Section 10

Other

Chapter

33

Sperm cryopreservation

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Introduction

Sperm cryopreservation, more commonly referred to as sperm banking, has long been a fertility preservation technique for men but had been limited to those men whose post-thaw specimen after cryopreservation yielded sufficient motile sperm for intrauterine insemination. With the advances in assisted reproductive techniques (ART), and in particular intracytoplasmic sperm injection (ICSI), cryopreservation has become an essential component of fertility treatment for men with oligozoospermia, cryptozoospermia, and even azoospermia. It has also become standard practice to cryopreserve at the time of testis biopsy for azoospermia as well as when semen quality declines in the face of standard treatment paradigms. Over the past decade, sperm banking has become an essential component of the treatment of the subfertile couple.

Impairment in male fertility can result from several causes. These include disease, anatomic, and/or functional issues (e.g., absence of the vas deferens, retrograde ejaculation or anejaculation), primary or secondary hormonal insufficiency, and frequently, from damage or depletion of the germinal stem cells resulting in impaired spermatogenesis.

There are many potential threats to spermatogenesis with disease treatment, often resulting in compromised sperm number, motility, morphology, as well as DNA integrity. Chemotherapy and radiotherapy for various cancers easily disrupt biochemical processes that occur during spermatogenesis [1]. In addition, non-chemotherapeutic drugs and herbal as well as non-herbal supplements can and do affect sperm quantity, quality, and fertility potential.

The popularity of sperm cryopreservation has been a direct result of patient demand. The majority of men who bank sperm have been afflicted by cancer at a young age and wish to preserve their future fertility. Although cancer survivors can become parents through adoption and gamete donation (using an anonymous or directed donor) most prefer to have biologic offspring [2]. In one study 48% reported that having children was an important issue to them after completing their treatment [3]. Similar results were reported by Schover et al. noting that **over 50% of male cancer survivors in the reproductive age group desire to preserve their future fertility** climbing to a rate of 77% in men who are childless at the time of cancer diagnosis [4,5]. Many of these men have concerns that their cancer or cancer treatment may result in birth defects or other health issues for their potential offspring [6]. Importantly, aside from inherited genetic syndromes, large registry studies have revealed no increased risk of genetic abnormalities, birth defects, or cancers in the children of cancer survivors [7,8,9,10,11,12].

This chapter follows the development of cryopreservation for mammalian sperm. It expands upon our earlier review of indications and protocols [13]. We also provide evidenced based answers to some of the questions our patients ask when seeking advice regarding sperm banking.

Historical perspective

Almost 250 years ago Lazaro Spallanzani, an Italian priest and physiologist, first reported that sperm became "motionless" when cooled by snow [14,15]. Advances in sperm banking were slow to progress. This was due not only to the technologic challenges required to reach and maintain the temperatures required to freeze sperm and ensure their post-thaw survival, but also to the ethical issues involved.

Research in the field first focused on veterinary applications. In the late 1930s and early 1940s researchers studied the preservation of sperm for use in artificial insemination in dairy cattle [16] and found that sperm could survive freezing lower than −321°F (−160°C) [17]. They also noted that survival was limited in the absence of a cryoprotectant such as glycerol [18]. Conception rates from thawed sperm averaged 65% in cows, a rate that has remained essentially unchanged for the past 30 years. The major advancements in mammalian cryopreservation have been in technique rather than an improvement in the understanding of cryophysiology.

The first human pregnancy with frozen and thawed sperm was reported in 1953 [19]. In their first foray into the cryopreservation of human spermatozoa, the authors demonstrated that spermatozoa were capable of fertilization and the subsequent induced development of normal progeny [20] after being frozen and stored in dry ice (−78°C). Moral and legal

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controversy surrounding use of "artificial" intrauterine insemination of previously frozen sperm prevented dissemination of this information and widespread use of cryopreservation. It was not until the 11th International Congress of Genetics in 1963 [21] that this breakthrough was reported and interest in human sperm banking began.

The introduction in 1963 of a method for freezing human semen in the vapor of liquid nitrogen and its storage at –196°C was followed by reports of normal births with its use [22]. The improvement of basic principles of technique over the past 40 years has made cryopreservation of human male gametes practical and effective. With the refinement in assisted reproductive techniques, especially intracytoplasmic sperm injection (ICSI) [23] in which a single sperm is injected into an oocyte, sperm cryopreservation has become an essential component in the treatment of the subfertile male. This has enabled men who have few sperm in their ejaculated semen, or even men with only rare sperm retrieved from their testes, to fertilize their partner's oocytes.

