Sperm Banking: Indications and Regulations

Alaa Hamada, Monika Wasik, Sajal Gupta, Ashok Agarwal

INTRODUCTION

Sperm banking is the process of freezing sperm at very low temperatures and storing it for future use in assisted conception. The process was first used in the 1950s to freeze and store bull semen in an effort to improve dairy cow breeding. In 1953, Schermann et al. were the first to apply the technology to human assisted conception. In their breakthrough study, artificial insemination utilizing human sperm, frozen in dry ice with glycerol (10%), resulted in three successful pregnancies with viable embryo development. It was not until 1964, however, when the first live birth was reported using thawed ejaculated sperm, persevered in glycerol freezing media and embedded in liquid nitrogen.

Currently, sperm banking is used to preserve fertility in a variety of men, including those with cancer. In fact, it has garnered a lot of attention in recent years largely because cancer cure rates have dramatically improved along with reproductive medicine technologies. As a result, a new field called ‘oncofertility’ has been created to focus on fertility preservation and quality of life issues for cancer survivors. Additionally, as male reproductive health has deteriorated during the past century, the indications for sperm banking have become more broadly implemented. It is now widely viewed as an effective method of fertility preservation for a variety of men.

CHAPTER OBJECTIVES

This chapter comprehensively describes the various aspects of sperm banking starting from patient counseling and ultimately describing the use of a thawed sample in assisted reproductive technology (ART) procedures. It aims to clarify common misconceptions and offer the most up-to-date information regarding semen freezing, otherwise known as cryopreservation. It is important to continuously update clinicians about fertility preservation options and innovations, so that they can apply this knowledge in their practice. When nonreproductive medicine specialists are familiar with these options, they can effectively counsel their patients to avoid the potentially devastating effects of fertility loss caused by toxic treatments or other confounding factors. Additionally, patients can learn about these topics and initiate the conversation regarding fertility preservation.
options with their healthcare provider. Sperm banking is generally considered to be a safe, cost-effective and useful means to preserve fertility and should be offered to all patients, who may benefit from it.

WHO CAN BENEFIT FROM SPERM BANKING AND WHY?

Cancer patients of reproductive age are most commonly referred to sperm banks in the United States—they comprise about 44% of all referrals. However, other male patients may benefit from this service including adolescents, pre-pubertal cancer patients, men with azoospermia, severe oligozoospermia or other rheumatic, neurodegenerative or hematologic diseases, those with occupational risks or limitations and biological males undergoing sex change surgery (Table 1).

In general, users of sperm banking are divided into two categories: autoconservation group and donors group. Patients who belong to the autoconservation group often preserve their sperm or testicular tissue for the purposes of fertility preservation or fertility treatment whereas, donors are recruited to become surrogate fathers for couples suffering from refractory male infertility problems or planning to prevent transmission of certain genetic diseases or infectious diseases in the husband or for single or lesbian women planning to mother a baby. These donors donate their semen to be preserved in sperm banks for future use in assisted reproductive techniques.

Referral rates in other countries tend to be lower than those in the United States, e.g. in China, the reported figure for the referred cancer patients is only 1.9%. These discrepancies reflect variations in the level of awareness, current emphasis on improving quality of life among cancer patients in western countries and probably limited financial resources allocated for sperm banking practice.

Sperm Donors Group

For sperm donor group see the Table 1.

Autoconservation Group

This group can be divided into two large subgroups based on the purpose of sperm cryopreservation: fertility preservation and fertility treatment.

Fertility Preservation Group Includes the Following

Adult oncology patients: Cancer and its effects on male fertility. In 2011, The American Cancer Society reported that the 5-year relative survival rate for all cancers was 68% (based on data gathered between 1999 and 2006). This is a significant improvement, up from 50% in the 1970s, and is due to the wide use of reliable screening procedures for earlier detection and successful application of aggressive cancer therapy. As such, more cancer patients are younger when first diagnosed and are more often cured, culminating in a larger number of cancer survivors than ever before. Thus, the landscape of patient priorities surrounding cancer management is changing as there is a greater concern for the treatment’s effects on fertility. Therefore, it is imperative that oncologists become familiar with fertility preservation options.

The most common cancers that affect males of reproductive age include testicular cancer, Hodgkin’s disease, acute leukemia, non-Hodgkin’s lymphoma and soft tissue tumors, such as sarcomas. Cancer affects the entire body in many negative ways, systemically and locally, and it particularly impairs spermatogenesis.

Meanwhile, cancer therapies are also quite toxic to reproductive health, hindering normal testicular function and decreasing semen quality. As such, high incidence of poor semen quality is observed among referred men with asthenospermia being the most common finding (64.2–86.3%). Other common semen abnormalities among cancer patients who are referred to sperm banking include oligozoospermia (49.8–53%), severe oligozoospermia (< 1 million sperm/ml) (22.6%), azoospermia (9.7–21%) and abnormal sperm morphology (22.8%).

Alternatively, a recent study showed that teratozoospermia is the most common abnormality (93.2%) among pretreatment cancer patients. Interestingly, most of these semen abnormalities often resolve after cancer therapy. Moreover, the chromosomal aneuploidy rate in germ cells (as detected by fluorescent in situ hybridization) is increased in men with testicular cancer and Hodgkin’s disease before they start therapy. Just how cancer affects male fertility and semen quality is still incompletely understood. Testicular cancers have local and systemic influences on spermatogenesis, perhaps because they are associated with the production of local paracrine factors, such as β-human chorionic gonadotropin (enhances estrogen production and suppresses gonadotropin levels) and cytokines, and the cancer itself disrupts the blood-testis barrier, resulting in abnormal immune sensitization against sperm. Meanwhile, male infertility and testicular cancer may coexist in males with undesended testes and males with a history of in utero exposure to xenoestrogens. Likewise, others suggest that testicular cancer and male infertility are the manifestation of fetal testicular insults culminating in testicular dysgenesis syndrome. This last theory is supported by a high incidence of carcinoma in situ (CIS) in testicular biopsies among infertile men (4%). CIS can transform into invasive cancer in 50% of cases.
## A. Fertility Preservation

<table>
<thead>
<tr>
<th>Patient Condition</th>
<th>What Makes them Eligible for Sperm Banking?</th>
<th>Sperm Collection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult cancer</td>
<td>Cancer and related therapies damage the gonads and impair spermatogenesis</td>
<td>Frequent ejaculation</td>
</tr>
<tr>
<td>Adolescent cancer</td>
<td>Same as above</td>
<td>Frequent ejaculation (when they are sexually mature)</td>
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<td></td>
<td>Penile vibratory stimulation</td>
<td>Electroejaculation</td>
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<tr>
<td>Cancer in prepubescent males</td>
<td>Cancer treatment negatively affects spermatogenesis; testicular tissue extraction is a promising experimental procedure</td>
<td>Testicular tissue freezing</td>
</tr>
<tr>
<td>Preoperative surgical procedure to treat or induce infertility</td>
<td>Bilateral varicocele ligation, prior to vasectomy</td>
<td>Frequent ejaculation</td>
</tr>
<tr>
<td>Nonmalignant disease</td>
<td>Systemic stress may impair spermatogenesis; gonadotoxic therapies affect semen quality</td>
<td>Frequent ejaculation</td>
</tr>
<tr>
<td>Occupational risk</td>
<td>Exposure to harmful chemicals may decrease fertility or cause chromosomal damage in the germ cells; facing hazardous situations that may result in an accident that causes infertility</td>
<td>Frequent ejaculation</td>
</tr>
<tr>
<td>Posthumous sperm cryopreservation</td>
<td>Performed at the time of brain death-based upon the patient's will or family request</td>
<td>Electroejaculation</td>
</tr>
<tr>
<td>Sex change/ gender reassignment</td>
<td>Hormone therapy damages spermatogenesis; and gender reassignment surgery sterilizes the male</td>
<td>Surgical removal of testes and epididymides</td>
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## B. Fertility Treatment

<table>
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<tr>
<th>Patient Condition</th>
<th>What Makes them Eligible for Sperm Banking?</th>
<th>Sperm Collection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe oligozoospermia (&lt;5 million sperm/ml)</td>
<td>Progressive decline in sperm production and high chances of azoospermia</td>
<td>Frequent ejaculation</td>
</tr>
<tr>
<td>Obstructive and nonobstructive azoospermic</td>
<td>Absence of sperm in ejaculate; benefit from using sperm extraction techniques for ART</td>
<td>Surgical retrieval techniques from testes or epididymis</td>
</tr>
<tr>
<td>Spinal cord injuries</td>
<td>Anejaculation and poor semen quality with difficulty in transportation</td>
<td>Assisted ejaculation and surgical sperm retrieval from testis and epididymis</td>
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<td>Donor semen insemination</td>
<td>Donor sperm use as therapeutic measure in a couple with sterile men, men with genetic or infectious diseases such as HIV and for insemination of single or lesbian women</td>
<td>Frequent ejaculation</td>
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<tr>
<td>Absence of the male factor during conduction of ART cycles</td>
<td>Frequently traveling husband</td>
<td>Frequent ejaculation</td>
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Systemic effects of malignant disease include fever (Hodgkin’s disease, non-Hodgkin’s disease, leukemia), malignancy-related malnutrition, abnormal immune response (lymphoma), altered hormonal milieu (testicular cancer, central nervous system tumor) and a generalized stress and inflammatory response leading to the production of cytokines, such as interleukins and tumor necrosis factor, all of which can have devastating consequences on testicular and sperm function. These substances damage sperm before treatment and may result in chromosomal aneuploidy in the germ cells. The precise mechanism by which tumors affect spermatogenesis is not fully known, and the clinical cancer stage has no relation with the severity of male infertility. Therefore, it is imperative for physicians to address each cancer patient’s fertility risk, no matter the clinical diagnosis or disease stage.

