

ID Checked by____

Type of Cells / Tissues Donated ________

Date of Examination_______________

Vitals: Height _______ Weight _______ Temperature _______ Pulse _______ Respiration _______ BP _______

Eyes ______ Normal _____Abnormal

- ❑ Any infection or redness of the eyes related to possible corneal abrasion or scarring consistent with vaccinial keratitis? _____Yes _____No
- ❑ Icterus? _____Yes _____No (if yes, answer may not result in donor ineligibility if cause of Icterus is other than infectious disease)

Throat _____Normal _____Abnormal

- ❑ Any oral thrush, white spots, or unusual blemishes? _____Yes _____no

Lymph nodes _____Normal _____Abnormal

- ❑ Any swollen lymph nodes in the neck, axilla, or groin or evidence of disseminated lymphadenopathy? _____Yes _____No

Abdomen _____Normal _____Abnormal

- ❑ Any tenderness or hepatomegaly? _____Yes _____No (if yes, answer may not result in donor ineligibility if cause of hepatomegaly is other than infectious disease)

Genital _____Normal _____Abnormal

- ❑ Any redness, edema, or physical evidence of genital ulcerative disease, herpes simplex, syphilis, genital warts, or chancroid? _____Yes _____No
- ❑ Any physical evidence of anal intercourse, insertion trauma, or perianal condyloma? ____Yes ____No

Skin _____Normal _____Abnormal

- ❑ Any needle tracks? _____Yes _____No
- ❑ Purple/blue spots consistent with Kaposi's Sarcoma _____Yes _____No
- ❑ Jaundice _____Yes _____No (if yes, answer may not result in donor ineligibility if cause of Jaundice is other than infectious disease)
- ❑ Rashes _____Yes _____No
- ❑ Large scab or necrotic lesion consistent with recent smallpox vaccination or vaccinia necrosum _____Yes _____No
- ❑ Lesions or eczema vaccinatum _____Yes _____No

Tattoo (s) _____Yes _____No

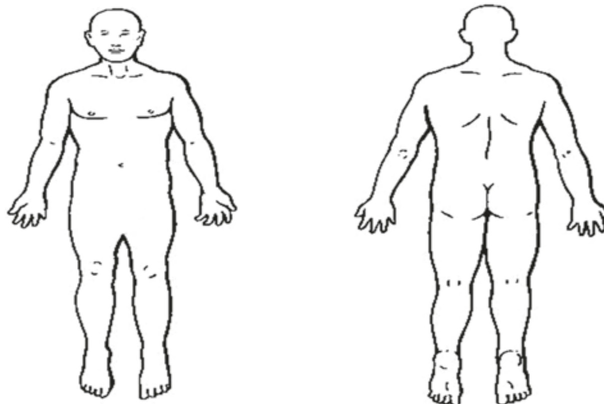
- ❑ Any evidence or recent (12 months) tattoo or home produced tattoo? _____Yes ____No

Body or Ear Piercing ____Yes ____No

Any evidence of recent (12 months) piercing? ____Yes ____No

Donor Physical Examination

Donor Name or Distinct ID Code__________________________



Please mark the location of any rashes, scars, lesions, tattoo(s), piercing(s), needle tracks, or hematomas.

Authorized Medical Provider Completing Form:

______________________	______________________	______________
Print Name & Title	Signature	Date

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Part IV
Current Practice and Future of Male Fertility Preservation

Chapter 18
Current Practice, Attitude, and Knowledge of Oncologists Regarding Male Fertility Preservation

Jenny Riley and Sara Barnato Giordano

Introduction

Many anticancer therapies pose a threat to fertility. In men, infertility from cancer treatments can occur through a variety of mechanisms including anatomic problems (for example, retrograde ejaculation from a retroperitoneal lymph node dissection), hormonal insufficiency, damage or depletion of the germinal stem cells, or most commonly decreased sperm production, known as azoospermia [1]. The risk of infertility or sterility with antineoplastic therapy is dependent on the patient's age and is specific to the type of chemotherapy or radiation field used [2, 3]. Established methods for FP in males include sperm cryopreservation, gonadal shielding, or gonadal transposition. Investigational strategies include cryopreservation of testicular tissue. With increasing cancer survivorship, there has been a shift to a comprehensive model of cancer care and discussion of FP became essential in treatment planning in order to improve a patient's quality of life [4]. Long-term effects of anticancer therapy resulting in infertility can have social, sexual, economic, and psychologic impacts on patients [5].

Cancer patients largely perceive education about FP as inadequate or untimely [3]. In an effort to improve overall quality of care for cancer survivors, ASCO and NCCN have developed guidelines for clinicians regarding FP [6, 7]. Discussions regarding the possibility of infertility and early referral to reproductive endocrinology are key components to both guidelines. However, health-care provider's lack of overall knowledge and lack of comfort in discussing FP as well as timely referral to reproductive endocrinology [5] prevent the guidelines from being used effectively. In addition to lack of physician knowledge and comfort level, multiple barriers exist that prevent patients from completing fertility preservation. Examples of some of these

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barriers include communication challenges, time constraints, insurance coverage, cost, and access to resources. Within current practice behaviors, attitudes vary between health-care providers regarding perception of importance of male versus female fertility preservation [5, 8], pediatric and adult clinicians [9], and certain patient populations [1, 10, 11]. It is essential to recognize and address these issues in order to provide optimal care for cancer patients.

Knowledge of Fertility Preservation Among Oncologists

While guidelines exist regarding FP, the practice among health-care providers is partially dependent on the clinician's knowledge about fertility preservation [12]. While many physicians are aware of the risk of infertility associated with certain chemotherapeutic agents and irradiation, two key barriers oncologists often face are knowledge of where to refer patients and knowledge of FP treatment options [4, 13]. Quinn et al. conducted a study of US oncologists and found that 25% of providers did not know where or how to refer a patient for FP options [5]. Specifically for male patients, physicians often report that it is difficult to know where to refer for sperm cryopreservation, even despite existence of online directories. Quinn et al. also cited that only 47% of health-care professionals routinely refer their patients of childbearing age to a reproductive endocrinologist. Similarly, in a nationwide survey of oncologists by Forman et al., 95% of providers routinely discuss a treatment's impact on fertility, but only 39% routinely referred patients to a specialist in reproductive medicine [14]. A multi-institutional survey of oncology physicians and fellows in the United States conducted by Schover et al. found that 91% of the respondents agreed that sperm banking should be offered to all men at risk of infertility; however 48% either never bring up the topic or only mention it to less than a quarter of eligible men and only 10% noted that they referred for sperm banking in routine practice. Private oncology clinics were more likely to inform patients about sperm banking followed by cancer centers and private hospitals [10, 15].

Lack of knowledge of FP treatment options is a barrier for many providers. A knowledge quiz was distributed to 162 providers who treat male oncology patients and on average physicians were able to answer only 10 of the 15 questions correctly [10]. More than half of the providers overestimated the number of sperm samples needed, did not know that males were more likely to experience infertility compared to females, and were unaware of the costs of sperm banking. In a survey of 37 pediatric oncologists in the Netherlands, 54–76% were aware of possibilities for fertility preservation; however less than 25% reported a moderate or high confidence in their knowledge of these techniques [13]. This gap in knowledge may partially be attributed to minimal or no formal training in FP during oncology fellowship apart from observing attending physician practice patterns [15]. While FP is an educational guideline required by training, fellows may vary in the depth of knowledge they acquire with this topic. Alternatively, some oncologists feel their primary responsibility to the patient is to treat the malignancy and that expertise in FP is in the domain of the repro-

ductive specialist. They are therefore reluctant to invest the time required to gain necessary knowledge in FP [16]. Lack of knowledge and responsibility may then result in avoidance and failure to discuss fertility preservation with the patient.