Over the last 40 years several key findings have resulted in sperm cryopreservation becoming a safe, effective, and reliable modality for preserving male fertility. **Storage in liquid or vapor phase nitrogen (–384°F/–196°C) has become the standard since it is readily available, inert, and can maintain cryopreserved sperm below the –150°C temperatures required. Cryoprotectants such as glycerol and dimethyl sulfoxide (DMSO), which protect sperm from damage from the freezing process, have become standard.** Extenders have also been improved. Extenders have several functions including: (a) optimizing the osmotic pressure and pH, (b) providing an energy source to prevent the sperm's undesirable use of its own intracellular phospholipid; (c) preventing bacterial contamination by including an antibiotic; and (d) allowing for dilution of the semen while offsetting the deleterious effect on survival produced by high dilution. Standardization of solutions and their availability through many vendors have added to the reliability of the process and have assured consistency in post-thaw survival. In addition, computer program-controlled freezing of specimens has helped standardize the freezing process.

Indications for cryopreservation

For the past 40 years sperm banking has been used for a growing list of indications including:

1. Patients with cancer in whom the treatment (e.g., chemotherapy, radiation) or the disease itself might impair the patient's sperm production or semen quality
2. Patients undergoing surgery on the testis, prostate or spinal cord, or retroperitoneal procedures that may disrupt antegrade ejaculation
3. Patients with severely impaired semen parameters to preserve their future fertility potential
4. Patients with physical disabilities who may require retrieval of sperm and pooling of specimens and/or timing of insemination with their partner's cycle
5. Patients involved in hazardous occupations may bank their sperm to preserve their fertility potential
6. Patients prior to undergoing a vasectomy
7. Cryopreservation of epididymal or vasal sperm for patients undergoing surgery to reconstruct an obstructed vas deferens, or ejaculatory duct in which specimens are cryopreserved for later use in in vitro fertilization procedures
8. Cryopreservation of testicular tissue obtained by testicular biopsy during the diagnostic evaluation of azoospermia
9. Pooling of cryopreserved specimens for IUI in patients with low sperm counts

The ability for a single, viable, yet immotile sperm to be used successfully for fertilization of an oocyte through intracytoplasmic sperm injection (ICSI) has greatly expanded the indications for sperm cryopreservation. Surgical retrieval of sperm from the testis, epididymis, and/or vas deferens is now standard therapy for the patient with cryptozoospermia or azoospermia.

Cryopreservation for malignant disease

One of the most common indications for preserving sperm is malignant disease. **Cryopreservation of sperm should be done prior to therapy for malignant diseases.** Cytotoxic chemotherapy, radiotherapy, and many surgical treatments for cancer may lead to testicular failure or ejaculatory dysfunction (Table 33.1). Freezing of spermatozoa before initiation of treatment is currently the best way to preserve future fertility. **Alkylating** and other chemotherapeutic **agents cause azoospermia in 90–100% of treated adult males.** Collection and cryopreservation of semen is feasible during chemotherapy, at least until azoospermia ensues [24], although the effects of chemotherapeutic agents on the ejaculated spermatozoa or retrieved testicular sperm is not known. Often, but not always, patients with cancer have few spermatozoa in their ejaculate and relatively few straws or vials can be made. Depending on the sperm concentration, motility, and partner's fertility potential, dilution of the specimen with commercially available solutions can increase the number of straws or vials cryopreserved. In addition, **vials can be preserved, thawed, and refrozen several times** [25] as an alternative method to increase the yield from a small sample. **Aliquots can even be scraped from a vial and used for IVF/ICSI.**

Although improved chemotherapeutic treatment regimens often result in recovery of fertility [26], the prevalence of azoospermia in patients after treatment is still high. In fact, only 20–50% of these men eventually recover spermatogenesis [24]. Most patients who survive their malignancy discontinue sperm banking because either they regain fertility or have improved semen quality. Utilization rates of cryopreserved sperm have been reported to be between 10 and 20% [27,28]. Because it is difficult to predict which category of cancer patients will require cryopreserved sperm after treatment, **sperm banking should be strongly recommended for all patients with**

Table 33.1

Disease	cccc	Effect on fertility	Dosage dependent	Reference
Hodgkins	MVPP Mustine, vinblastine, procarbazine, prednisone	Azoospermia >10years post-chemo	Yes	[1–3]
	MOPP Mustine, vinblastine, procarbazine, prednisolone	Chronic azoospermia with return to normal in 10 years	Yes	[3,4–6]
	ChlVPP Chlorambucil, vinblastine, procarbazine, prednisolone		Yes	[1,3,7]
	COPP Cyclophosphamide, vincristine, procarbazine, prednisolone	Permanent azoospermia	Yes	[1,3,5]
	ABVD Adriamycin, bleomycin, vinblastine, dacarbazine	Acute azoospermia with return to near baseline approx. 18 months. Less gonadotoxic	Yes	[1,3–9]
Non-Hodgkins	CHOP Cyclophosphamide, adriamycin, vincristine, prednisolone	Acute azoospermia with return to near baseline approx. 5 years post treatment	Yes	[1–2]
	VAPEC Vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide	Acute azoospermia with return to near baseline post treatment	Yes	[10]
	VACOP-B Vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide–bleomycin	Acute azoospermia with return to near baseline, post treatment	Yes	[1]
	MACOP-B Methotrexate, doxorubicin, prednisolone, etoposide, cyclophosphamide–bleomycin		Yes	[1]
	VEEP Vincristine, etoposide, epirubicin, prednisolone		Yes	
Testicular cancer	Cisplatin, carboplatin	<2 years for near baseline post treatment	Yes	[1,7,11–14]
Non-seminomatous germ cell tumors	BEP Bleomycin, etoposide, cisplatin	Return to near baseline post treatment in approx. 5 years		[12–18]
	Etoposide	Acute azoospermia	Yes	[7,13]
	Ifosfamide	Permanent azoospermia, but used in conjunction with other agents	Yes	[7,10]
	Mesna	For use in conjunction with ifosfamide for unwanted side effects		
			Function	
ALL	Gleevec	None	Tyr kinase inhibitor	[7,19]
	Daunorubicin	Temporary oligo and/or azoospermia	See above	[1,7]
	Etoposide	Temporary oligo and/or azoospermia	See above	[7,13]
	Vincristine	See above	See above	See above
	Methotrexate	Reversible	See above	[1,7,20]
	Cyclophosphamide	Prolonged azoo or oligospermia	Affects DNA replication	[5,7,10,15,20–22]