In addition to the cancer itself, modern oncologic treatments are also highly toxic to the body and the testes. Gonadotoxic therapies are particularly harmful to the early stages of spermatogenesis, specifically to proliferating and differentiating spermatogonia, and could cause permanent damage. Oncologists should strive to maintain a balance between the toxic effects of cancer therapy and the benefits they procure, as cancer treatment acts as a double-edged sword having harmful effects on a man’s fertility as a byproduct of its therapeutic activity.

In general, the effects of oncologic treatments on fertility are quite unpredictable. A common side effect of toxic treatment is temporary or permanent azoospermia and severe oligozoospermia. Although spermatogenesis can resume after treatment, practitioners do not know how long that may take if and when it does happen. For instance, in men with testicular cancer, it takes at least 2 years after cessation of (etoposide + bleomycin) therapy for spermatogenesis to recover. Ultimately, recovery occurs in 80% of patients within 8 years of treatment. While normal parameters may recover, the ability to fertilize an oocyte may still be affected as has been observed with the use of vincristine. Due to the uncertain nature of cancer treatment, it is prudent to bank sperm before the start of treatment to insure against potentially permanent loss of fertility. It has been widely accepted that pretreatment sperm cryopreservation is the most effective method for preserving fertility in young cancer patients.

Chemotherapy, which is generally considered a highly toxic treatment, specifically affects spermatogenesis. Chemotherapy regimens consisting of either a single or multiple therapeutic medications have variable effects on testicular function-based upon the toxicity profile of each agent, cumulative dose, coexistent radiotherapy and treatment duration. Alkylating agents, such as nitrogen mustard, cyclophosphamide, ifosfamide, busulfan, procarbazine and chlorambucil are notorious for having the most harmful effects on the body organs including the testes. The more toxic regimen used in therapy for Hodgkin’s disease, which consists of nitrogen mustard, vincristine (Oncovin), procarbazine and prednisone (MOPP) is less commonly used in the United States and is reserved mostly for treatment of advanced disease and in cases where there is a risk of cardiac toxicity that precludes the use of adriamycin, bleomycin, vinblastine, dacarbazine (ABVD). Six cycles or more of MOPP that contain two alkylating agents, mustard and procarbazine, results in a high incidence of prolonged azoospermia in 83–97% of patients post therapy. Fortunately, the less toxic ABVD, which is the treatment more commonly used today, causes significantly less damage to the reproductive organs. In testicular cancer, 53% and 44% of men receiving a regimen consisting of BEP and cisplatin—the most commonly used regimen, develop oligozoospermia and azoospermia, respectively, after 2–4 cycles.

Radiotherapy is similarly harmful to spermatogenesis. While damage to the testicles depends on the dose and method of exposure, the risk is high when treatment is targeted directly at the testicles (e.g. testicular irradiation for CIS or total body irradiation prior to bone marrow transplantation) or when there is a scatter effect (e.g. radiotherapy for pelvic organs). Radiotherapy occasionally causes persistent sperm DNA fragmentation and reduced semen volume and damages cells in all areas of the male reproductive organs. Moreover, radiotherapy can obstruct the ejaculatory ducts and access to mature sperm.

The amount by which spermatogenesis is affected depends on the dosage. Four Gy appears to be the threshold for permanent azoospermia, as anything less presumably only causes temporary variations in semen parameters. Radiation-induced transient alterations in spermatogenesis may disappear within 2 years. For Leydig cells, the toxic dosage is 20 Gy or more for prepuberal boys and more than 30 Gy in adult males, which will render men testosterone deficient. Predictably, the detrimental effects of chemotherapy and radiotherapy are increased when the two are used in combination. During combined therapy, the body is exposed to a variety of toxins that affect the male gonads differently depending on whether the treatment is localized or general.

**Pediatric and adolescent cancer patients:** Sperm banking is also a viable option for many adolescent cancer patients whose fertile years are still ahead of them at time of diagnosis. Cure rates for pediatric cancers have dramatically improved over recent years and are currently approaching 80%. With the increasing number of cancer
survivors living today, long-term quality of life concerns are becoming an important part of pretreatment discussion. As a result, adolescent and pediatric cancer patients are increasingly being provided with opportunities for sperm cryopreservation. Adolescents, who have already achieved sexual maturity (at least Tanner stage 2, and a testicular volume of 5 ml) are interviewed regarding their ability to masturbate. If masturbation is not feasible, other methods are offered, such as penile vibratory stimulation and electroejaculation under general anesthesia. Semen quality is also influenced by cancer in the same way as in adults and probably through the same mechanisms.

When azoospermia is encountered, onco-testicular sperm extraction (TESE) is a reasonable choice. In prepubertal males, fertility preservation is a challenging situation as there are no haploid sperm or even spermatids in testicular tissue. In these cases, testicular tissue freezing opens the door for preserving fertility after iatrogenic sterilization, hopefully through stem cell isolation and transplantation or through in vitro maturation and induced spermatogenesis.

Regardless, fertility preservation options should be discussed before treatment is started. Indeed, the American Society of Reproductive Medicine and the American Society of Clinical Oncology (ASCO) support this practice for adolescent cancer patients. Patients’ parents are also quite receptive to sperm banking, even though some of the procedures such as, immature testicular tissue freezing are considered experimental in young boys. Ginsberg et al. showed that parents’ attitude towards sperm cryopreservation procedures is noteworthy and deserve undertaking the opportunity, even though there are no guarantees. It can therefore be concluded that fertility preservation options are indicated for young cancer patients.

**Impaired fertility resulting from other disease-related factors:** Patients with other diseases can also benefit from sperm banking before serious treatment or before the disease becomes so debilitating that it disrupts spermatogenesis. For example, men with lupus, rheumatoid arthritis, ulcerative colitis, multiple sclerosis or hematologic diseases that are treated with bone marrow transplants may also consider sperm banking early in the disease process. Men with such conditions should be aware of the possible effects of their disease on spermatogenesis and future ability to father a child. Many experts suggest that health care providers offer sperm cryopreservation to patients with nonmalignant diseases and urological pathologies, such as bilateral varicocele, testis torsion or necrosis, particularly when they are about to undergo surgery. A systemic disease that damages the entire body is likely to affect testicular function. Men who are awaiting surgery, such as a urogenital procedure or vasectomy should preserve their sperm as a precautionary measure as described below.

**Occupational risks:** Some healthy men may wish to consider using sperm banking for fertility preservation to counteract certain occupational risks. Gupta et al. recommended that men who work with toxic chemicals, ionization radiation or biological hazards “consider banking sperm as these exposures may jeopardize their reproductive potential”. Radiation, a known carcinogen, may damage the germ cells and cause birth defects in offspring. Working with glycol ethers also poses a threat to male fertility and is associated with a low motile sperm count. To avoid such outcomes, men in high risk occupations should bank sperm before these negative effects accumulate.

Sperm banking is increasingly being promoted to men in the United States Armed Forces. These men often travel extensively and are away from home for extended periods of time when deployed. If a military man is having trouble conceiving with his partner, he may want to consider banking his sperm to have it available for use in ART procedures during the female’s time of ovulation. Sperm banking would also act as insurance against severe combat-related injury or chemical exposure that may impair spermatogenesis or even death. While there are few studies published on this topic, the practice has garnered media attention, as there is a growing number of cryobanks offering services to armed forces personnel. Many cryobanks have noticed an influx of military personnel, who wish to bank semen before deployment. One such place is the California Cryobank which discounts sperm storage costs for military men. The Florida Fertility Institute allows military service members going overseas into combat to store their samples completely free of cost. In some places, the service is even being expanded to first responders or men who work in security and are often in dangerous situations. The California Cryobank also offers services for civilians, such as firefighters and emergency medical technicians.