Another barrier to an oncologist's knowledge of FP lies in the lack of up-to-date information, including journals and meetings, geared more toward the reproductive specialists. A survey of practitioners at Memorial Sloane Kettering Cancer Center (MSKCC) revealed that 92% of respondents agreed that patients should be informed of FP options; however only 69% reported access to information and fewer respondents consistently discussed risks of infertility with male (32%) or female (49%) patients. No significant difference existed in practice or knowledge of FP between providers who were practicing for less than versus more than 5 years [12]. Despite the existence of national guidelines, hospital-based groups and private practice guidelines often do not exist and therefore physicians may not always be equipped to address FP with each patient [4]. In the study conducted by Quinn et al. of US oncologists, less than 25% were aware of or distributed educational materials to their patients despite their existence through the Fertile Hope organization, the Lance Armstrong Foundation, and the Oncofertility Consortium [5]. This study also revealed that while 60% of oncologists are aware of ASCO guidelines for fertility preservation, less than 25% of respondents said that they follow them on a regular basis. Pediatric oncologists stated that they had little resources to counsel their patients and 92% found educational resources not completely sufficient [13].

Current Practice Guidelines

In order to assist health-care providers address FP among cancer patients, ASCO published a clinical practice guideline in 2006 for both pediatric and adult patients [2]. Following a systematic review of new literature, these guidelines were updated in 2013 and recommendations essentially remained the same [6]. The ASCO guidelines addressed the four following questions: (1) Are patients with cancer interested in interventions to preserve fertility? (2) What is the quality of evidence supporting current and forthcoming options for preservation of fertility in males? (3) What is the quality of evidence supporting current and forthcoming options for preservation of fertility in females? (4) What is the role of the oncologist in advising patients about fertility preservation options [6]?

The ASCO guidelines support the concept that people with cancer are interested in discussing fertility preservation. ASCO recommends that health-care providers caring for adult and pediatric patients with cancer (including medical oncologists, radiation oncologists, gynecologic oncologists, urologists, pediatric oncologist, surgeons, and others) should address the possibility of infertility as a potential risk of therapy as early as possible before treatment starts. Health-care providers should refer patients who express an interest in FP (and patients who are ambivalent) to reproductive specialists. Another discussion

and/or referral may be necessary if pregnancy is being considered. Providers should be able to answer basic questions about whether fertility preservation may have an impact on successful cancer treatment. If patients experience distress about potential infertility, they should be referred to psychosocial providers. The guidelines also comment that all of these discussions should be documented in the medical record [6].

Current evidence supports that for adult male patients, sperm cryopreservation (sperm banking) is the only established FP method. ASCO does not recommend hormonal gonadoprotection as hormonal therapy in men as it has not proven to be successful in preserving fertility. The ASCO guidelines state that patients should be informed of other experimental methods and should be encouraged to participate in registries. It is strongly recommended that sperm be collected before initiation of treatment because the quality of the sample and sperm DNA integrity may be compromised after a single treatment session. Men should be advised of a potentially higher risk of genetic damage in sperm collected after the initiation of chemotherapy. Although sperm counts and quality of sperm may be diminished even before initiation of therapy, and even if there may be a need to initiate chemotherapy quickly such that there may be limited time to obtain optimal numbers of ejaculate specimens, these concerns should not dissuade patients from banking sperm. Intracytoplasmic sperm injection allows the future use of a very limited amount of sperm; therefore even in these compromised scenarios, fertility may still be preserved [6].

ASCO supports the discussion of FP with parents or guardians of children and adolescents undergoing treatment. Established methods of FP should be used (semen cryopreservation and oocyte cryopreservation) for postpubertal minor children with patient assent (if appropriate) and parent or guardian consent. For prepubertal minor children, the only FP options are ovarian and testicular cryopreservation, which are investigational. Testicular tissue cryopreservation does not require sexual maturity and is used for the purpose of future reimplantation. Experimental methods should be encouraged when opportunities are available [6].

NCCN has also developed guidelines regarding FP for oncology patients [7]. Similar to ASCO, NCCN supports the notion that FP as well as sexual heath and function should be an essential part in the management of young patients with cancer who are at risk for infertility with treatment. The discussion of risk of infertility, FP, and contraception should be held prior to the initiation of therapy [7]. Specifically, men should be advised that they are most commonly at risk for azoospermia following therapy, which may or may not resolve over time. Referral to FP clinics should be done within 24 h for all interested patients. Referral to a mental health professional may be appropriate in certain situations to assist with complex decision making. NCCN supports that sperm banking should be discussed with male oncology patients through a local sperm bank or an online sperm banking kit [7].

Table 18.1 Potential barriers to fertility preservation (FP)

Patient	• Stage of diagnosis
	• Performance status
	• Psychological distress
	• Access to resources
	• Health literacy
	• Cost
Provider	• Knowledge base
	• Inadequate education in training
	• Inadequate ongoing education
	• Low referral rates to reproductive specialists
	• Timing of discussions with patients
	• Attitudes toward certain patient populations
	• Time restraints in clinic
Institution	• Insurance coverage
	• Lack of adequate resources
	• Lack of education materials

Barriers to Fertility Preservation

Barriers exist at multiple levels and prevent effective FP prior to cancer therapy (Table 18.1). These barriers exist at the patient level, the provider level, and a socio-economic level. At the patient level, communication barriers are present that prevent clinicians from relaying the appropriate information. Patients and families who have psychological distress regarding their cancer diagnosis may be less interested or focused on receiving information regarding FP prior to treatment [17]. However, as mentioned previously in the ASCO guidelines, most patients with cancer are interested in discussing the topic. Studies indicate that patients have found banking sperm as a positive factor to help them cope with their diagnosis even if the samples were never used [5, 18, 19].

Poor health literacy may complicate education regarding FP [4]. Not only do clinicians have to relay the information so the patient can understand, but educational materials also need to be on the literacy level of each patient. Schover et al. found that men who banked sperm were younger, more likely to have been childless, and more highly educated [11]. Patients presenting with late-stage disease with a poor prognosis can make communication of FP more challenging [4]. Goals of therapy with advanced disease are often palliative and focused on symptom management rather than future childbearing potential. Patients may also be too physically ill to bank sperm or explore FP options [17]. Select patients are interested in posthumous parenting where a patient stores sperm or embryos and then uses the

stored material for assisted reproduction with a partner after the patient's death; however this remains controversial [4]. Some patients require more urgent treatment at the time of diagnosis and clinicians are hesitant for them to have a treatment delay to pursue FP [5]. Peddie et al. identified in a survey of both patients and health-care providers that one of the primary barriers to pursuing FP was the "urgent need for treatment" conveyed by staff [20].

Pediatric and adolescent providers face different challenges compared to adult oncology providers. First, communication of FP may be difficult based on the patient's age and understanding of reproduction. While providers are looking to follow the patient's wishes, they ultimately have to obtain legal approval from the guardians [4]. Second, providers and/or families may be uncomfortable with the discussion of sexuality, reproduction, and techniques of FP (specifically ejaculation) in this population. Vadaparampil et al. collected data from 24 pediatric oncologists in Florida at 13 children's cancer centers and found that half of physicians believed that parents wanted information about FP while the other half thought that parents were either uncomfortable with the discussion or solely focused on their children's treatment plan [18]. Some of the physicians looked for cues from parent's body language to gauge their comfort with the discussion and others pushed through trying to educate on the importance of FP [18]. In this study, 5 of the 24 physicians described clinical scenarios where the parents wanted the patient to sperm bank; however the patient refused. No one reported the opposite scenario where the patient desired sperm banking and the parents refused consent. Achille et al. interviewed 20 adolescent cancer survivors of either Hodgkin's lymphoma or testicular cancer and 18 health-care professionals and found that patients did not typically view sperm banking as a difficult procedure and that having a supportive parent was an enabling factor to bank sperm [4, 21].

Pediatric and adult providers also face similar challenges discussing fertility preservation. One provider barrier previously discussed in detail is lack of knowledge and availability of patient education material. Second, providers have time constraints with each patient due to high patient caseloads. A majority of the initial visits are focused on discussing the cancer diagnosis and treatment plan and this leaves little time to discuss future considerations of cancer survivorship, including FP. Caprice Knapp points out in the chapter, "Healthcare Provider Perspectives on Fertility Preservation for Cancer Patients," that current guidelines do not address that discussions about FP may not be completed in one session and need to be ongoing. The discussions should not be viewed as a "one-time task to be checked off on a care plan, but as an evolution of health information exchanges [4]."