Table 33.1 (*cont.*)

Disease	cccc	Effect on fertility	Dosage dependent	Reference
	Cytarabine	Oligo and/or azoospermia, reversible 1–5 years post treatment	Affects S phase	[1,7,9,23]
AML	Cytarabine	Transient oligospermia with dose-dependent recovery	Inhibits DNA polymerase	[9,23]
	Daunorubicin/doxorubicin	Temporary oligospermia	Anthracycline	[23]
	Thioguanine	Temporary oligospermia		[7]
	Vincristine	See above	See above	See above
	Etoposide	See above	See above	See above
	Prednisone	See above	See above	See above
	Mercaptopurine	Temporary when used alone		[7]
CML	Imatinib	See above	See above	See above
	Interferon	No affects	No affect	[7]
	Busulphan	Prolonged azoospermia and/or oligospermia	DNA breaks	[5,7,10,15,20–22]
	Hydroxyurea	Temporary, but if used in conjunction, then azoospermia		[7]
CLL	Fludarabine	Temporary		[7]
	Cyclophosphamide	See above	See above	See above
	Rituximab	No known effect	Monoclonal antibody	[7]
Prostate cancer	Radiotherapy	Possible return to near baseline depending on treatment dosage and prior chemotherapy	Interferes with cell replication	[1,8,13,16,24]

Chemotherapy	Type of agent	Risk	Mechanism of action	Reference
Cyclophosphamide Busulfan Ifosfamide	Alkylating	Azo and/or oligospermia, prolonged	DNA single and double-stranded breaks	[5,7,10,15,20–22]
Procarbazine	Alkylating	Prolonged azoospermia	DNA single and double-stranded breaks	[1,4,5,7,24–26]
Cisplatin	Alkylating-like agent. Has no alkyl group	<2 years for recovery	Increased apoptosis in germ cell with DNA breaks. Platinum leads to DNA chain breaks	[1,7,11–14]
Doxorubicin	Anthracycline	Azoospermia, acute, if used alone. In conjunction with other agents, may cause prolonged azoospermia	Apoptosis of spermatogonia and primary spermatocytes -Intercalates DNA	[1,10,27,28]
Vincristine Vinblastine	Vinca alkaloids	Azoo and/or oligospermia if used alone. Azoospermia, acute, if used alone. In conjunction with other agents, may cause prolonged azoospermia	Affects microtubules for DNA replication	[1,7,22]
Dactinomycin	Antibiotic with chemotherapy property	Acute oligo. In post treatment, near baseline	Inhibits transcription. Interferes with DNA replication	[1]
Methotrexate	Cytotoxic to S phase of active replication	Acute oligo with return to near normal post treatment Reversible	Antifolate	[1,7,20]
Cytarabine	Antimetabolite	Oligo and/or azoospermia	Affects S phase of cell cycle	[1,23]
Radiotherapy	Radiation	Possible return to baseline depending on treatment dosage	Interferes with cell replication	[8,13,16,24]
Prednisone	In conjunction with chemotherapeutic drugs	No known effect on spermatogenesis		[7]
Busulfan				

Table 33.1 (*cont.*)

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malignant diseases who may wish to have children, even if they eventually decide that the specimens are not needed [28].

Effects of chemotherapeutic drugs on spermatogenesis

Drugs used for chemotherapy have a marked effect on spermatogenesis (Tables 33.1 & 33.2). In addition, malignancy itself may impact male fertility well before chemotherapy has even started [29]. Many authors have reported a decline in semen quality before therapy from diseases such as Hodgkin's lymphoma and testicular cancer. It has been suggested that attenuation of sperm quality is related to the direct effects of the tumor and the production of tumor related factors (e.g., estradiol, βhCG or other factors) by some neoplastic histotypes. In Hodgkin's disease, a decrease in semen quality may also be linked to the presence of constitutional symptoms, such as fever and weight loss, which accompany the disease [30].