**Sex change and gender reassignment:** Biological males, who are undergoing gender reassignment through hormonal or surgical therapy may also benefit from sperm banking. Estrogen has been shown to have detrimental effects on spermatogenesis and overall semen parameters, and sex change surgery includes orchiectomy and penisectomy, which renders the male sterile, as they are no longer able to ejaculate. After male-to-female sex change surgery, two-thirds of
Section 4  In Vitro Fertilization

patients identify as lesbian and some may want to have biological children with their partner—either via the biological female partner or a surrogate. For this reason, it would be beneficial to counsel such patients on fertility preservation before irreversible biological changes are made with surgery.

Prior to undergoing surgical procedures for treating or inducing infertility: Certain surgeries performed to cure infertility, such as bilateral varicocele ligation and surgical relief of seminal duct obstruction, may be complicated postoperatively by reduced testicular blood supply or inadvertent vasal transaction, particularly when the surgery is performed on both sides of the testes. Fertility preservation may also be an option for men planning to do surgical contraception via bilateral vasectomy as they may change their mind regarding having children in the future.

Fertility Treatment Group
This group includes the following indications:

Severe oligozoospermia: Severe, very severe and extreme oligozoospermia are defined by a very low sperm count of less than 5 million/ml, less than 1 million/ml and less than 100,000 million/ml, respectively. Technically, cryobanking is conducted in these patients to preserve existing spermatozoa to ensure an adequate sperm supply for multiple ART cycles and to eliminate the possibility of not finding fresh sperm in the ejaculate on the day, it is needed for ART. Moreover, recent reports have suggested that a significant proportion of men with severe oligospermia will ultimately become azoospermic and experience a loss of testicular germ cells, which further supports the use of sperm banking in this population.46,47

Azoospermia: Azoospermia is the condition of not having sperm in the ejaculate. There are two kinds of azoospermia: obstructive and nonobstructive. Obstructive azoospermia (OA) is usually caused by post-testicular congenital or acquired obstructive lesions. In such conditions, spermatogenesis is often normal. For this reason, viable sperm can still be obtained through various extraction methods from the testes or epididymides, and testicular sperm retrieval is successful in almost 100% of cases. Nonobstructive azoospermia (NOA), on the other hand, usually implies the presence of a sperm production defect, and its cause is often more difficult to identify and treat. For men with NOA, TESE and microsurgical TESE are the preferred methods for obtaining sperm. Men with temporary azoospermia (perhaps due to a systemic disease or acute testicular insult) may wish to cryopreserve their semen as soon as possible to ensure against further loss of fertility.48 If the transient azoospermia is due to disease, it is recommended that semen be banked before the start of any potentially gonadotoxic treatment. Furthermore, men who experience periodic azoospermia and are undergoing ART procedures with their partners may wish to bank sperm before the procedure, so that the sample is viable on the day it is needed, thereby minimizing the chances of any delays or fertilization failure.

Absence of male factor during assisted reproductive technology cycles: this indication applies for men, who are absent from home for long periods because of military service or frequent travels overseas.

Ejaculatory dysfunction and spinal cord injured men: more than 90% of men with spinal cord injury are infertile and have poor semen quality due to a combination of adverse factors, such as anejaculation, retrograde ejaculation, semen stagnation, frequent genital infections and increased levels of seminal oxidative stress.

COUNSELING AND ETHICAL CONSIDERATIONS

Counseling patients on fertility preservation can prove to be difficult, especially at the time of an initial oncologic diagnosis. When a patient first discovers that he has a disease or has been exposed to agents that have endangered his fertility, he may initially be reeling with the implications of this news. Who is the best candidate then, for counseling this patient during such a difficult time? Many studies on cryopreservation in cancer patients have observed that the first line of information on fertility preservation is often the oncologist or physician delivering the diagnosis.49 The difficulty with counseling cancer patients stems from patient’s excessive distraction and concern about his primary diagnosis. The important task of the oncologist at this time is to clearly explain, in a compassionate manner, the disease, possible lines of therapy and probable implications of the disease and its effects on male infertility. As fertility preservation issues garner more attention, it is important to identify the “role of healthcare practitioners have in patient decision-making with regard to fertility preservation.”38 To facilitate this added responsibility for the physician, the authors recommend early and open communication with patients and the implementation of a multidisciplinary oncofertility team.
Who Should Counsel?

Because cancer and cancer treatment both adversely affect spermatogenesis, it is part of an oncologist’s duty to discuss the effects treatments can have on fertility and fertility preservation options, with an emphasis on cryopreservation. Whose responsibility is it to keep oncologists up to date on the advances in reproductive technologies? Van Casteren argues that it is in fact the reproductive medicine specialist’s responsibility to ensure that other physicians are aware of the new possibilities in fertility treatment and relatively good pregnancy outcomes using the cryopreserved semen.49 It is imperative that physicians outside reproductive medicine are educated enough to know when to refer a patient to a fertility specialist and to discuss sperm banking and subsequent future fertility options.49 Oncologists should also be aware of current ART procedures and use descriptions of promising advances in reproductive medicine to inspire optimism in their patients regarding future fertility options.

One organization, www.savemyfertility.org, whose mission is to increase awareness of fertility preservation options among both providers and patients, provides informative materials that facilitate and stimulate discussions on the importance of sperm cryobanking.50 Specifically, the website provides handouts and tips on fertility preservation counseling, tailored information for patients on how to initiate this discussion with their care provider and take-home information to review after the shock of an initial potentially devastating diagnosis has tapered.50 These outside organizations also encourage patients to take initiative and responsibility for their future fertility. Generally, the authors recommend that physicians initiate and guide sessions on fertility preservation with their patients. An informed nurse and other healthcare provider staff, however, may be helpful in providing continuing support throughout oncologic treatment. After the initial discussion, the patient should be referred to a skilled reproductive medicine specialist. The oncologist should follow-up with the patient to prevent failure to follow on the referral and discuss the patient’s decision to remain informed (Figure 1).

Barriers to Counseling

Despite the effectiveness of cryobanking, barriers to counseling patients on fertility preservation, however, continue to exist. One study emphasizes that sperm cryopreservation remains underutilized for a variety of reasons,7 including lack of time during a patient’s visit, anxiety at the time of diagnosis, conflicting cultural or religious views, loss to follow-up during referral to a specialist, an inadequately communicated sense of seriousness regarding fertility loss and lastly, a physician, who is poorly informed about fertility preservation options.7 Discussing potential fertility loss can cause patients to become very anxious. Specifically, discussing gamete cryobanking at the time of diagnosis may prove challenging, as the patient can feel overwhelmed, and his or her mind is primarily flooded with questions relating to survival rates, treatment strategies and an uncertain future. For example, in a retrospective 2008 study, Anderson observed that up to 58% of patients felt that their levels of anxiety had affected their ability to think about fertility. Consequently, it may be beneficial instead for physicians to discuss fertility preservation options initially at the time of diagnosis, and again perhaps a week later but still before the start of treatment. Cultural or religious views that oppose masturbation or artificial insemination, for example, may also prevent fertility discussions from occurring productively.38 Fortunately, the interdisciplinary nature of the growing field of oncofertility aims to counter such problems and increase the understanding between the patient and physician on these issues.38

Some authors, on the other hand, believe that physicians who do not work in reproductive medicine may have a suboptimal knowledge of the effects of cancer and its treatment on fertility. As such, this factor is considered to be the main reason why many patients do not receive fertility counseling.51 Currently, most oncologists do not automatically consider fertility preservation options as part of the standard workup of males with a newly diagnosed cancer.65 Correcting this ‘discrepancy’ will also require an adjustment in thinking on the part of oncologists and other physicians. The ASCO included discussions on fertility preservation as part of standard care for malignancies in their 2006 guidelines. Although this recommendation was voiced approximately 5 years ago, it is still not implemented as often as it should be. Presumably, it will take time for physicians to become accustomed to initiating these difficult discussions with their patients and integrate new knowledge regarding fertility preservation into their practice.

Counseling adolescent patients poses an additional challenge to healthcare providers. Common barriers include patient age, experimental techniques, advanced disease progression and inadequate banking facilities. Most commonly, the physician will broach the topic of fertility preservation. Sometimes, the parents will ask about options; the patient does so least often.51 Very young patients and/or those with a high level anxiety at diagnosis and at discussion have a more difficult time producing a semen sample. Doctors must work to alleviate patient anxiety by considering biological issues and acknowledging the psychological needs and
Section 4  In Vitro Fertilization

Figure 1  Counseling a reproductive-aged male at risk for fertility loss

individual situation when counseling each younger cancer patient, which can be quite a daunting task. Uncertainty on how to navigate these legal and ethical issues may discourage them from appropriately counseling these patients.