Barriers exist at a socioeconomic level that prevent effective FP. Patients undergoing cancer treatment are subject to high costs of therapy even with adequate insurance coverage due to large co-pays. For males, sperm cryopreservation is rarely covered by insurance [22, 23]. ASCO guidelines on FP estimate the cost of sperm banking at approximately $1500 for three samples stored for 3 years with a storage fee for additional years [6, 22]. Some studies suggest that potential cost is a barrier for providers to enthusiastically support FP [4, 24, 25]. Organizations, such as Fertile Hope, offer programs that include discounted nationwide sperm banking

and fertility preservation services [22]. Cultural and geographic barriers also exist where certain patient populations may not endorse and even discourage artificial assistance with reproduction. This may also then prevent providers from initiating a discussion on the topic. In other cultures and religions where family and children are of high priority and adoption is prohibited, FP may be essential [8].

Fertility Preservation Perceptions and Attitudes Among Oncologists

The overall consensus across multiple surveys indicates that oncologists have a favorable perception toward FP for cancer patients and believe that this discussion is important. Adams et al. performed a national survey of 100 oncologists in the United Kingdom and found that 95% of providers reported usually or always checking with their patients regarding the personal importance of future fertility and 91% reported taking the patient's desire for future fertility into account when planning the treatment regimen [1]. Similarly, Ghazeeri et al. reported in a survey of Lebanon oncologists that 94% of oncologists agreed that FP should be discussed with a patient prior to their cancer treatment [26]. Overbeek et al. found of 37 pediatric oncologists in the Netherlands that 97% of providers found it largely or entirely their responsibility to discuss infertility risk with the patient and family [13]. Despite providers reporting interest in discussing FP, this is not always done in the clinical setting.

There are certain patient populations where oncology providers are less likely to discuss and/or refer for fertility preservation. In the national survey of oncology physicians and fellows by Schover et al., providers reported that they would be less likely to offer sperm banking to men who were homosexual and HIV positive or had a poor prognosis [10]. Adams et al. found in a survey in the United Kingdom that 21% of responding oncologists reported that if a patient was homosexual it may influence their decision to discuss or refer the patient for FP [1]. Schover et al. found that factors that did not correlate significantly with sperm banking included ethnicity, marital status, or religious activity level [11].

Attitudes vary among oncologists regarding male versus female FP and even among male and female providers. Arafa et al. performed a cross-sectional study addressing the knowledge and practice of oncologists toward FP for male and female cancer patients in Saudi Arabia [8]. Of the 103 respondents, nearly 45% were not familiar with female FP options and 32% did not have such options at their institute. 92% of the providers believed that patients would benefit from a referral and 76% perceived cryopreservation as important but complicated. In order to determine FP attitudes and practice patterns from pediatric oncology specialists in the United States, the Survey for Preservation of Adolescent Reproduction (SPARE) was developed [9]. Kohler et al. found that 86% of respondents agreed that all pubertal males should be referred to a FP specialist and 66% of them do so whereas

73% of respondents agreed that all pubertal females should be referred to a FP specialist prior to cancer therapy; however only 23% do so [9]. Quinn et al. found that the likelihood of a referral for FP was predictive by the sex of the oncologist, with females more likely to refer than male oncologists [5]. However there is little research examining the differences in these practice patterns. Quinn et al. suggest that this practice pattern may exist as female physicians are more in tune with a patient's desire for family and fertility-related issues and are more willing to discuss sexuality [5, 27].

Conclusion

With increasing survivorship, oncofertility is an essential component of treatment planning. Young cancer survivors find this topic to be of high priority. It is the physician's responsibility to incorporate the topic of FP in patient discussions and continue to have ongoing conversations over the course of multiple visits. Many barriers do exist that may prevent completion of FP (Table 18.1), but health-care providers must work together to overcome these obstacles for the purpose of improving patient satisfaction and long-term quality of life.

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Chapter 19
Psychological Impact and Barriers to Fertility Preservation in Male Cancer Patients

Angela K. Lawson

Young men and women diagnosed with cancer often face the possibility of an uncertain fertile future as they may be exposed to gonadotoxic chemotherapies and/or radiation. They may also undergo surgeries or other treatments which may impair their ability to have genetic children. Increasing survival rates in pediatric and some reproductive-aged cancer patients however have led to increased interest in fertility preservation treatment (FP) [1–8]. Although survival rates among cancer patients aged 15–29 remain largely unchanged for the last several decades young cancer patients and their families are also interested in FP [9]. Indeed, many cancer survivors express regret, anger, and sadness if they were not counseled about fertility preservation prior to their cancer treatment [10–14]. Fertility preservation counseling (FPC) by medical professionals has therefore been strongly recommended by multiple medical societies [2, 6, 7, 15–18]. Unfortunately however, it appears that there is inconsistency in counseling male and female cancer patients regarding options for fertility preservation [13, 19–28]. It is also problematic that although a large number of studies examine the experiences of female cancer patients, the experiences of male cancer patients with fertility preservation and fertility preservation counseling are less well understood.

Rates of Fertility Preservation Counseling and Referral to FP

What little research exists regarding fertility preservation counseling by an oncology specialist indicates that although many female cancer patients do not receive FPC, a larger number of men may receive such counseling. Two such studies found

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that greater than half of males (57–68%) in the studies received FPC and information about FP whereas only 14–28% of females received such counseling. Male cancer patients also appear to be referred for fertility preservation treatment more often than female patients [8, 29, 30]. These apparent gender disparities in FPC and referral to FP may account for the large difference in numbers of men versus women who complete FP (e.g., 54% vs. 2%) [30, 31]. Additional studies show similar disparate results in completion of FP. In perhaps the largest study to date of gender differences in FPC and FP, 225 men and 325 young adult cancer survivors diagnosed between 2006 and 2012 (a subset of participants who had completed the 2012 Livestrong Survey) were asked about their experiences with fertility preservation. The results of the study were that significantly more men (49%) than women (22%) reported having completed FP [19].

Although rates of FPC among men may be higher than for women, for some men, FPC and referral for FP may be somewhat limited in nature and entail simply the scheduling of an appointment for sperm banking without the patient asking that such appointments be scheduled and without the patient's full informed consent. The automatic scheduling of FP for male cancer patients appears to assume that it is a given that all men will desire FP whereas for women this is not the case [32]. Examples of the automatic scheduling of male patients for FP are evident in multiple qualitative studies of men's experiences with FPC and referral to FP [14, 33–35]. For example, in a study by Armuand et al. (2015) [32] one male cancer patient stated:

> I didn't really perceived it [sperm banking] as an option really … it was more like 'You're going, on Wednesday morning at eight you'll be there, and freeze [sperm]' … Then of cause, people can choose by themselves for sure. But it wasn't something … it wasn't like a question, 'Do you want to or not?' rather 'It's already booked' and all that, to go up [to the fertility clinic]. […] I thought it was good. Then you don't have to think about it and to make a choice [32].

Another example of the normalization of FP appointments for male cancer patients is evident in Eiser et al.'s (2014) [36] qualitative study of male cancer patients who had previously banked sperm:

> We kept going back and forth to Hospital and then next thing I know they said right we're going to take you over to store some sperm before we start with the chemotherapy. So I just thought it were like normal practice you know before … [36].

Despite the apparent higher rates of FPC among male cancer patients as compared to female cancer patients, it appears that as many as 49% of male cancer patients exposed to gonadotoxic treatments may not receive FPC [14, 19, 33, 37–40]. It is also estimated that only 33–56% of male cancer patients choose to undergo FP [19, 30, 41]. As with research on barriers to FPC among female cancer patients, there are multiple potential individual and institutional barriers to FPC and FP for men (see Table 19.1).

Table 19.1 Barriers to male fertility preservation (FP)

Individual	Institutional
• Cost	• No FPC by a physician[a]
• Insufficient time	• No referral for FP by a physician[a]
• Scheduling concerns	• Medical staff discomfort
• Religious/ethical concerns	• Lack of knowledge
• Embarrassment	• Lack of training
• Concern about future use of gametes	• Disparate FPC
• Anxiety/distress	• Disparate referral for FP
• Lack of familial support	• Assumptions about patient desire
• No interest in having children	

[a]Two of the most important barriers. *FPC* =fertility preservation counseling

Patient-Based Barriers to FP

One of the greatest barriers to cancer patients' completion of FP has been argued to be the cost of FP. Indeed, the cost of FP for women is often several thousand dollars in out-of-pocket costs and is significantly greater than for men, and as such FP cost has been found to be a barrier for women. However, cost has also been found to be a barrier for men in as many as 38% of male cancer patients [14, 19, 31, 35, 42]. Additionally, greater time is required to complete FP for women (approximately 2 weeks [43]) than for men. Although having insufficient time (or the perception of insufficient time) to complete FP prior to beginning cancer treatment has been found to be a greater barrier for FP among female cancer patients than for male cancer patients (39% vs. 25%), male cancer patients also frequently report such barriers to FP [14, 19, 31, 39, 44, 45].