The likelihood of rapid recovery of spermatogenesis following gonadotoxic insult is related to the agent(s) used and the doses received [31]. The use of chemotherapy may lead to gonadal dysfunction affecting both the endocrine and exocrine compartments of the testis [32]. Often the first sign of gonadal dysfunction is the decrease in serum testosterone and the sudden rise of serum FSH and LH occurring shortly after the start of chemotherapy. This may be due to the reduced negative inhibition of testosterone at the level of the hypothalamic-pituitary reflecting a degree of impairment by the Leydig cells [32].

The primary gonadotoxic chemotherapeutic drugs used today are the alkylating agents. Among them, **cyclophosphamide and procarbazine have been known to result in prolonged azoospermia** in the patient. Although very effective in treating

Table 33.2 This table represents a compilation of clinical experience and current research on common cancer treatments that may impact reproductive function in men

Degree of risk	Treatment	Common usage
High risk Prolonged azoospermia post treatment	Total body irradiation (TBI) Testicular radiation dose >2.5 Gy in men	Bone marrow transplant/stem cell transplant(BMT/SCT) Testicular cancer, acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma (NHL)
	Testicular radiation dose ≥ 6 Gy in boys	ALL, NHL, sarcoma, germ cell tumors
	Protocols containing procarbazine: COPP, MOPP, MVPP, ChlVPP, ChlVPP/ EVA, MOPP/ABVD, COPP/ABVD	Hodgkin lymphoma
	Alkylating chemotherapy for transplant conditioning (cyclophosphamide, busulfan, melphalan]	BMT/SCT
	Any alkylating agent (e.g., procarbazine, nitrogen mustard, cyclophosphamide) + TBI, pelvic radiation, or testicular radiation	Testicular cancer, BMT/SCT, ALL, NHL, sarcoma, neuroblastoma, Hodgkin lymphoma
	Cyclophosphamide >7.5 g/m^2	Sarcoma, NHL, neuroblastoma, ALL
	Cranial/brain radiation ≥4O Gy	Brain tumor
Intermediate risk	BEP × 2–4 cycles (bleomycin, etoposide, cisplatin)	Testicular cancer
	Cumulative cisplatin dose <400 mg/m^2	Testicular cancer
Prolonged azoospermia	Cumulative carboplatin dose ≤2 g/m^2	Testicular cancer
not common at standard dose	Testicular radiation dose 1–6 Gy (due to scatter from abdominal/pelvic radiation)	Wilms' tumor, neuroblastoma
Low risk Temporary azoospermia post treatment	Non-alkylating chemotherapy: ABVD, OEPA, NOVP, CHOP, COP Testicular radiation doses 0.2–0.7 Gy	Hodgkin lymphoma, NHL testicular cancer
Very low/	Testicular radiation dose <0.2 Gy	Multiple cancers
No risk	Interferon-α	Multiple cancers
No effects on sperm production	Radioactive iodine	Thyroid
Unknown	Irinolecan	Colon
Risk	Bevacizumab (Avastin)	Colon, non-small cell lung
	Cetuximab (Erbitux)	Colon, head & neck
	Erlotinib (Tarceva)	Non-small cell lung, pancreatic
	Imatinib (Gleevec)	Chronic myeloid leukemia (CML), gastrointestinal stromal tumor [GIST]

Hodgkin's lymphoma and non-Hodgkin's lymphoma, procarbazine is thought to affect spermatogenesis either by destroying spermatogonial stem cells or by damaging the paracrine mechanisms thereby interfering with the production of sperm [33].

Cyclophosphamide, used both in Hodgkin's and non-Hodgkin's lymphoma, affects the DNA–DNA structure on normal cells leading to single-strand breaks, cross links, and template dysfunction [34]. Its effects also may result in prolonged to permanent azoospermia.

Other agents that have a significant effect on male fertility are anthracyclines, vinca alkaloids, and the antimetabolites. The extent of the illness as well as the duration and dosage of these medications will determine the overall effect on spermatogenesis. Even with these medications, there is a return of sperm to the ejaculate after chemotherapy. The time after chemotherapy for sperm to appear in the ejaculate may take from18 months to 5 years or longer [35]. **Up to 50% of patients treated with cisplatin-based therapy recover from its effects in the first 2 years and 80% recover after 5 years** [31].