Finally, if a patient has a very poor prognosis for survival, it is unlikely that fertility preservation will be considered important. It may be beneficial, however, for the care-provider to have a discussion regarding fertility preservation regardless of how seemingly hopeless the situation may be. By looking to the future, a patient may be able to foster an optimistic outlook to better focus on fighting the cancer. Anderson et al. noticed that many patients and their families make use of open and spontaneous discussion about fertility. Such discussions can encourage patients to look to the future and reassure them that the aim of cancer therapy is to cure. It is possible, of course, that a patient may be so sick and have a prognosis so poor that he will be physically incapable of producing a sample—in this case, sperm cryobanking would not be worth the effort. Such possibilities again emphasize the need to discuss fertility preservation options on a case-by-case basis.

Ethical Issues Associated with Sperm Banking/ Difficulties Associated with Counseling

The topic of sperm cryobanking is often a very sensitive one for many patients. Future infertility, however, is not a problem of the individual, but of the couple and a product of a family. As a result, decisions regarding fertility preservation can impact others besides the patient. One particularly difficult case can occur when counseling an adolescent or pediatric cancer patient on fertility preservation. As minors, these patients have little autonomy to decide to bank sperm without their parents’ approval. Some families may not be comfortable discussing masturbation, sexuality or reproduction with their son. The boys themselves may feel particularly uncomfortable talking openly about these issues or providing a sample via masturbation or they may not fully understand the future implications of a more immediate loss of fertility, if they are too young to be thinking about having children. Asking them to produce a sample when accompanied by a parent may unduly embarrass them.

Another serious ethical issue surrounds is the post-mortem period in cases where the patient did not use his semen sample. To whom does the sample belong? While the details of ensuring that the sample is properly handled is described in the section on ‘disposing of the sample’ below, it is important for physicians to be aware of this possibility and to address these conundrums. Because of the ethical dilemmas that may be encountered when discussing fertility preservation options, oncologists should carefully make sure to broach the topic with patients. Each patient and his family should be approached with specific consideration as to what the family will likely feel comfortable discussing, as well as the patient’s given situation, as they have a right to be aware of fertility-threatening side effects and
preventive options. Each clinical circumstance is unique and each individual patient’s diagnosis, prognosis, current desires and future hopes, relationship status and (specifically in adolescents) maturity level must be taken into account when discussing fertility preservation options.

**PROCESS OF CRYOPRESERVATION**

Sperm cryopreservation is a tactful process that must be carefully performed. There are a variety of methods for obtaining the sample, and these range from masturbation to more invasive techniques. Freezing and thawing should be done in such a way as to minimize sperm damage. The field and associated methods of cryobiology are continuously changing, as evidenced by the variety of techniques currently in use. Finally, the cryobank facilities should be able to store the samples in a low-risk manner for long periods of time. There is little room for error in cryopreservation from beginning to end, as the samples being frozen are often a patient’s only hope for being able to father a biological child.

**Screening of Patients Prior to Banking**

All sperm donors are required to complete a physical examination, including a genital exam, no more than 12 months prior to the first storage appointment. Most sperm banks also require every potential donor to schedule a physical semen analysis prior to sperm retrieval and storage. Donors are screened for infectious diseases with blood tests for HIV-1/2, HTLV-1/2, hepatitis B, hepatitis C, syphilis and sometimes CMV. Genetic testing of the sperm donor is required by certain states only hope for being able to father a biological child. The next set of methods is essentially assisted masturbation. These include penile vibratory stimulation and electro ejaculation and are most useful for men with spinal cord injury, anejaculation or young boys. For younger boys, whose testes are producing sperm but do not yet masturbate or for adolescent patients, who are anxious about producing a sample, penile vibratory stimulation or electroejaculation should be performed under general anesthesia to minimize psychological discomfort.34

If the patient is azoospermic, more invasive extraction and aspiration techniques should be contemplated. There are a variety of methods that can be used to extract sperm from the testes, including: fine needle aspiration, such as TESA (testicular sperm aspiration), TESE, micro-TESE. For men with OA, percutaneous epididymal sperm aspiration (PESA) or micro-epididymal sperm aspiration (MESA) can be performed.

For pre-pubertal boys, there is currently only one option for fertility preservation. A sample of testicular tissue, most often obtained during a testicular biopsy, can be cryopreserved for future use. Although the procedure is still considered experimental, the isolated stem cells from the tissue could be reimplanted again into the patient once thawed or they could be used to enhance *in vitro* maturation of spermatogenesis. Such procedures have been conducted in animal studies and will take some time to be translated into commonplace clinical practice.36,55,56 A 2010 study, conducted at a children’s hospital, showed that parents of pediatric cancer patients are willing to pursue this technique, despite any unknowns.36

**Freezing the Sample and Cellular Damage During Freezing**

Sperm cryopreservation is a controlled procedure. If not done properly, it can damage the sample, leaving it unusable for subsequent ART procedures post-thaw. Four kinds of clinical samples are cryopreserved for the purpose of sperm banking: ejaculated semen, aspirated epididymal fluid containing sperm, mature testicular tissue and immature testicular tissue.

Cryopreservation damage or cryoinjury to any cell is due to a combination of four factors: osmotic stress/dehydration, oxidative stress, intracellular ice formation and cryoprotectant toxicity (Figure 2). These factors are...
responsible for a 25–75% loss of sperm motility, decrease in sperm cryosurvival and DNA fragmentation after thawing. During cryopreservation and subsequent thawing, sperm are exposed to various challenging osmotic and oxidative situations that the sperm must overcome to survive. Sperm freezing and thawing results in osmotic stress, extracellular and intracellular ice crystals, production of reactive oxygen species (ROS), and resultant negative downstream effects on sperm motility, lipid phase change, membrane integrity, mitochondrial function, DNA integrity, cell signaling and metabolism. Apoptosis and necrotic cell death can also occur. Due to the relative stability of the frozen state, most of these negative effects mainly occur during thawing.

To minimize or avoid these effects, osmotically active cryoprotective agents (CPA) are added to the semen sample before it is frozen. The occurrence of excessive osmotic stress and intracellular ice formation depend on which cooling rate is selected. During rapid cooling rate, most intracellular water remains inside the cell and thereby forms intracellular ice whereas slow freezing dehydrates the cell resulting in shrinkage. Similarly, in response to the slow thawing process, after dilution of extracellular fluid, the cell swells in volume because of intracellular fluid hypertonicity, precipitating another form of osmotic stress (Figure 3).

A single spermatozoon has minimal and maximal cell volume limits between which the cell can withstand increased extracellular and intracellular fluid hypertonicity, respectively. These volume limits should be strictly considered when selecting the proper regimen of sperm freezing. Otherwise, the exerted osmotic stress can result in irreversible cellular damage (Figure 4).

Intracellular ice formation is usually lethal to the sperm as it culminates in cell rupture. Both osmotic and oxidative stresses have been shown to induce ROS in sperm and contribute to cellular damage that may be lethal or sublethal.
Figure 3  Cryodamage, survival, and cell volume changes during cryopreservation with and without CPA


Other sources of sperm damage during freezing can arise from exposure of sperm to cryoprotectants. Permeating cryoprotectants, such as glycerol, are foreign intracellular substances that can certainly poison the cell. Such toxic effects might be masked or diminished by slow cell metabolism at very low freezing temperatures. Nonetheless, at relatively high temperature, such as 0°C, when very high concentration of these agents is used, substantial sperm toxicity effects might be evident.

Semen Sample

Semen samples destined for cryopreservation are usually obtained from two sources, either from the patient himself (autoconservation) or from the donor. Semen taken from a donor is used to inseminate single or lesbian women, treat couple with inability to find sperm in testicular biopsy or with frequent intracytoplasmic sperm injection (ICSI), failure due to sperm factors or prevent transmission of husband’s known genetic or infectious diseases to the offspring. The whole
Section 4  In Vitro Fertilization

**Figure 4** Effect of permeating CPA on cell volume  

Laboratory work is shown in the flow diagram (Figure 5). At first, semen analysis is undertaken by experienced personnel after an adequate period of sexual abstinence (2–5 days) to check for adequate semen parameters, such as sperm count, motility and morphology. Although donors with a low sperm count or poor motility are primarily excluded from undergoing a cryopreservation protocol, this is not the case for those referred for autoconservation. Computerized assisted semen analyzer (CASA) can also be used for prefreezing and even post-thawing semen analysis (count and motility). CASA and computerized assisted sperm motion analysis can also provide additional information about the kinetic properties of prefreezing and post-thawing sperm, such as average path velocity (VAP), straight line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), straightness (STR) and linearity (LIN) (Figure 6). These kinetic measurements correlate well with frozen sperm fertilization outcomes using ART.69

Should sperm be washed before freezing? Given the high incidence of poor semen quality among cancer patients and infertile men, some authors prefer to subject semen samples to washing procedures before the samples are frozen. Sperm washing techniques aim to separate ejaculated spermatozoa from the seminal plasma and eliminate dead spermatozoa along with exfoliated epithelial cells, cellular debris, leukocytes and amorphous material.62,63 Washing the sample not only improves post-thaw parameters, it also removes debris and some infectious diseases, such as HIV and improves acrosomal status.44 It also allows for sample processing, which isolates the most viable and highly functioning sperm present in the sample, which is likely to improve ART success rates. Poor-quality samples are more likely to be damaged in the freeze-thaw process. As such, in patients with oligozoospermia and/or asthenospermia, semen washing is done to improve post-thaw motility and vitality.67 For these reasons, the removal of seminal plasma is strongly recommended prior to freezing.