There are multiple other patient-based concerns that may limit a male cancer patient's participation in FP. For example, males (or their partners or families) may have ethical or religious concerns about the use of cryopreserved gametes for fertility treatment in the future, they may be concerned about the risks to children conceived with cryopreserved sperm or about the risk of cancer to children conceived with their gametes, [14, 40, 46–48] they may be concerned about the future cost of fertility treatments [49, 50], and/or they may be concerned about the complexity of scheduling FP [14]. Additionally, male cancer patients may avoid FP if they find the provision of sperm to be embarrassing or awkward [14, 32–34, 44], they may desire to avoid thinking about the possibility of infertility [14, 32], they may be anxious to complete their cancer treatment, and/or some males may not desire children (or additional children) in the future [14, 31, 40, 42]. Friends and family members of the patient may also influence the completion of FP particularly if parents of minor male children do not consent for FP or FPC [14, 39] or pressure minor children to undergo FP [11, 34] or if partners of adult males are not supportive of FP [14, 40].

Institutional Barriers to FPC and FP

Although patient barriers to FP have been identified, it may be that the greatest barriers to FP and certainly to FPC are institutional in nature. In particular, it appears that without FPC and referral for FP from a medical provider, many patients do not complete FP. This appears to be particularly true for male cancer patients. For example in Achille et al.'s (2006) [14] qualitative study of male cancer patients, one patient stated:

> If the doctor had told me do it, I would have done it, that is clear. But because he did not insist [. . .] it did not seem that important to do it [14].

In another study of adult survivors of childhood cancer and their parents, parents also expressed their belief that they would have encouraged their sons to participate in FP if their physician had encouraged it:

> If they had said something about [fertility preservation], we would have thought more about it [10].

It appears that if FPC and referral for FP are initiated by a physician, a large majority (as many as 99% of male cancer patients) may go on to complete FP [11, 34, 45, 51]. For example, a large study of male cancer patients referred for FPC and FP in France (N = 4345) between the ages of 11 and 20 years found that 99% (n = 4314) of patients attempted to completed FP via masturbation with only a small number of men (n = 31) who refused FP and only 7% of men (n = 310/4314) who were unsuccessful in providing a sample. Of the 93% of males who completed FP, 83% had a sample of high enough quantity and quality to cryopreserve and 81% were able to provide two or more samples [51]. Although a large majority of men in the study were able to participate in FP, it should be noted that in this study cost was unlikely to be a barrier to treatment as costs are covered by national insurance; thus rates of FP may be lower in populations where FP costs are out of pocket for patients. Further, given that 17% of men were unable to have a sample cryopreserved it is important that male cancer patients understand that the successful completion of FP varies as some men are unable to produce samples and some men produce poor-quality samples [4, 11, 52, 53]. It is also important that male FP candidates be aware that successful sperm cryopreservation does not guarantee future live-born offspring and thus should not be referred to as an insurance policy for future fertility [5, 42].

Another example of the role of FPC and referral for and completion of FP comes from a study of 4881 men who were diagnosed with cancer between the ages of 18 and 55. Of study participants, 411 men were offered a consultation for formal FPC and 75% (n = 306) of these men participated in FPC. Ultimately 87% (n = 266) of those who received FPC underwent FP [53]. Other studies however have found much lower rates (18–56%) of FP among male cancer patients who received FPC [5, 40–42, 54]. It may be that the age of the studied patient populations (or other sample differences) and insurance coverage for FP are driving the higher acceptance of FP as it appears that younger adult males and men with insurance coverage for FP are more likely to participate in FP [5, 40–42, 53–55].

Yet another barrier to FPC and referral for FP may be medical staff discomfort with such concerns. In a study of pediatric physicians and nurses specializing in pediatric oncology, approximately 20% of physicians and advanced-practice nurses and nearly 50% of nurses reported some discomfort in talking about FP with their patients. Additionally, greater than half of each group lacked sufficient knowledge regarding fertility treatments and nearly all participants (93%) indicated that they received no training on FPC [15]. Similarly other studies of oncology specialists (primarily oncologists) in the USA and the UK have found that as many as 39–58% of medical providers lacked sufficient knowledge of various fertility treatments as well as national guidelines on FPC [8, 14, 29, 37, 39, 56, 57].

Additional barriers to FPC and referral for FP from medical providers may arise when providers have medical or cancer-treatment-related concerns such as a patient's poor prognosis [9, 14, 37, 39] or the provider's concern that there is insufficient time to bank sperm prior to the onset of cancer treatment [29, 37, 39, 44]. Medical providers may also however provide disparate FPC and referral for FP based on their assumptions about patient desire for FP. Multiple studies have found that providers base the provision of FPC and referral to FP on their own assumptions about a patient's desire for FP based on the patient's age, sexuality, desire for children, or other assumptions about the patient such as their inability to afford treatment [8, 14, 29, 37, 57].

Although not assessed in identified studies of FPC among male cancer patients, given the evidence of disparate FPC to patients based on their sexuality, it is likely that disparities in FPC and referral to FP may also exist among transgender female cancer patients (born biologically male). Physicians may incorrectly assume that transgender women do not want children or that their children are at risk of psychological harm as a result of their identity; however research finds that transgender individuals have similar desires for parenthood as cisgender individuals whose socially assigned gender identity and biological sex are consistent [58–60] and there appears to be little to no risk for children of transgender parents [61]. Unfortunately, research showing low rates of FPC for noncancer transgender patients supports these concerns [59, 60].

Emotional Consequences of Disparate FPC and Referral to FP

Evidence of disparate FPC and referral to FP among biologically male cancer patients is concerning as such disparate treatment has been associated with increased risk of negative emotional outcomes for patients. Feelings of shock, sadness, anger, and a lowered self-esteem are not uncommon among male cancer patients who did not receive FPC [62]. Additionally, Stein et al.'s (2014) [10] qualitative study of adult childhood cancer survivors beliefs about FP revealed that regret associated with lack of adequate FPC was the most common theme among all respondents. Parents of survivors in this same study expressed feelings of guilt that they did not

raise concerns about their son's future fertility at the time of their cancer treatment. For example, two parents expressed feelings of parental failure by not facilitating their engagement in FP:

> I should have protected him [10].
>
> I did nothing, and then I lived with torment for years [10].

In the same study by Stein et al. (2014) [10], both survivors and their patients believed that the lack of FPC was a failure on the part of oncologists who should have been responsible for raising the issue. Given that previous research has found that greater than half of young men diagnosed with cancer desire future children, failure to discuss FP will also likely result in psychological distress should these men not be able to reproduce [10, 63].

Regret related to FPC may also arise for male survivors of cancer who received FPC, as they have been found to report regret that insufficient time was dedicated to FPC. For example, one male survivor of pediatric cancer stated:

> I was only 16 years old, and you just weren't that concerned about it at the time. Looking back I kind of wish they had set aside a time or an appointment, more than just like 2 minutes [10].

It appears that medical providers may have limited time available to discuss FP with newly diagnosed cancer patients as one study of oncologists indicated that nearly half (46%) of these physicians spent only 0–5 min on FPC with patients [29]. Research finds that there are a myriad of psychological issues involved in FP decision making that are unlikely to be sufficiently addressed in as little as 5 min.

First, patients who are newly diagnosed with cancer are emotionally preoccupied with news of their diagnosis [64, 65] and are often worried about their long-term survival. Cancer patients also frequently report body image concerns and concerns regarding future sexual functioning (particularly for patients who undergo orchiectomy [66, 67]) which may affect the development or continuation of romantic relationships. Men who are told that their fertility may be affected by their cancer treatment have also been found to worry about their future desirability as a partner [33, 48, 62, 68, 69]. For example, in a qualitative study of male childhood cancer survivors by Nilsson et al. (2014) [68] one survivor (age 23, diagnosed with cancer at age 8) stated:

> I have a follow up question! At what time in a new relationship should you tell your new partner 'I might not be able to have children because of my disease [68].