Combination therapy is frequently used in patients undergoing cancer treatment. Spermatogenesis is often compromised, particularly with combinations containing alkylating agents. Some combinations are more gonadotoxic than others. **All agents in MOPP** (mustine,vinblastine, procarbazine, prednisone) therapy, with the exception of prednisone, **are gonadotoxic. COPP** (cyclophosphamide, vincristine, procarbazine, and prednisolone) therapy **also has a significant effect on spermatogenesis**. Although these combinations are very effective in treating several diseases, they result in impaired future fertility. One method to mitigate this is to eliminate the alkylating

agents, such as cyclophosophamide, procarbazine, etc. from the regimen**. ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) is often used for treatment of Hodgkin's disease** due to its effectiveness in treating the disease as well as its minimal impact on spermatogenesis. ABVD combination regimen results in short-term azoospermia, **with males generally returning to near baseline roughly 18 months after the last chemotherapeutic treatment.**

For patients suffering from testicular cancer, impaired sperm production and reduced Leydig cell function may contribute to infertility before treatment has started [36]. Two forms of therapy that have been used are cisplatin based (cisplatin, etoposide, and bleomycin) or carboplatin-based (carboplatin, etoposide, and bleomycin). Both treatment regimens improved the survival rate of testicular cancer; however, both resulted in oligo/azoospermia with cisplatin having the more profound effect. The sperm recovery rate ranged from 2 to 5 years, 48% and 80%, for cisplatin and carboplatin, respectively [31], with recovery from carboplatin well within 2 years [37].

Effects of radiotherapy for malignant disease on spermatogenesis

Radiotherapy has significant detrimental effects on spermatogenesis and therefore mandates discussion of sperm banking with the patient prior to therapy. There are several putative reasons why the testis has an absorbed dose of radiation despite being outside the primary area of treatment [38,39,40,41,42,43,44]: (1) leakage through the treatment head of the machine; (2) scatter from the collimators and beam modifiers; and (3) scatter within the patient from the treatment beams.

Fractionated radiation doses to the testis greater than 35 cGy cause azoospermia while **more than 200 cGy can result in irreversible azoospermia** [45]. Stovall et al. [11] evaluated the radiation dose to the gonads in children undergoing treatment. They found the range to be 1 to 700 cGy with a median dose of 7 cGy. More than 49% of the gonadal doses were >10 cGy and 16% were >100 cGy. In cases where radiation therapy is used, the recovery rate of having sperm in the ejaculate may be longer. The amount of Gy administered usually determines how long the recovery period may be. The time to recovery can range from months to 5 years or greater.

Protection against radiation damage to the testis by pretreatment with FSH [46] and GnRH [47] has been shown experimentally in rodents, but not in primates. The use of hormonal suppression to protect the testis in man is presently under study [48].

What advice can we give our patients as to when it is safe to conceive after treatment for malignant disease?

This will be covered in more detail towards the end of this chapter. The damage of sperm DNA is seen most commonly post-chemotherapy and radiation therapy. Similar damage to sperm DNA may also be seen, but to a lesser degree in normal males who have not undergone treatment. The duration, dosage, and type(s) of the drugs used can determine how long it will take before there is a return to near baseline (pre-chemotherapy). There have been studies published investigating the return of sperm to the ejaculate, however, few have studied the effect of these agents on the offspring.

Fertility after the treatment of cancer is of great concern, especially with alkylating agents. At lower doses, recovery may occur within 3 years, whereas it may take considerably longer to restore spermatogenesis after treatment with higher doses [49]. Despite the recovery of spermatogenesis, the ability for fertilization to take place naturally may still be hindered. Studies, such as the sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and the single-cell gel electrophoresis assay (COMET) assay, have been conducted to measure DNA fragmentation and to predict the viability of natural fertilization [32,34,36]. Since assisted reproductive procedures, such as ICSI and IVF, bypass the biologic control system, there is new concern for increased transmission of defective DNA to human offspring. Animal studies have been conducted with adult male rats treated with short- and long-term doses of combination chemotherapeutic agents, such as CHOP [49]. In this study, evidence has shown embryo loss occurring both pre-implantation and post-implantation. Another study by Beiber et al. [50] used animal studies with the combination treatment of BEP. Early infant mortality was shown with a prolonged course of the regimen; however, pre- and post-implantation viability were not affected.

Owing to the detrimental effects of chemotherapy on spermatogenesis together with the 2- to 3-month cycle of spermatogenesis, patients are often advised to delay attempting conception for 4 cycles, approximately 1 year, after their last treatment of chemotherapy. However, this is based on subjective data rather than being substantiated by studies [35].

Cryopreservation for non-malignant disease and surgical procedures

Cryopreservation of sperm is also recommended for men of reproductive age with non-malignant, systemic disease or treatment regimens that may cause infertility [26]. These include men with autoimmune disorders, kidney disorders, diabetes, ulcerative colitis, and heart transplants. Many of these patients require immunosuppressive or cytotoxic therapy. Although the pre-treatment quality of semen in these men is inferior to that of healthy donors, semen samples are within the normal reference range of the World Health Organization, providing adequate specimens for cryopreservation [26].