There is a number of semen washing protocols. In the swim-up technique, the centrifuged sperm pellet or uncentrifuged semen is layered below proper culture medium, Earle’s balanced salt solution or sodium hyaluronate solution for 2 hours. This allows highly motile sperm to migrate inside the culture medium.
Chapter 29  Sperm Banking: Indications and Regulations

The supernatant containing the best sperm is aspirated, centrifuged and resuspended again to be stored.

In the density gradient method, two types of dense solutions—colloidal silica and a tri-iodinated derivative of benzoic acid—are commonly used to create the gradient either in a continuous fashion (a single layer of specific concentration of the dense solution) or in a discontinuous fashion (two layers of the same dense solution with different concentration). Semen is placed on the top of the dense solution and centrifuged for 30 minutes. Highly motile sperm penetrate the dense solutions and precipitate as pellets in the bottom of the tube while the seminal fluid and other cells remain on top. Sperm pellets are resuspended in a proper medium and prepared for the next step.

Magnetic activated cell sorting (MACS) is an alternative to semen washing that removes apoptotic cells and selects motile, viable, morphologically normal spermatozoa with high post-thaw survival rates and subsequent fertilization potential. In this procedure, microbeads attached to annexin V—a phospholipid-binding protein, are used. Annexin V, in the presence of an adequate concentration of calcium, avidly binds with phosphatidylserine. This is an early marker of apoptosis and is expressed on the outer leaflet of the plasma membrane of apoptotic cells. The bound apoptotic sperm are then removed, leaving behind viable, intact sperm (neat sample). This method is still experimental and has not been used clinically. Nonetheless, most laboratories perform cryopreservation on neat semen even though the quality is not optimal. The advantage of using a neat sample is that the natural antioxidant enzymes (superoxide dismutase, catalase) and scavenging molecules (such as, ascorbate, urate, and albumin) are still present to protect against free radical damage during freezing.

Addition of cryoprotective agents: The freeze-thaw cycle can induce cryodamage in sperm, resulting in poor vitality and motility post-thaw. To reduce such damage, CPAs are added to semen samples before freezing. Slow freezing initially induces extracellular ice crystal formation, which promotes cellular dehydration and shrinkage (osmotic stress). Rapid freezing, on the other hand, results in the propagation of intracellular crystal formation depending on the availability of intracellular water. Therefore, it is imperative to select...
the appropriate freezing rate and synchronize it with selecting a substance which can resist the damaging influences of freezing.

Cryoprotectants are substances that are added to pre-frozen neat semen samples or the prep sperm to counteract extra- and intracellular ice formation during freezing. The mechanism of action is not entirely understood. However, these osmotically active agents lower the freezing point, reduce the size of ice crystals and prolong the time required for ice formation. CPAs are divided into one or two groups: penetrating or non-penetrating. Penetrating CPAs are small, nonionic molecules that are lipophilic and highly miscible with water, giving them the ability to easily penetrate the plasma membrane. Examples include glycerol, dimethyl sulfoxide, propylene glycol, ethylene glycol and methylformamide.

Penetrating agents slowly permeate the cell and equilibrate within the cytoplasm, decreasing the volume of intracellular water without overtly dehydrating the cell. Furthermore, because CPAs are highly miscible with water, they act as solvents for other solutes in the remaining intracellular water, reducing their concentration and providing a buffer against freezing related salt-induced stress. These properties are attributed to the colligative nature of CPAs, which are responsible for the freezing point and osmotic pressure of solutes mixed in a solution.

Glycerol is the prototype of all penetrating CPAs and is most successfully used in the cryopreservation of human spermatozoa and that of other species including horse, pig, sheep, dog, and rabbit. A final concentration of 5–10% has been shown to be an optimal concentration of glycerol for a freezing solution.

Examples of nonpenetrating CPAs include mono-, disaccharide, polysaccharide and long-chain polymers (glucose, galactose, sucrose, trehalose, methylcellulose, raffinose, polyethylene glycol and hyaluronan)—these agents are unable to permeate the plasma membrane. Nonpenetrating CPAs can stabilize the lipid bilayer, form hydrogen bonds between hydrophilic sides, increase viscosity and lower the freezing point of the extracellular fluid even though they promote fast cellular dehydration. Egg yolk, which is composed of phospholipid, lipoproteins (low density lipoprotein), cholesterol and antioxidants, soy lecithin (phospholipid isolated from egg yolk and other sources), milk and albumin, are also considered nonpenetrating CPAs that are included in every cryoprotective medium as they protect sperm membranes from cold injury. The mechanism of protection is unknown but it is thought that they maintain plasma membrane fluidity and constituents during freezing. Nonpenetrating CPAs have been used successfully as the sole cryoprotectant in the cryopreservation of red blood cells. However, they are of limited value when used by themselves in the cryopreservation of nucleated cells, such as sperm. Alternatively, these high molecular weight CPAs are used synergistically with other penetrating CPAs to reduce the toxic concentration of penetrating CPAs.

Cryoprotective medium (CPM) is a solution used to dilute semen or prepared sperm—it is essentially composed of permeating CPAs and one or more of non-permeating CPAs. Other components of CPM include plasma membrane protective agents, such as egg yolk, milk or soy lecithin, chelators, buffers and antilipid peroxidative agents (Table 2).

More than ten different CPMs are used in sperm banking. There are generally no specific advantages of one medium over another (Table 3). For a particular patient, different CPMs are used until the one that best suits his needs and the best post-thaw sperm parameters is identified. For donor cryopreservation, donors are selected based on their good post-thaw semen parameters in a particular single cryopreservative protocol and medium.

Nevertheless, the most common CPMs are Human Sperm Preservation Medium, Ackerman’s Medium, Enhance Sperm Freeze and those containing strong pH buffer systems, such as zwitterions in Test yolk.