In another qualitative study of male cancer patients' cancer-related fertility concerns, men reported not only being concerned about the effect of their infertility on finding a future partner, but also the effect that it could have on their parents:

> Before I started on chemotherapy they didn't take any sperm sample, didn't freeze any sperm. So to be told as an 18-year-old guy, 'You're not gonna have children,' that's devastating, that's really sad. And also for my parents to know that they're not gonna have any grandchildren by me and I can't imagine you know that's, and how do I go into a relationship and say, 'I can't have children,' am I gonna face instant rejection? [33].

The emotional toll of a cancer diagnosis is so significant that it is frequently associated with symptoms of depression and/or anxiety [70–80]. Given the often short time period between diagnosis and decision making for FP it is not surprising that a newly diagnosed cancer patient's emotional state has been associated with decision making. Indeed, previous research has found that the majority (58%) of male cancer patients acknowledge that anxiety negatively affected their decision regarding pursuing FP [52]. For example, in one study of survivors of testicular cancer one patient reported:

> Diagnosis was an emotional time. It was not really possible to think clearly about the desire for future children at the time of diagnosis [42].

In another qualitative study of recently diagnosed male cancer patients, one man expressed his regret at the way in which his cancer-related anxiety affected FP decision making:

> They did offer sperm banking but I actually declined it simply because I was in a bit of a state. I'd had a real shock and this was something that was making it worse really, to have to go and do that [bank sperm]. And I was feeling pretty awful. Well I declined and it's a decision which [pause] I think was the wrong decision now, but it's easy to look back and say that it was the wrong decision, when you're in that situation, you know, maybe it is [33].

It appears that the time-sensitive nature of decision making regarding FP leaves little time for emotional recovery from news of a cancer diagnosis and that the patient's preexisting cancer-related anxiety may be increased as a result of the need to balance desire to begin cancer treatment with desire for future fertility [14, 33, 44]. Distress may be further increased among patients who are unable to afford FP and/or patients who are unable to participate in FP due to religious, ethical, or other concerns.

For patients who chose to undergo FP, there are multiple other potentially anxiety-provoking or otherwise emotional decisions which need to be made both at the time of FP and in the future. These largely pertain to the disposition of cryopreserved gametes. Patients who pursue FP often do so for emotional reasons, to prevent future regret if they are later found to be infertile but desire genetic children [41]. However, little to no research exists which has assessed the degree to which patients were counseled about or held realistic expectations regarding the use of cryopreserved gametes to achieve live birth nor their emotional reactions should their frozen gametes fail to result in a live-born child. Additionally, it is unclear how men who choose to pursue FP only to not return to use their gametes will feel about their decision.

Research finds that similar to women who pursue FP, few men return to use their gametes or make other disposition decisions [4, 5, 53, 81–83]. It is unclear why men who pursue FP do not return to use their gametes. It may be that men who do not return to use their sperm as they are too young [4] do not understand the potential effects of cancer treatment on their ability to have children, desire to avoid thinking about the possibility of infertility for fear of how that knowledge would affect them emotionally, are not yet ready to have a child, cannot afford to begin fertility treatment, and/or are not ready to make a disposition decision regarding their sperm

[36]. Further, some men who complete FP will unfortunately die as a result of either their disease or some other factor [81, 84]. Men who complete FP must make a decision about the disposition of their genetic material in the event of their death. For these men sensitive discussion of their beliefs and desires regarding posthumous assisted reproduction should be conducted at the time of FP and may result in increased anxious or depressive thoughts about their mortality and/or ethical concerns [85, 86]. Failure to appropriately counsel patients regarding gamete disposition could result in future distress for patients and their partners or families. It is unclear if such detailed discussions are occurring at the time of FPC or FP as one male cancer survivor expressed his distressed realization after a breakup that he consented to allow his partner to use his gametes in the event of his demise:

> At the time I was diagnosed I was in a long term relationship and I put her name on it and got up to probably a year afterwards when I split up with her. So like maybe a couple of years after I'd been diagnosed, had my treatment and everything. Then it dawned on me that she would have quite a lot of power if anything was to happen to me, if she said yes I want to have his children then my mum and sister wouldn't be able to say 'no psycho' [36].

Recommendations for Psychological Counseling of Male Cancer Patients Considering FP

Decisions regarding the pursuit of FP are psychologically complex in the context of the patient's current cancer diagnosis and have potential emotional ramifications that extend beyond completion of cancer treatment [85]. Consistent with multiple professional medical guidelines and with recommendations for female cancer patients [7, 16, 56, 85] it is therefore recommended that pretreatment psychological counseling by a mental health professional with specialty in reproductive medicine be strongly recommended (if not required) for all male cancer patients who receive FPC (see Table 19.2). Further, in light of the influence of family members and partners on decision making for FP, a patient's partner and/or when appropriate their parents should be included in counseling with the mental health professional. The inclusion of an appropriately trained mental health professional in FPC allows for greater opportunity for discussion of relevant psychological issues as mental health professionals typically are able to see patients for consults ranging as long as 50 min or more.

Mental health counseling of FP patients should include a sensitive discussion of patients' (and their partner's or parent's if appropriate) preexisting and current psychological status as well as the role of a patient's, their partner's, or their parent's current distress on decision making. Giving the possible role of familial/relationship influence or coercion in decision making, relationship status and presence of coercion should be evaluated. Further, it is recommended that the mental health professional assess and discuss the degree to which patients hold realistic expectations regarding their ability to successfully complete FP and father genetic children in the future. If not already understood, patients should be counseled that FP should

Table 19.2 Recommendations for psychological counseling in male fertility preservation (FP)

• Pretreatment counseling [of patient and partner/parent(s) if appropriate] by a MHP with specialty in reproductive medicine is strongly recommended for all patients who receive FPC
• Counseling should include discussion of:
• Current/past mental health history
• Influence of all relevant parties' distress on decision making
• Relationship status and influence on decision making
• Realistic treatment expectations
• Current and future religious/ethical concerns about FP
• Beliefs regarding posthumous reproduction
• Future emotional and financial risks/costs of FP
• Decision making and regret
• Openness to alternative family building

FPC = Fertility preservation counseling, *MHP* = Mental health professional.

not be viewed as an insurance policy which guarantees future family building. Patients should also be counseled about the potential future emotional and financial costs and ethical concerns associated with the use of their gametes with assisted reproductive technologies. Patients who complete FP only to be unable to afford or otherwise engage in appropriate future fertility treatments are at risk of emotional distress. Additionally, patient's religious or ethical concerns associated with masturbation, the disposition or destruction of cryopreserved gametes, and the use of genetic testing in fertility treatment should also occur prior to the completion of FP. Mental health counseling regarding the emotional consequences and ethical concerns associated with the disposition of gametes should also include a sensitive discussion of patients' beliefs about posthumous assisted reproduction [85]. Although many male cancer patients appear to find posthumous reproduction an acceptable means of gamete disposition [87], a thorough discussion of the relevant psychological and ethical issues is warranted. Finally, it is recommended that mental health professionals discuss patients' decision making in relationship to future regret. Men may regret delaying cancer treatment for FP if they later experience a recurrence of their disease or some other negative medical outcome or if they are unable to achieve future live birth with their gametes. They may also experience regret if they decline FP only to later discover that they are infertile. Thus for men who decline FP, openness to alternative family building strategies (e.g., donor sperm, adoption) should be discussed. Finally, mental health counseling regarding regret should be careful to avoid emotional coercion which could serve to pressure patients into completing FP [85].

It cannot be stressed enough that all patients who will be exposed to gonadotoxic treatment should be provided FPC and referral for FP by an oncology specialist regardless of their medical provider's beliefs about the appropriateness of FPC or assumptions about a patient's desire for FP or ability to parent. FPC from an oncology specialist can and should include a discussion of any relevant medical

risks associated with delaying cancer treatment to pursue FP. These discussions should also allow the patient the opportunity to actively participate in decision making as counseling from an oncology specialist is associated with future emotional well-being; the addition of counseling from a fertility specialist has been found to incrementally increase this positive outcome. Regardless of patient engagement in FP following FPC, patient participation in FP decision making plays an important role in reducing the risk of future feelings of regret [20]. Disparities in FPC and referral for FP on the other hand have the ability to cause significant harm to patients.