Spermatogenic effects of medications used for non-malignant disease (Table 33.3)

Antibiotics may have a negative effect on male fertility. An in vitro protocol studied five antibiotics and each had a different

Table 33.3

Category	Drug name	Indications	Effect of drug on fertility	Reversibility	Ref.
Antibiotics	Co-trimoxazole	Antibiotic	Only high concentrations (500 µg/mL) impaired rapid motility *in vitro*	No	[1]
	Erythromycin	Antibiotic	Significant decline in motility increasing with concentration of drug *in vitro*	No	[1]
	Amoxycillin	Antibiotic	No effect on motility or concentration *in vitro*. At very high concentrations, decreases viability	No	[1]
	Tetracycline	Antibiotic	Very low concentrations reduce the motility significantly *in vitro*. Inhibits acrosome reaction	No	[1]
	Chloroquine	Antimalarial	At low concentrations, enhances rapid motility. At high concentrations, inhibits rapid motility *in vitro*	No	[1]
Antiretroviral	Antiretroviral treatment	HIV-1	Ejaculate volumes, percentages of progressive spermatozoa, total sperm counts, and polymorphonuclear cell counts were significantly decreased. Mitochondrial toxicity. Multiple DNA deletions	Unknown	[2]
Antineoplastic/ non-malignant disease	Hydroxyurea	Sickle cell disease	Impairs spermatogenesis, resulting in testicular atrophy, a reversible decrease in sperm count, and abnormal sperm morphology and motility. Furthermore the chromatin structure of germ cells is also affected, mainly in preleptotene spermatocytes and apoptosis is increased, essentially in spermatogonia and early spermatocytes, while stem spermatogonia do not seem to be affected, resulting in the repopulation of seminiferous tubules	Not to initial levels	[3]
Gastrointestinal	Sulfasalazine	IBS, UC, and CD	Doses of 2–4 g/day showed a significant decrease in sperm count and motility as well as an increase in abnormal morphologic forms. Characteristic "megalo" head form	After 3 months of drug withdrawal	[4]
	Infliximab	IBS, UC, and CD. A chimeric monoclonal antibody to tumor necrosis factor alpha	Increase in volume (possibly due to better overall health), normal sperm concentration, below normal sperm motility, normal forward progression, increase in abnormal forms	Unknown	[5]
	Mesalamine agents	IBS, UC, and CD	Normal sperm concentrations	Unknown	[5]
	Azathiorine	IBS, UC, and CD	Normal sperm concentrations	Unknown	[5]
Herbs	St. John's wort	Depression	Anorgasmia, decreased libido orgasmic delay, erectile dysfunction, and inhibition of sperm motility. Potent inhibition of sperm motility unrelated to changes in pH, as well as compromised sperm viability	Yes	[6,7]
	Saw-Palmetto	Enlarged prostate	Metabolic changes in treated sperm	Yes	[7]
	Echinacea purpura (high doses)	Immune system booster	Interference with sperm enzymes	Yes	[7]
	Mucuna pruriens	Stress reducer	Administration to infertile men for 3 months resulted in general improvement in sperm count and motility as well as reducing psychologic stress. It is a rich source of L-DOPA and its metabolites. An increase in dopamine level may induce the activation of sexual behavior and also increase plasma	Unknown	[8]

Table 33.3 (*cont.*)

Category	Drug name	Indications	Effect of drug on fertility	Reversibility	Ref.
			testosterone levels. Psychologic stress increases oxidant production and long-term exposure to stress may lead to peroxidation of polyunsaturated fatty acids of sperm membrane, resulting in unfavorable alterations of sperm structure and function		
Neurological	Phenobarbital	Antiepileptic, epilepsy	Affects ion membrane conduction at high concentrations. In vitro, sperm motility was inhibited. Concentrations were much higher than they would be therapeutically. Sperm motility may be impaired under therapeutic concentrations due to prolonged exposure. In vivo, poor motility was observed even though the serum levels were within therapeutic ranges. AEDs are highly lipid soluble and can cross the blood–testicle barrier into the genital tract. Interference with sperm membrane function may be the underlying mechanism. Spermatogenesis did not seem to be altered by long-term therapy	Yes	[9]
	Carbamazepine	Anticonvulsant, epilepsy	Serum SHBG levels increase progressively during long-term treatment. Serum testosterone levels remained unchanged; consequently, the SHBG level changes result in a continuously decreasing serum-free androgen fraction. May contribute to development of premature aging of the reproductive endocrine system, and these drug-related hormonal changes may be a major cause of hyposexuality and impotence. Affects ion membrane conduction at high concentrations. In vitro, sperm motility was inhibited. Concentrations were much higher than they would be therapeutically. Sperm motility may be impaired under therapeutic concentrations due to prolonged exposure. In vivo, poor motility was observed even though the serum levels were within therapeutic ranges. AEDs are highly lipid soluble and can cross the blood testicle barrier into the genital tract. Interference with sperm membrane function may be the underlying mechanism. Spermatogenesis did not seem to be altered by long-term therapy.	Unknown, Yes	[9, 10]
Neurological	Phenytoin	Antiepileptic, epilepsy	Serum SHBG levels increase progressively during long-term treatment while testosterone levels remained unchanged. The SHBG level changes result in a continuously decreasing serum-free androgen fraction. May contribute to development of premature aging of the reproductive endocrine system, and thus may be a major cause of hyposexuality and impotence. Modification of the neuronal membranes to a state of relative insensitivity to depolarization. Mediated by inhibition of the large transient increase in the permeability of the membrane to sodium and calcium ions. In vitro, sperm motility was inhibited. Concentrations were much higher than they would be therapeutically. Sperm motility may be impaired under therapeutic concentrations due to prolonged exposure. In vivo, poor motility was observed even though the serum levels were within therapeutic ranges. AEDs are highly lipid soluble and can cross the blood testicle barrier into the genital tract. Interference with sperm membrane function may be the underlying mechanism. Spermatogenesis did not seem to be altered by long-term therapy.	Unknown, yes	[9, 10]