<table>
<thead>
<tr>
<th>Table 2 Constituents of cryoprotective medium</th>
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<tbody>
<tr>
<td>Permeating cryoprotection agent: glycerol, ethylene glycol, dimethyl sulfoxide, propylene glycol and methylformamide</td>
</tr>
<tr>
<td>Nonpenetrating cryoprotective agents: long chain polymers or sugars (methylcellulose, sucrose, raffinose, trehalose)</td>
</tr>
<tr>
<td>Plasma membrane protective agents: egg yolk, milk, soy lecithin and albumin.</td>
</tr>
<tr>
<td>Chelating agents: EDTA and citrate chelate</td>
</tr>
<tr>
<td>pH Buffers: Glycine, sodium citrate, TRIS or zwitterionic buffers, such as HEPES or TES</td>
</tr>
<tr>
<td>Free radical scavenger (anti-lipid peroxidation agents): Butylated hydroxytoluene, glutathione and dithiothreitol have been reported to provide protection against peroxidation during cryopreservation, with improvements in post-thaw motility and acrosomal integrity</td>
</tr>
<tr>
<td>Antibiotics: gentamicin</td>
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</tbody>
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422
<table>
<thead>
<tr>
<th>Cryopreservative medium</th>
<th>Penetrating CPA</th>
<th>Nonpenetrating CPA</th>
<th>Plasma membrane protecting agents</th>
<th>Buffer</th>
<th>Free radical scavenger</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test yolk buffer TBY (Irvin)</td>
<td>Glycerol 12%</td>
<td>Fructose</td>
<td>Egg yolk 20%</td>
<td>TES, TRIS</td>
<td></td>
<td>Gentamicin Sulfate or Penicillin-streptomycin</td>
</tr>
<tr>
<td>Enhance sperm freezing medium</td>
<td>Glycerol 15%</td>
<td>—</td>
<td>HSA</td>
<td>HEPES</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Sperm freezing medium (Cooper Surgical, Trumbull, CT)</td>
<td>Glycerol</td>
<td>Sucrose</td>
<td>Glucose</td>
<td>HSA</td>
<td>HEPES</td>
<td>Gentamicin</td>
</tr>
<tr>
<td>Ackerman medium (Cryobiosystem)</td>
<td>Glycerol 12%</td>
<td>Anhydrous</td>
<td>Egg yolk 20%</td>
<td>Citrate</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Human sperm preservation medium Mahadevan and Tounson</td>
<td>Glycerol 15%</td>
<td>Sucrose</td>
<td>—</td>
<td>Glycine</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Sydney sperm cryopreservation buffer (Cook Medical)</td>
<td>Glycerol</td>
<td>Glucose</td>
<td>Sucrose</td>
<td>HSA</td>
<td>HEPES</td>
<td>Gentamicin</td>
</tr>
<tr>
<td>Tardigrade medium (Cryobiosystem)</td>
<td>Glycerol 24%</td>
<td>Glucose</td>
<td>Sucrose</td>
<td>HSA</td>
<td>HEPES</td>
<td>—</td>
</tr>
<tr>
<td>Spermstore (Gynemed)</td>
<td>Glycerol 15%</td>
<td>—</td>
<td>HSA</td>
<td>HEPES</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Sperm freezing medium (Life Global)</td>
<td>Glycerol 15%</td>
<td>Glucose</td>
<td>Sucrose</td>
<td>HSA</td>
<td>HEPES</td>
<td>—</td>
</tr>
<tr>
<td>Cryosperm (Medicult)</td>
<td>Glycerol</td>
<td>Glucose, Raffinose</td>
<td>—</td>
<td>HEPES</td>
<td></td>
<td>Penicillin, streptomycin</td>
</tr>
</tbody>
</table>
yolk buffer is a combination of TES [N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid, pK 7.5] and Tris [(hydroxymethyl) aminomethane] and is combined with fresh egg yolk, dextrose and penicillin-streptomycin to form the cryobuffer known as TEST (TES and Tris)-yolk buffer (TYB). TYB is the preferred cryoprotectant for men with good or poor semen quality as opposed to glycerol alone. TYB containing 12% glycerol is associated with higher post-thaw sperm motility and survival rates than glycerol alone. TYB also promotes the recovery of a high percentage of motile sperm with normal shapes (p = 0.04), resulting in improved capacitation and sperm penetration. Furthermore, researchers at Cleveland Clinic compared TYB with other commercially available media, such as Sperm Freezing Medium (Cooper Surgical, Trumbull, CT) and Enhance Sperm Freeze (Conception Technologies, San Diego, CA), and found that the sperm cryopreserved in TYB had the greatest longevity. Three possibilities may explain such findings: (a) sperm membranes may be stabilized by an altered phospholipid-cholesterol ratio imposed by the egg yolk content of TYB, (b) egg yolk has free radical scavenging properties resulting in less peroxidative damage to the sperm and, (c) TYB has a nearly optimal concentration of glycerol. Because sperm have critical volume limits, CPM should be added slowly and in a step-wise fashion with shaker assistance to reduce the risk of osmotic stress (Figure 7).

Options for packaging of the sample: semen samples are generally stored in cryovials and plastic straws. Syringes were traditionally used as they were easy to fill and could be prepared for insemination rather quickly. However, they are now obsolete due to their large size. Moreover, they are difficult to seal, which increases the risk of contamination. Cryovials are made of plastic material with either polypropylene or polyethylene screw caps which are manufactured as either an external or internal seal onto or into the vial. Cryovials are easily filled in an aseptic technique with 1 ml of semen plus cryoprotectant. However, there are two problems that arise with cryovial use. First, the heat exchange that occurs in the center of the vial is not equal to that in the periphery. Secondly, the seals can leak resulting in liquid nitrogen ingress and a potential for explosion on vial removal and thawing.

Straws or paillettes, on the other hand, are small radius tubes that provide a uniform heat exchange system, efficient seal and less risk of contamination. At first, classic straws were made of polyvinyl chloride (PVC) or polyethylene terephthalate glycol (PETG). Polyvinyl chloride straws were withdrawn from the market in 1998, because the PVC disintegrated under radiation sterilization. Therefore, PETG straws replaced the old ones. Such straws can hold 0.25–0.5 ml of semen and are usually sealed from both ends by ultrasonic welding, solid plastic plugs or hematocrit tube sealant. These straws are easily filled with a sterile nozzle and are
free from contamination. Air space must be left to allow for expansion during cooling. Ionomeric resin straws made by Cryo Bio System (called high security straws) are characterized by enhanced tensile strength on cooling and secure sealing achieved via a thermal welding device (SYMS sealer) made by the same company.

Which freezing method is appropriate? There are a variety of methods used today to freeze semen samples, including slow freezing, rapid freezing and vitrification. Because there is debate over whether there is truly any considerable difference between the various freezing methods, each will be discussed here.

**Slow freezing:** Slow freezing allows the sperm cells to gradually adjust to new conditions and it places less osmotic strain on the cellular membrane. Cell membranes have a certain amount of flexibility when allowing fluids to cross in and out—which is referred to as the ‘osmotic tolerance limit’.76 The slow-freezing method used at Cleveland Clinic involves the gradual addition of cryoprotectants and step-wise freezing of the sample to increasingly colder temperatures before final storage in –196°C liquid nitrogen tanks. Some studies have shown that this method is superior to rapid freezing in regards to sperm kinematics.51

After the CPM has been slowly added to the samples (washed or raw semen), to achieve the final vol./vol. ratio of 1:1, they are loaded into straws or cryovials where they are exposed to a freezer temperature of –20°C for 8 minutes and to –96°C in liquid nitrogen vapor for another 2 hours. They are then placed in liquid nitrogen in –196°C for storage. This slow freezing rate can be achieved by manual freezing adjustment or by using the automatic freeze programmer.

**Rapid freezing:** Rapid freezing aims to minimize toxicity of the cryoprotectant and reduce osmotic membrane damage attributed to the formation of extracellular ice crystals. The most commonly recognized method is the Irvine Scientific Method during which the entire volume of freezing medium is added at one time—1:1 (vol./vol.) of freezing medium to ejaculate. The aliquots are placed in cryovials and then in nitrogen vapors at –96°C for 2 hours. The samples are finally immersed in liquid nitrogen at –196°C. Nallella et al. showed that compared with the slow freezing method, rapid freezing leads to better post-thaw motility and overall survival.77 However, the same study also found that the slow freezing method was associated with better kinematics parameters, such as VCL; VSL; VAP; ALH in the slow freezing method.

**Vitrification:** Vitrification is a technique that rapidly cools sperm at a rate greater than 1,000°C per minute. This technique induces an extreme elevation in solution viscosity leading to glass-like solidification without ice crystal formation. Vitrification causes minimal cell damage, but the technique is quite difficult to execute for a number of reasons—it is difficult to cool the cell so rapidly, the cryoprotectants must be extremely concentrated and the procedure has not yet been standardized, leading to considerable variability depending on who is performing the technique.52 Although this method is ideal for men with poor semen quality or for those in whom sperm retrieval yielded a very low number of sperm, a high concentration of cryoprotectant is harmful by itself to the cells.

Isachenko et al. introduced a new method of sucrose-based sperm vitrification utilizing 50 μl plastic capillaries containing 10 μl of prep specimen without any penetrating CPA.77 This method was associated with better post-thaw sperm motility, plasma membrane integrity and acrosomal integrity than the conventional slow freeze method. Nevertheless, more studies are needed before this method can be widely implemented.

**Epididymal Sperm**

To obtain a large number of motile sperm in men with OA, MESA is generally used. It is preferable to PESA and carries a lower risk of blood contamination although it is more expensive. Epididymal aspirate is first checked for motile sperm with an inverted microscope, and when an adequate number of sperm number is found, the aspirate undergoes centrifugation at 1,800 gm for 5 minutes. The resultant pellets are resuspended in a nutrient-rich medium, such as human tubular fluid (HTF), and 4–6 samples of 0.5 cc are prepared for each patient. Each sample is mixed slowly with CPM such as Test-yolk buffer. At the authors’ institution, the slow freezing method is applied. The samples should be placed in cryostorage within 1.5 hours of aspirate collection.

Peng et al. have conceptualized a method for preserving a few sperm cells at a time in a droplet of cryoprotectant for long-term storage.78 In this study, the authors showed that a very small number of sperm are lost with high post-thaw sperm motility. It will be interesting to see that this method translated into clinical practice, as it seems particularly worthwhile for oligozoospermic men and patients, who are only able to bank a small number of cells.

**Testicular Sperm**

Testicular sperm are retrieved in men with OA and NOA. If surgeons are unable to aspirate sperm from the epididymis in patients with OA, then they should turn to the testes and perform TESA. Unfortunately, the
Section 4  In Vitro Fertilization

aspirate usually yields a very low number of sperm and the sample might be contaminated by blood.