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Chapter 20
Germ Cell Transplantation and Neospermatogenesis

Aya Uchida and Ina Dobrinski

Introduction

Male infertility is a significant problem in various fields such as human medicine, livestock reproduction, and wildlife conservation. Nowadays almost 7% of men are suffering from infertility [1] and genetically or economically valuable animals do not always naturally produce sperm.

As described in previous chapters, some reproductive techniques such as sperm cryopreservation and intracytoplasmic sperm injection (ICSI) are currently applied to fertility preservation [2]. However, such techniques are only applicable for post-pubertal males with some sperm in their testes, and prepubertal individuals at risk of azoospermia have hardly any option to preserve their fertility. Especially, pediatric cancer patients who will undergo gonadotoxic chemotherapies or irradiation are waiting for novel reproductive technologies to preserve their potential to father their own genetic children in the future [3].

Hereafter, we will introduce germ cell transplantation (GCT) as a promising countermeasure against the risk of infertility [4]. This technique is feasible for both prepubertal and postpubertal males, and is expected to overcome current limitations of fertility preservation. The key elements in GCT are spermatogonial stem cells (SSCs), which are testis-specific stem cells at the foundation of spermatogenesis and male fertility [5]. By transplanting germ cell populations, including SSCs, the infertile recipient testis can undergo donor-derived spermatogenesis originating from the transplanted SSCs [6]. Up to the present, GCT has provided valuable insights into the mechanism of SSC maintenance, worked as an efficient assay system for the stem cell potential of SSCs, and opened up novel approaches to fertility preservation.

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In this chapter we first briefly review mammalian spermatogenesis, review the development of GCT and the resulting neospermatogenesis in the recipient testis, and describe SSC culture as a procedure preceding GCT. As an alternative method to GCT, we also introduce testis tissue grafting. We will then review current applications of GCT in various fields, and discuss possible future applications of GCT. While the majority of work to date has been performed in mouse models, we will also refer to non-rodent animal models considering the actual applications for biomedical, agricultural, and conservation fields.

Spermatogonial Stem Cell Niche

Spermatogenesis is an intricate and well-organized process to continuously generate large numbers of sperm throughout the reproductive life span. In mammalian species, spermatogenesis originates from spermatogonial stem cells (SSCs) located within the basal compartment of the seminiferous tubules. Like other tissue-specific stem cells, SSCs are characterized by two distinct abilities, the ability to self-renew and the ability to differentiate. While a subpopulation of SSCs self-renew to maintain a certain SSC population, some differentiate into sperm as they move through seminiferous epithelia, from basal to adluminal compartment [7].

Spermatogenic linage development is regulated by various secreted factors, transcription regulators, and endocrine systems. The balance between self-renewal and differentiation of SSC is mainly regulated by several secreted factors. For example, glial cell line-derived neurotrophic factor (GDNF) and fibroblast growth factor 2 (FGF2) promote self-renewal of SSCs [8–11], while bone morphogenic protein 4 (BMP4), activin A, and retinoic acid stimulate differentiation of SSCs [12–14]. Therefore, the growth factor milieu in the basal compartment is essential for SSC proliferation. Such microenvironment is called “niche,” and within this niche environment, SSCs are believed to undergo self-renewal to expand their population [15].

The SSC niche is relatively well defined in invertebrate species such as *Drosophila* [16], but in mammalian testis, SSCs appear to be located randomly along the basement membrane throughout the seminiferous tubule. Recent study has suggested a preferential distribution of SSC to the vascular system and interstitial cell population in vivo [17]. Furthermore, the niche-like environment was detected at the terminal segment of seminiferous tubules [18]. However, the nature of the mammalian SSC niche still remains an enigma.

Germ Cell Transplantation

In 1994, Dr. Ralph Brinster and colleagues developed the method of germ cell transplantation (GCT) in mouse testis and demonstrated the functional identity of SSCs for the first time [4]. In this technique, germ cell populations including SSCs are

isolated from the donor animal testis and microinjected into the seminiferous tubules of infertile recipient testes, via efferent ducts [19] or the rete testis [20, 21], and then the recipient testis undergoes donor-derived spermatogenesis to produce sperm (Fig. 20.1). To increase the efficiency of donor-derived spermatogenesis, suppression of endogenous spermatogenesis in recipient testes can be achieved by genetic mutation (W/W^v mouse; [22], Sl/Sl^d mouse; [23]), focal irradiation [24, 25], or gonadotoxic drug administration (busulfan; [4]). In order to distinguish the transplanted cells in recipient testis, transgenic animals are preferred as donors for experimental purposes. For example, transplanting transgenic donor germ cells into the wild-type recipient testis will facilitate visualization of donor-derived spermatogenesis by fluorescence microscopy.

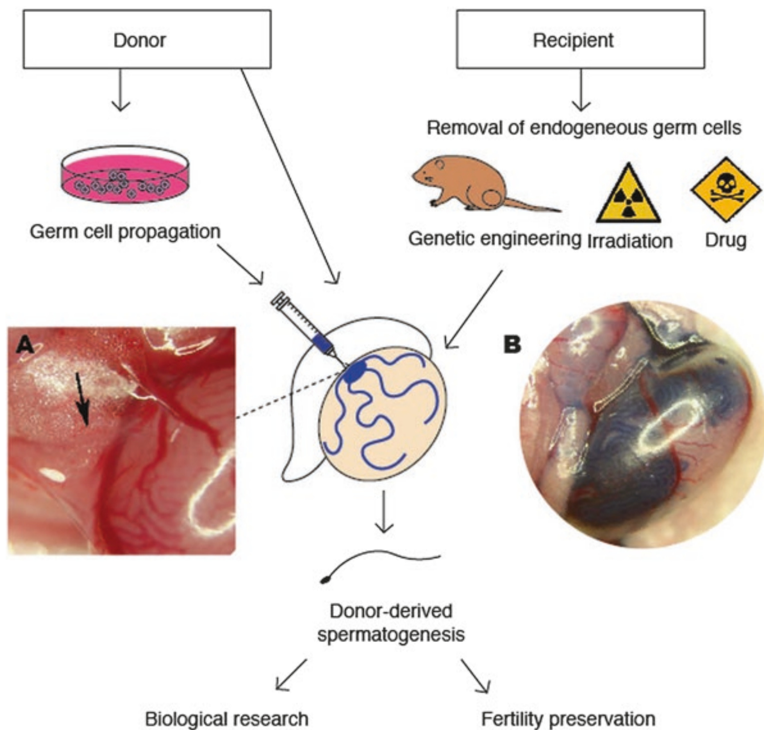

Fig. 20.1 Germ cell transplantation. Germ cells obtained from a donor testis are transplanted into the recipient testis either directly or after propagation in vitro. To improve donor cell colonization efficiency, the recipient testis should have undergone endogenous germ cell depletion prior to the transplantation by either genetic engineering, irradiation, or cytotoxic drug treatment. The recipient testis gives rise to donor-derived spermatogenesis after germ cell transplantation, which can be used for biological research and fertility preservation both in human medicine and in animals. (**a**) Donor germ cells are transplanted into the recipient testis through an efferent duct (*black arrow*) via microinjection. (**b**) Appearance of a recipient mouse testis after germ cell transplantation. Trypan blue was added to the germ cell suspension before transplantation in order to visualize filling of the seminiferous tubules with the donor cell suspension

Successful GCT has also been reported in non-rodent animal models such as bulls [25], cats [26], dogs [27], sheep [28], monkeys [29, 30], goats [31], and pigs [32], and autologously in human [33]. Allogenic GCT trials were also performed between mouse and various animal species such as rats [19, 34], hamsters [35], rabbits and dogs [36], primates [37], bulls [38], and cats [26]. Transplanted germ cells from rat and hamster undergo complete spermatogenesis in host mouse testis, while germ cells from other animal species result in incomplete spermatogenesis. This is presumably because the mouse somatic cells lack the ability of supporting germ cells from phylogenetically distant species.