Table 33.3 (*cont.*)

Category	Drug name	Indications	Effect of drug on fertility	Reversibility	Ref.
Neurological	Valproate	Antiepileptic, epilepsy	Reversibly depresses the activity of all excitable tissues or cells, although its true mechanism of action on the membrane is still not known. In vitro, sperm motility was inhibited. Concentrations were much higher than they would be therapeutically. Sperm motility may be impaired under therapeutic concentrations due to prolonged exposure. In vivo, poor motility was observed even though the serum levels were within therapeutic ranges. AEDs are highly lipid soluble and can cross the blood–testicle barrier into the genital tract. Interference with sperm membrane function may be the underlying mechanism. Spermatogenesis did not seem to be altered by long-term therapy. Men on this drug also had significantly lower free carnitine/total carnitine, which may have implications for sperm motility. They also had higher insulin and C-peptide concentrations.	Yes	[9,11]

IBS = irritable bowel disease; UC = ulcerative colitis; CD = Crohn's disease.

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effect on fertility [51]. Co-trimoxazole impairs sperm motility at high concentrations in vitro. Sperm treated with erythromycin in vitro showed a significant decline in motility increasing with concentration. Tetracycline, even in very low concentrations, reduced the motility significantly in vitro. Furthermore, in vitro exposure to tetracycline inhibited the acrosome reaction of the sperm. Chloroquine, an antimalarial drug, inhibits rapid motility at high concentrations in vitro. Amoxicillin has a slightly different effect on fertility. In vitro, amoxicillin has no effect on motility or concentration. However, at very high concentrations, sperm viability decreases. In this in vitro study, the effects of these drugs were not reversible [51], but the reversibility was only tested on the same sperm specimen, not the patient.

Antiretroviral drugs can have a negative effect on fertility as well. One study shows significant decreases in ejaculate volumes, percentages of progressive spermatozoa, total sperm counts, and polymorphonuclear cell counts. Furthermore, it was found that antiretroviral treatment, in particular HAART, causes mitochondrial toxicity and multiple DNA deletions [52].

The treatment for sickle cell disease, hydroxyurea, has also been shown to impair spermatogenesis, resulting in testicular atrophy, a reversible decrease in sperm count, and abnormal sperm morphology and motility [53].

In the treatment of Crohn's disease and ulcerative colitis, mesalamine agents and azathiorine were not found to have detrimental effects on sperm quality [54], while sulfasalazine and infliximab did impact sperm quantity and morphology. **Sulfasalazine therapy resulted in a significant decline in sperm count and an increased occurrence of specific morphologic aberrancies** [55]. Infliximab therapy was shown to increase semen volume but it decreased overall sperm motility and increased the number of aberrant forms [54].

Anti-epileptics such as valproate, carbamazepine, and phenytoin may reduce sperm motility and testicular volume due to their effects on endocrine function (increasing levels of sex-hormone-binding-globulin, thereby decreasing free testosterone) and axonal transport [56,57,58,59].

Herbal supplementations used in alternative medicine have also been correlated with decreased semen quality. **St. John's**

Wort is shown to decrease sperm motility and viability within 1 week of treatment [60] unrelated to changes in pH. Ginko, however, has been shown not to have detrimental effects on sperm motility. High concentrations of *Echinacea purpura* interfere with sperm enzymes, and Saw-Palmetto demonstrates metabolic effects [61]. *Mucuna pruriens*, interestingly, was found to increase both sperm count as well as motility when administered to infertile men [62].

Cryopreservation for assisted reproductive (ART) procedures

Cryopreservation of human semen is a standard practice for anonymous, directed donor (i.e., known donor) and husband/partner (i.e., client depositor) sperm of good quality prior to artificial insemination or IVF [63]. Storage, pooling, and concentration of many oligospermic samples from one partner can increase numbers of progressively motile cells inseminated in AIH. Also, preservation can be done prior to intrauterine insemination (IUI) or in vitro fertilization (IVF) in the event of inability to produce a specimen or unexpected azoospermia on the day of the procedure [13]. In fact, many recent applications of sperm banking have paralleled advances in IVF. Through the use of intracytoplasmic sperm injection (ICSI) of a single sperm into an oocyte, one routinely obtains fertilization and pregnancy [64]. Therefore, no matter how impaired the quality and quantity of ejaculated sperm, sperm can usually be retrieved from the ejaculate, vasal fluid, epididymal fluid or testis and cryopreserved for future use in IVF using ICSI [65,66].