Sperm retrieved from the testes are frozen in the same manner as described for epididymal sperm. After mixing the aspirate with human tubal fluid (HTF), the mixture is centrifuged, and the pellets are resuspended with HTF. Cryovials are then filled with this suspension and an equal volume of CPM (e.g. Test-yolk buffer) is slowly added.

For sperm retrieval by TESE, the procedure is somewhat different from that used for testicular aspiration. In this case, tissue biopsies are taken and shredded into small pieces with a sterile 25-gauge needle or fine scissor, mincer or enzymatic digestion using type IV collagenase, trypsin and trypsin inhibitor. This allows the permeating cryoprotectant, such as glycerol, to fully penetrate testicular homogenate. Each tissue sample is assessed for the presence of motile spermatozoa with an inverted microscope. If there are no motile sperm in the testicular biopsy, further biopsies may be taken from the ipsilateral and contralateral testes until mature sperm are found.

The effluents and shredded biopsy tissue can be centrifuged. The pellet is resuspended in culture medium and prepared for freezing. CPM is added slowly in a drop-wise fashion to the testicular homogenate to allow proper mixing before the sample is frozen.

In many men with NOA, testicular biopsies often reveal a very low density of poorly motile sperm, and the cryopreservation method usually culminates in further deterioration of the harvested sperm. Alternatively, three other methods have been used in such cases that involve inserting testicular sperm inside: (1) Hamster egg’s zona pellucida, (2) Nylon microloops or, (3) Vehicles made from the algae species Volvox globator and then freezing. All three of these methods are experimental and future studies are required to refine these techniques.

Immature Testicular Tissue Preservation

As mentioned before, this procedure is used for children and prepubertal boys with cancer before receiving aggressive chemotherapy or radiotherapy. Gonadotoxic therapy can not only affect the rapidly proliferating differentiated spermatogonia but also the stem cell pool. In some cases, testicular tissue is biopsied whereas in others, whole testes might be removed. Controlled slow-rate freezing with penetrating CPA such as 5% dimethyl sulfoxide proves to be the best approach to preserve human immature testicular biopsies. Nevertheless, it is still an experimental procedure and there is currently no standard protocol. Extensive animal and human research is currently being performed in the field of fertility restoration including, stem cell transplantation, post-thaw testicular tissue transplantation and in vitro stimulation of sperm production.

Storing the Sample

Once the sample has been properly frozen, it must be safely stored for successful long-term (in practice, indefinite) preservation. Liquid nitrogen tanks are useful for short-term storage (Figure 8), while bulk refrigerators can be used for longer periods of time (Figure 9). Using liquid nitrogen tanks is reliable because the temperature remains constant. It is preferable to store sperm at temperatures lower than –96°C to prevent sperm damage. Long-term storage at –196°C is ‘superior’ to storage at any other temperature. It is of the utmost importance to ensure that samples are sealed well to prevent cross-contamination. To further decrease the risk of contamination, samples should be tested for infectious diseases prior to freezing.

Thawing the Sample

The thawing process must be performed carefully to minimize sperm damage. The objective of the thawing process is to obtain viable and motile sperm from the frozen specimen. Two important steps are followed. In the first step, the cryovial or straw is removed from the storage medium and allowed to warm. Although some authors prefer to adjust the rate of rewarming, so that it is in concordance with the freezing speed, others prefer rapid rewarming as it results in better recovery of motile sperm. This step is usually achieved by placing the cryovial or straw in room temperature for 5 minutes and then under water bath at 37°C for another 20 minutes. In the second step, the CPA is slowly removed from the cells along with the other constituents of the CPM, such as the buffer system. This step is performed using sperm washing medium, such as modified Biggers-Whitten-Whittingam (BWW) medium (HEPES-Biggers-Whitten-Whittingam, Irvine Scientific, Santa Ana, CA) (1:2, vol./vol.) enriched with 5% of human albumin or modified human tubal fluid. The sperm washing medium is added slowly over the rewarmed semen, followed by centrifugation at 350 g for 5–10 minutes. The pellets are resuspended and subjected to another round of centrifugation, resulting in sperm-concentrated pellets. Finally, the contents of these pellets are aspirated and resuspended in nutrient-rich media.

Semen analysis for the post-thawed frozen sample is conducted 24 hours after freezing to check the quality of frozen semen and measure the number of motile sperm which withstand the stress of freeze thaw cycle. Based on these results, patients will be asked to provide more samples to be banked.
Chapter 29  Sperm Banking: Indications and Regulations

The thawing process can damage cell membranes and intracellular compartments, affecting metabolic activities and leading to decreased sperm motility. It also can cause DNA damage, possibly because the process increases production of ROS. To assess viability following thawing, hypo-osmotic swelling tests should be conducted, particularly in the presence of immotile sperm. Poor sperm count and motility post-thawing may necessitate the addition of pentoxifylline and/or performing density gradient centrifugation to improve the quality of the thawed specimen in terms of sperm count and motility.

Utilization Rates

In discussing the usefulness and importance of sperm cryopreservation, it is important to examine utilization rates of banked samples. Overall, sperm cryopreservation remains underutilized for a variety of reasons. Studies report utilization rates ranging anywhere from 4.7–7.5%. Despite these low rates, it is still recommended that all male cancer patients be counseled about this option, as sperm cryopreservation is a form of insurance for potentially irreversible health problems. The specific reasons why many men choose not to use their banked sperm or discard it, will be discussed at the end of this section. When looking at the rates of use of banked sperm, however, it is important to remember that without storage, none of these patients would have the opportunity to biologically father a child. Within this mindset, any rate of utilization can be considered a success.

Use of Cryopreserved Sperm in ART

Once the sample has been successfully thawed and the post-thaw semen parameters have been evaluated, the sperm is ready to be used in ART. Depending on each patient’s situation and the quality of his sperm, different methods can be used to help him and his partner conceive. The success and type of the ART procedure will depend on a variety of factors; including the type of the sample (ejaculated semen, epididymal or testicular sperm) and the post-thaw sample quality. The highest-quality post-thaw ejaculated semen with adequate parameters can be used for intrauterine insemination.
Section 4  In Vitro Fertilization

(IUI). This procedure is closest to natural conception, as sperm is injected into the woman’s uterus. Poorer quality semen, as indicated by post-thaw parameters, epididymal sperm and testicular sperm should be used in assisted-fertilization procedures, such as in vitro fertilization (IVF) or Intracytoplasmic sperm injection (ICSI).

Fresh versus frozen ejaculated sperm and ART outcomes: Despite the paucity of well-designed controlled trials on IUI outcomes using frozen ejaculated sperm, several studies have found that the fertilizing capability of frozen sperm is lower than that of freshly ejaculated sperm. This finding can be interpreted on the basis of cryopreservation-related sperm dysfunction. However, one single study reported identical pregnancy rates between frozen and fresh ejaculated sperm used in artificial insemination when there was a sufficient number of progressively motile sperm in the post-thaw specimen and in absence of severe teratozoospermia and asthenospermia.

Several cases of allergic reactions to hen’s egg yolk have been reported by women undergoing IUI.

For conventional IVF, the data are also controversial. While Cohen et al. and Mahadevan et al. failed to show any difference in the fertility outcomes between cryopreserved ejaculated sperm and fresh sperm, others have identified significant differences. Nonetheless, good post-thaw semen quality has always correlated well with adequate outcomes.

Lastly, the 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. Meanwhile, the authors reported higher ongoing pregnancy rates per cycle with fresh sperm (23.7%) versus frozen (35.2%).

Fresh versus frozen epididymal sperm: The first pregnancy resulting from the use of fresh epididymal sperm (taken from a man with OA) occurred in 1995. This breakthrough has since helped many infertile men, who were previously thought to be sterile, to father biological children. Comparative studies between frozen and fresh epididymal sperm regarding ICSI outcomes have not detected major differences in regards to fertilization rates and ongoing pregnancy rates, particularly when motile sperm were used.

Fresh versus frozen testicular sperm in ICSI outcomes: In general, there are no differences in ICSI outcomes in terms of fertilization rates, clinical pregnancy rates or ongoing pregnancy rates between fresh and frozen motile testicular sperm. However, implantation rates are slightly better with the use of fresh motile testicular sperm. Others have reported that such findings are more evident in men with OA than with NOA. Motile testicular sperm are generally preferred for use in ICSI but when the testicular sperm are immotile, a common occurrence, it is important to verify their vitality. Viable sperm can be selected using the hypotonic swelling test, via laser assisted immobilized sperm selection or by adding motility enhancing agents, such as pentoxifylline.