Even now, GCT is the only way to conclusively identify the stemness of SSC. Importantly, GCT provides a functional and quantitative assay system of SSC activity and population. Considering the principle that only an SSC has the ability to generate and maintain a spermatogenic colony in the infertile recipient testis, each colony is thought to arise from a single SSC [39, 40]. Therefore, total number of transplanted SSCs can be roughly estimated by counting the number of colonies formed in the recipient testis.

Neospermatogenesis

After transplanting germ cells, the recipient testis undergoes donor-derived spermatogenesis [4]. Elucidating the mechanism of neospermatogenesis originating from the transplanted SSCs is important for the improvement of GCT efficiency and for further analyses of regulatory mechanisms in SSCs. The transplanted SSCs move towards the basal compartment of the seminiferous tubule to colonize within the SSC niche. This movement of SSCs is called "homing," and the homing efficiency determines the number of donor-derived SSC colonies in the infertile recipient testis [41]. In order to home into the niche environment, SSCs have to interact with Sertoli cells, migrate from lumen to the basal compartment of seminiferous tubule, and attach to the basal membrane of the seminiferous tubule [41].

Colonization efficiency of transplanted SSCs is assumed to be 5–10% in mouse and even lower in other animal species [41, 42]. This is mainly because of the difficulty of passing through the tight junctional areas between Sertoli cells which is called blood-testis barrier (BTB), and this low homing efficiency of transplanted SSCs has prevented the clinical applications of GCT. To improve the homing efficiency to overcome this limitation, work has been directed at uncovering the mechanism of SSC homing. It has been shown that in the absence of small G proteins called *Rac1,* SSCs lack the ability to migrate through the BTB, but retain their ability to reinitiate spermatogenesis in the testis. Rac1 modulates the expression of tight junction-associated proteins such as claudin 3 [43]. Secreted chemotactic factors from the niche environment, such as GDNF and C-X-C chemokine ligand 12 (CXCL12), were also implicated in promoting migration of transplanted SSCs from the tubular lumen to the basal compartment of the seminiferous tubule [44, 45].

Also, the adhesion molecule beta-1 integrin on both SSCs and Sertoli cells plays a critical role in the localization of SSCs to the basal membrane [46].

Most of the factors required for successful homing are also crucial in the maintenance of SSCs in vivo. For example, deficiency of beta-1 integrin, Rac1, and CXCL12 signaling via its receptor C-X-C chemokine receptor type 4 (CXCR4) in SSCs impaired the maintenance of SSCs, leading to loss of the germ cell population [43–47]. These findings underscore the importance of the niche environment on germ cell homing and survival. In mice, this homing process occurs in the first week after transplantation, and subsequently donor-derived spermatogenic colonies expand [41]. Due to the conserved homing mechanism among various animal species, homing of SSCs happens even in xenotransplantation. However, species-specific differences in spermatogenic regulatory mechanism prevent complete spermatogenesis from xenotransplanted SSCs [26, 27, 36–38].

In mice, after SSCs reach to the basal membrane, transplanted SSCs proliferate to generate monolayered colonies in 1 week–1 month posttransplantation. After 1 month of transplantation, colonized SSCs undergo both mitosis and the resulting cells enter meiosis to generate sperm while expanding their colonies in longitudinal axis of the seminiferous tubule [42, 48, 49]. Thus the length of each colony increases with the passage of time, but the number of the donor-derived colonies decreases gradually after 2 months of transplantation [42]. Most of the homing and colonization mechanisms of transplanted SSCs still remain an enigma, but further understanding of these mechanisms will ultimately lead to successful and efficient clinical applications of GCT.

GS Cell and Testicular Tissue Organ Culture

The culture system of germ cells is well established in mouse, and has provided an SSC propagation system and valuable insights to understand basic regulatory mechanism of SSCs. Cultured germ cells were termed germ-line stem (GS) cells, which were shown to include SSCs as a subpopulation by GCT. GS cells are inducible both from neonate and adult mouse [50, 51], even in feeder-free culture conditions [52, 53]. A crucial role of GDNF for maintaining SSCs in culture was established [50, 51] and soluble GDNF family receptor alpha-1 (GFR alpha-1) is known to enhance the trophic effect of GDNF on GS cells [54]. Recent study has shown that FGF2 can maintain and expand the SSC population in vitro without GDNF [10]. Germ cell purification by FACS or MACS sorting and differential attachment of germ cells and somatic cells to tissue culture plates are effective to prevent the contamination with somatic cells and enable stable long-term GS cell culture.

Up to the present, GS cell culture systems were reported in various animal species including rats [54, 55], hamsters [56], rabbits [57], bulls [25], pigs [58, 59], primates [60, 61], and human [62, 63]. However, it has not been achieved to generate fertile haploid cells from GS cell culture in any animal species. Thus it is

still required to transplant GS cells into recipient testes as previously described in order to generate fertility-competent sperm.

While it remains challenging to produce complete spermatogenesis from GS cells all in vitro, an organ culture system with liquid-gas interface can generate functional sperm from neonatal mouse testis in vitro, even after testicular tissue cryopreservation [64]. Long-term spermatogenesis in organ culture from neonatal mice is feasible by using microfluidic devices to mimic in vivo condition [65]. However, the efficiency of spermatogenesis from adult testis is low even in mouse [66], requiring further investigation and system improvements before clinical applications in human patients.

Strikingly, both mouse embryonic stem (ES) cells and induced pluripotent stem (iPS) cells can be induced into primordial germ cell-like cells (PGCLCs) which are capable of spermatogenesis and produce functional sperm when transplanted into infertile recipient mouse testis [67, 68]. Although further study is required to elucidate the properties of PGCLCs, this breakthrough expanded the future possibilities of clinical applications. By translating these results to applications, infertile individuals may be able to regain their fertility in the future.

Testicular Tissue Grafting

Testis tissue xenografting is a feasible method to generate complete spermatogenesis from large animal species in a mouse host. In 2002, complete xenogenic spermatogenesis between large animal species and mouse was first reported by xenografting testicular fragments [69]. In this technique, testicular pieces with a diameter of 0.5–1 mm are obtained from donor and embedded under the back skin of immunodeficient adult mice (Fig. 20.2a). The testicular xenografts increased in size and developed to form seminiferous tubules with all stages of spermatogonic cells (Fig. 20.2b, c). The grafts are then excised, minced, and dispersed to isolate sperm that can be used to obtain viable offspring by ICSI (Fig. 20.2d) [70, 71]. After grafting, testicular tissue grows capillaries to construct a functional circulatory connection between the graft and the recipient mouse [72]. The grafted testicular tissue interacts with hypothalamus and pituitary gland, and undergoes continuous steroidogenesis [24]. Testis xenografting was successfully performed in various animal species, including rodents, carnivores, ungulates, and primates [73]. Although the efficiency of spermatogenesis is different for different donor species, testis grafting is considered an applicable technique for a wide range of animal species.

Importantly, not only fresh testis tissue but also refrigerated or cryopreserved testis tissue can develop complete spermatogenesis when grafted into mice [69]. Therefore, cryopreservation of testicular tissue from human childhood patients, valuable livestock, or endangered species is important for fertility preservation. Some studies have also demonstrated that testicular tissue xenografting is useful to study gonadotoxicity of drugs [74] and endocrine disruptors [75].

Human testicular xenografts from infants or prepubertal boys have been revealed to survive and proliferate with structural integrity of seminiferous tubules in mouse

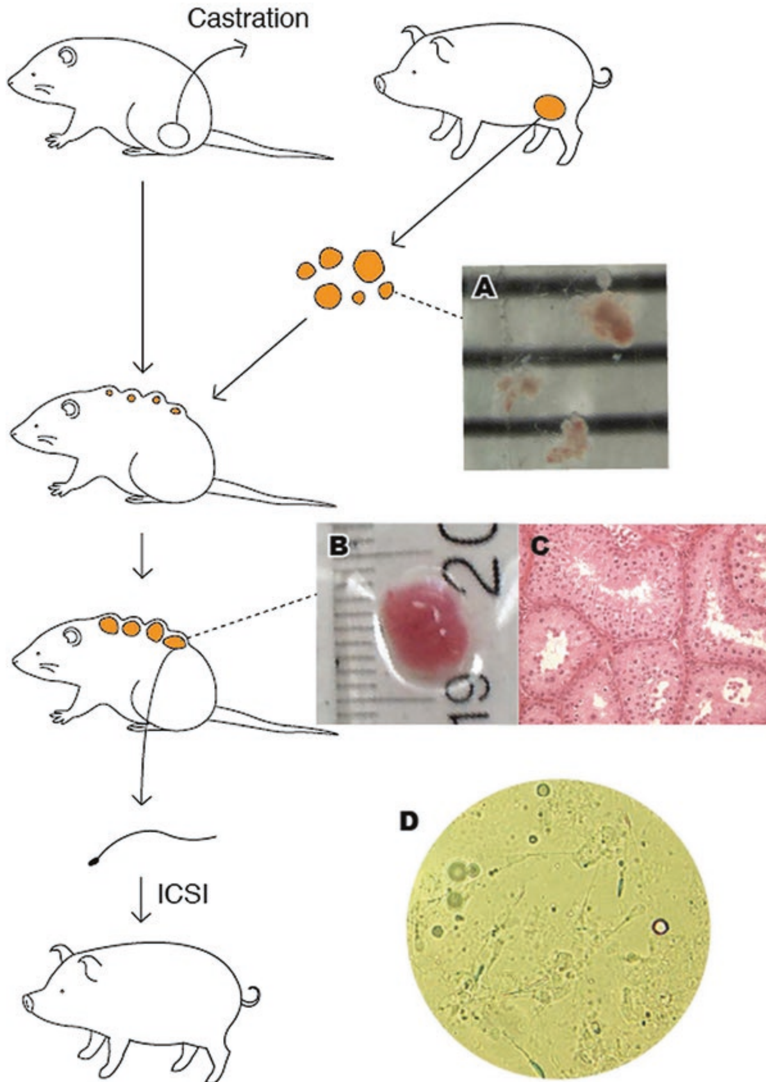

Fig. 20.2 Testicular tissue xenografting. To improve graft development, the recipient mouse is to be castrated before testicular tissue grafting. Testis tissue obtained from the donor (here illustrated for a porcine donor) is fragmented into small pieces (1 mm^3; (**a**)) and subsequently grafted into the subcutaneous tissue under the dorsal skin of the recipient mouse. After a certain period, the grafted testicular tissue develops and increases in size (**b**) and shows donor-derived spermatogenesis within the tubular structures (**c**). Sperm obtained from the grafts (**d**) can generate embryos by intracytoplasmic sperm injection (ICSI) into eggs and these can result in healthy offspring after transplantation to surrogate females

for a long period (6 months), even after cryopreservation, and promote spermatogenesis up to pachytene spermatocyte [76, 77]. In the xenografts from adult human, SSCs and spermatogonia occasionally survived, but no spermatocytes were observed and sclerosis of the xenografted tissue progressed in time [78, 79]. Based on these findings, testicular xenografting can be a powerful tool to examine the early period of gametogenesis in human, as well as to preserve germ cell population from infants or prepubertal individuals, rather than adults. Potentially, this model can also be used for fertility recovery of cryptorchid (undescended testes) individuals, considering that a previous study showed that cryptorchid testis tissue recovered spermatogenesis partially in prepubertal human and completely in horse when grafted into immunocompromised mouse [80, 81].

Testis tissue xenografting provides highly valuable options to explore testicular growth, spermatogenic development, endocrine regulation, and toxicity of various substances on spermatogenesis as well as to maintain and propagate male germ cells for the purpose of fertility preservation.

Applications of GCT

GCT technology can be leveraged into applications in various fields. As mentioned before, GCT serves as a functional and quantitative assay system for SSCs. GCT has contributed not only to identify SSCs, but also to uncover biological features of SSC and mechanism of spermatogenic regulation. Transplantation of gene-manipulated germ cells is considered as an especially useful method for such analysis.

Previous studies demonstrated the feasibility of genetic manipulation of cultured germ cells by using viral vectors in rodents, and the genetically modified germ cells generated fertile transgenic sperm upon transplantation [82–85]. The genetic manipulation of GS cells is also feasible in non-rodent animal species such as goat [86], dog [87], pig [88], and bull [89]. In consideration of these results, transplantation of genetically modified GS cells can provide a novel approach to transgenesis and gene editing of non-rodent animal species.

Transgenic large animal models play an important role in biomedical research, agriculture, and human disease modeling and are also used to produce biopharmaceutical proteins. Currently, transgenic large animal models are mainly produced by somatic cell nuclear transfer [90, 91]. However, this method has low efficiency and frequently leads to fetal deformity and neonatal mortality associated with nuclear reprogramming [92, 93]. GCT can provide an alternative to overcome these problems and generate transgenic sperm in significantly shorter time compared to somatic cell nuclear transfer. Gene-manipulated germ cells transplanted into the testis subsequently colonize the recipient testis, undergo spermatogenesis, and continuously generate fertile transgenic sperm, which can be used to generate transgenic embryos by in vitro fertilization (IVF). Up to the present, production of transgenic sperm through GCT was reported in goat and pig, following viral transduction and

nonviral transfection, respectively [31, 88]. These studies have provided the foundation of future practical applications of GCT for animal transgenesis.

GCT can also be applied to fertility preservation. GCT technology is expected to provide a fertility preservation option for the patients of Klinefelter syndrome, cryptorchidism, and childhood cancers. Klinefelter syndrome is a disease that causes progressive loss of germ cells along with sexual maturation [94]. Preservation of their SSCs before puberty may give patients the option to obtain their own children in the future. People with cryptorchidism may obtain fertile sperm through GCT as long as SSCs can be successfully extracted and preserved [80]. Prepubertal boys at the risk of infertility due to cytotoxic treatment for cancer may have the option to preserve their fertility by cryopreserving their SSCs in preparation for future GCT. While cancer itself can be the direct cause of infertility [95], chemotheraphy or radiotherapy as a cancer treatment often affects germ cells and causes infertility, especially in childhood cancer survivors [3]. Considering that semen cryopreservation cannot be applied to prepubertal cancer patients at the risk of infertility who require gonadotoxic therapies, testis biopsy and germ cell cryopreservation for future GCT can be an effective alternative to their fertility preservation [96, 97]. Testicular tissue banking is currently available in some programs such as the Fertility Preservation Program of Pittsburgh (http://www.fertilitypreservationpittsburgh.org).

Discussion

Autologous GCT is a potential approach to restore fertility, especially for childhood cancer survivors who have become infertile due to cytotoxic therapies to treat cancer. However, the safety of this strategy has to be evaluated before translating GCT to the human clinic. First, elimination of potential contamination of donor germ cells with malignant cells is necessary before GCT can be considered a safe option [98]. Magnetic or fluorescence-activated MACS/FACS cell sorting systems have been explored for their usefulness to remove cancer cells from the donor cells [99, 100], though an efficient depletion system for the malignant cells is not sufficiently established yet [101]. Also, due to the current limitation of human germ cell isolation and enrichment in vitro, it is difficult to obtain sufficient numbers of germ cells from the donor to use for transplantation. Therefore, the development of in vitro propagation systems for human germ cells has a high significance for the future application of GCT. To date, concerns about safety have prevented the application of GCT in humans, although the production of functional sperm through GCT has already been shown in primate models [29]. To minimize the risk to reintroduce malignant cells into the patient, testis tissue xenografting is a promising method to preserve fertility, as it allows to obtain functional sperm from the xenografts [69]. However, safety concerns regarding xenotransplantation, such as potential for contamination with endogenous retroviruses carried by the recipient, still need to be addressed [102]. To prepare

for the further development and future clinical applications of GCT and testis tissue xenografting, testis tissue banking is suggested for children at risk of infertility.

Genetic manipulation of germ cells followed by generation of functional sperm through GCT has been achieved in several animal models and has been suggested as an option for people with heritable genetic mutations to correct the mutation in germ cells, to produce sperm to father healthy children. While this strategy for genetic modification is useful and feasible in livestock, its application to germ-line gene therapy in humans remains controversial because of the ethical issues and possible risks for the offspring.

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