Disposing of Samples

While ART procedures with cryopreserved sperm are often successful, not all men who bank sperm return to use their samples, as evidenced by the generally
low utilization rates described above. Often, a cancer survivor who banked sperm will dispose of his sample following cessation of treatment—particularly, if he regains his fertility or his semen quality improves.49 Others may wish to actively dispose of the banked samples for a variety of reasons, including patient death, financial restrictions or a lack of desire to father children.103 Patients may also be concerned about whether they will be healthy enough in the future to be a good parent.104

Having a banked sample requires some amount of caution. While the patient is alive, he has full control over how the preserved sperm are used. If he dies unexpectedly or without ensuring the proper precautions to protect his sample, however, the situation could become fraught with legal and ethical problems. Who does the sample belong to when the patient passes away? It is therefore of the utmost importance that the patient protects his sample, however, the situation could become fraught with legal and ethical problems. Who does the sample belong to when the patient passes away? It is therefore of the utmost importance that the patient signs a form stating the ownership and destination of the banked semen in case of his death.49 Some important points to include in any such consent form include whether or not the sample should be disposed of after death and who else, if anyone is permitted to use it in ART procedures.

**Additions of Antioxidants**

Sperm are redox cells that can produce ROS. Meanwhile, sperm and seminal plasma possess enzymatic and nonenzymatic antioxidants that can scavenge ROS to prevent the precipitation of oxidative stress and cellular damage. Sperm are particularly susceptible to oxidative stress because their plasma membranes contain a large amount of polyunsaturated fatty acids and have a limited intrinsic reserve of cytoplasmic antioxidant enzyme systems. Therefore, sperm predominantly depend upon the antioxidant support of seminal plasma. As such, sperm preparation for cryopreservation, which often involves the removal of seminal plasma by centrifugation, increases the susceptibility of spermatozoa to oxidative stress. In addition, studies find that the antioxidant activity of the spermatozoa themselves may be decreased by cryopreservation.105,106 Freeze-thawing of equine, human and bovine sperm was associated with an increase in ROS generation.107-111 Recent studies on Rhesus Macaque cryopreserved sperm have shown that frozen-thawed sperm not only experience excessive ROS generation during the process but also severe DNA and chromosomal damage.112 More recently, Gadea et al. identified decrease in the total seminal level of reduced glutathione (GSH) in human semen undergoing freezing-thawing cycles.113

Oxidative stress usually lowers sperm motility and viability and leads to ionophore-induced acrosome reaction and sperm-oocyte fusion. Hydrogen peroxide appears to be the primary ROS responsible for these adverse changes, and sperm plasma membrane lipid peroxidation is postulated to be an important mechanism of action.114,115-118

When plasma membrane lipid peroxidation occurs, the membrane loses the necessary fluidity and integrity required to participate in membrane fusion with an oocyte during fertilization. Moreover, lipid peroxidation can also damage DNA, resulting in chromatin cross-linking, base changes and DNA strand breaks.111

A recent study conducted by Kalthur et al. found that adding antioxidants to CPM in the form of Vitamin E during freezing significantly enhanced the total motility and progressive motility in post-thaw samples.122 Such improvements in freezing procedures may ultimately lead to an increased conception rate in ART cycles.121 Other authors have reported reduced oxidative stress markers with the use of antioxidants, including Gadea (who used reduced GSH) and Li et al. (who used ascorbate and catalase separately).113 These significant findings should encourage the companies that produce CPM to add proper antioxidants supplementation, whether enzymatic or nonenzymatic, to their commercial preparations to enhance post-thaw sperm quality.

**THE RISE OF ONCOFERTILITY AS A FIELD**

The field of oncofertility has been growing rapidly since its official inception in 2006. It is a discipline that combines the fields of oncology and reproductive medicine which, until now, were kept separate. In fact, the field is a conglomeration of bioethics, social sciences, pediatrics and a multitude of other areas resulting in a highly interdisciplinary approach.36,38,54 The union of these previously disjointed areas of study allows healthcare providers to help resolve difficult ethical and legal issues that often arise in fertility preservation discussions. The field is developing in response to the discrepancy between the attention given to cure cancer and quality of life concerns and because infertility treatments have often been considered an enhancement rather than a necessary treatment that is covered by health-care insurance. This approach shifts reproduction from a privilege to a human right, acknowledging and addressing that, the desire to have a child is an overwhelming human emotion.38 Oncofertility specialists also aim to raise awareness among patients about the detrimental effects of cancer on fertility and options for fertility preservation. The continual evolvement of this field will undoubtedly ease patient concerns regarding fertility during the already difficult time following a cancer diagnosis.


Section 4 In Vitro Fertilization

**AVAILABLE RESOURCES ON FERTILITY PRESERVATION**

Because of the growing awareness of fertility preservation both among providers and patients, there is an increasing body of knowledge on the topic as well as available informational resources. Oncologists treating reproductive-aged males would benefit from ready-to-use online provider handouts on counseling patients on fertility preservation. Likewise, patients can browse these websites to help ease diagnosis-associated anxiety and initiate a conversation with their provider on what fertility preservation option would be best for their specific case. Some organizations that provide these valuable resources include, the Oncofertility Consortium, Fertile Hope and the Livestrong Foundation.

**CONCLUSIONS AND FUTURE RESEARCH**

Although enormous strides have been made in the technologies of assisted reproduction, cryopreservation protocols, cancer research and in the new field of oncofertility, there are still many unknowns to be determined. Primarily, there is still much to be learned about what causes semen quality to decrease in patients with cancer and the mechanisms of how cancer affects spermatogenesis both locally and systemically. Ultimately, it is crucial to develop cancer therapies that are less harmful to fertility. This includes developing new therapies and ensuring that new chemotherapeutics are tested for gonadotoxicity prior to clinical prescription, as well as focusing on developing medications that have gonadoprotective properties.\(^\text{36}\) The Oncofertility Consortium also strives to decrease the incidence of disease-related fertility loss by finding noninterventional or medical ways to eliminate a young person’s risk of losing his fertility due to cancer treatment.\(^\text{36}\) Experimental procedures, such as testicular tissue extraction and freezing for prepubescent cancer patients, require more formidable translation from animal research to real clinical human practice.\(^\text{36}\) Finally, the field of oncofertility needs to continue gaining momentum, ensuring that all physicians are fully informed about fertility preservation options available to their patients. It is time for the awareness of the toxic effects of cancer treatments on future fertility to coincided with the rates of referral to sperm banks.

As the side effects of cancer therapy receive more attention and the field of oncofertility blossoms, the search continues for modern treatments that are less harmful to the body. Researchers in this area are generally looking at how to lower doses, use different treatment regimens and reduce ‘drug-related toxicity without compromising cure rates.’ For example, some specialists are looking at ways to make radiation therapies more localized to invasive cells, thereby minimizing damage to the rest of the body. One method of achieving this is to protect the gonads during treatment, to prevent radiation scatter and ‘minimize the time required for spermatogenetic recovery following radiotherapy’.\(^\text{26}\) In addition, researchers are looking at inhibiting spermatogenesis with hormone therapy prior to anticancer therapy to preserve fertility during treatment. Others are attempting to invent a molecular target therapy to combat cancer. For example, in lymphomas, researchers are looking at whether they can substitute epidermal growth factor or vascular endothelial growth factor for the toxic conventional chemotherapy to avoid their adverse effects. All of these projects are preliminary but promising nonetheless.\(^\text{26}\)

Systemic diseases and chemotherapy disrupt fertility in general and spermatogenesis specifically. Kim et al. focused on hormonal disruptions by targeting the hypothalamic-pituitary-gonadal axis.\(^\text{123}\) Specific endocrine therapy can be used to balance these changes and preserve fertility during treatment. A gonadotropin-releasing hormone analog can be administered either during or after treatment with procarbazine or radiotherapy.\(^\text{123}\)

More research is still needed with larger groups using drugs that are used more commonly in humans. Overall, there is still much room for the improvement of cancer therapies. Ideally, these currently highly toxic treatments would become harsh enough to properly attack the cancerous, invasive cells while sparing the rest of the body, specifically, the gonads and fertility.

**RECOMMENDATIONS**

- Many men in various stages of life could benefit from utilizing sperm cryopreservation.
- It is advisable for any physician to engage in a discussion on cryobanking with any patient, who may be exposed to gonadotoxic factors at work, is severely 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.
- Such discussions can prevent patients’ misconception about the sperm banking and regretting not taking the necessary precautions against potential fertility loss.
• It is preferable to offer the patient the option of fertility preservation rather than precluding his chance of fathering a biological child.

• Patients should have time to consider these issues rather than have their health care provider make decisions on their behalf.

• It is of the utmost importance to remember that specific fertility preservation recommendations must be made on a case-by-case basis.

• Sperm cryobanking has formally become part of the standard of care for reproductive-aged oncologic patients.

• Sperm cryopreservation is the most effective and the most strongly recommended option for fertility preservation prior to any treatment or occupational exposure that may pose a threat to a man’s fertility.

REFERENCES


Section 4  In Vitro Fertilization


Chapter 29 Sperm Banking: Indications and Regulations