Sperm can be cryopreserved before surgical infertility treatment, such as varicocele ligation in men with severe oligospermia. Sperm can also be cryopreserved at the time of diagnostic transrectal ultrasound (TRUS) with seminal vesicle aspiration, prior to transurethral resection of the ejaculatory ducts (TURED). This provides insurance in case azoospermia ensues post-operatively. Though not performed as commonly as initially anticipated, **sperm can also be cryopreserved preceding vasectomy.** This provides the opportunity for possible future potentially successful assisted reproduction, if circumstances change regarding desire for progeny.

Intraoperatively, cryopreservation is indicated at the time of primary procedures performed for obstructive azoospermia. These include **microsurgical epididymal sperm aspiration (MESA)** [65]**, percutaneous epididymal sperm aspiration (PESA), electroejaculation (EEJ) procedures, and TURED.** Sperm can also be cryopreserved when found at the time of complex surgical reconstructive procedures including **vasovasostomy and vasoepididymostomy** [67].

When performed at the time of **testis biopsy/testicular sperm extraction (TESE)**, cryopreservation of testicular spermatozoa from men with severe spermatogenic failure but with focal spermatogenesis may obviate the need for repeated invasive procedures. It also allows ovarian stimulation of the female partner to be timed and avoids the expense and frustration of having an ICSI cycle end unsatisfactorily with no sperm on the day of egg retrieval [66,68].

Post-operative cryopreservation can be performed after successful vaso-epididymostomy, vasovasostomy, TURED, and varicocelectomy in previously azoospermic men. This can provide insurance against a late stricture and re-obstruction after an early period of having sperm in the ejaculate.

Posthumous sperm cryopreservation

Pregnancy with ICSI using spermatozoa from a deceased partner [69] **has been achieved.** The posthumous use of frozen spermatozoa to attempt to achieve a pregnancy has been available for cancer patients since the commencement of sperm cryopreservation, but only recently has assisted reproduction technology offered a realistic chance of success for those in need. Issues of informed consent arise in this setting, often requiring legal consultation and ethical considerations [70].

The retention of fertilizing capacity of a partner or donor in his temporary or permanent absence is possible with the use of cryopreservation. Sperm can be cryopreserved prior to entry into military service or anticipation of toxin exposure.

Although sperm retrieval from deceased or incompetent individuals may be achieved readily, it is incumbent on the practitioner to consider the legal and moral implications of these procedures before proceeding [71]. A set of guidelines addressing postmortem sperm retrieval has been developed by at least one institution [71], and a position statement has been made by the ASRM [72]. The guidelines provide the following exclusion criteria: (1) only the wife may provide consent for postmortem sperm retrieval; (2) the couple must have been planning a family prior to the death of the husband; (3) the death was sudden and not due to any disease known to affect spermatogenesis or effect disease transmission; (4) available resources exist to retrieve sperm within 24 hours after death; and (5) the wife must consent to a 1-year period of specimen quarantine, so that additional counseling may be provided after the initial bereavement period has passed. Additionally, many states have regulations that will determine whether and under what circumstances posthumous sperm may be retrieved, stored, and used. For example, New York State requires that the facility storing client depositor sperm (i.e., sperm from a donor who is a sexually intimate partner of the recipient) have a written informed consent signed by the donor documenting his willingness to participate in the storage program and "…. include the male client depositor's specific instructions for disposition of frozen semen upon his Death" [73]. If another type of legal document is acceptable to the regulatory authority it should specify not only that sperm can be retrieved but also that it can be stored and released for the purpose of conception of a particular individual.

It is therefore incumbent upon the storage facility that a consent document be signed at the time of cryobanking, prior to treatment for malignancy or other medical illnesses, which

clarifies who is allowed access to the sperm in the event of death of the male partner. In many states this can only be the sexually intimate partner of the client depositor. The person identified in this document then becomes the only responsible party and can choose to discard the sperm if she so chooses at a later date. Signing such a document at the time of banking avoids any later legal controversies over the right to have access to the sperm of the deceased individual.

Cryopreservation methodology

Source of male gametes

MESA/ICSI

In selected ideal cases of unreconstructable azoospermia, elective open microsurgical epididymal sperm aspiration with cryopreservation yields pregnancy rates similar to that employing fresh sperm. Abundant high-quality sperm can be cryopreserved in a single procedure for all future attempts at IVF/ICSI [65]. With the advent of ICSI, only a small number of motile spermatozoa are required for successful fertilization [23]. Unlike in the past when poor specimens would not have been cryopreserved, they are now routinely cryopreserved and used successfully with ICSI. The use of fresh sperm obtained from microsurgical epididymal sperm aspiration (MESA) has dramatically increased the likelihood of embryo formation when used in conjunction with ICSI. Typically, sperm and oocyte harvesting are performed simultaneously, providing fresh sperm for ICSI. However, Oates et al. achieved excellent fertilization rates (37% per oocyte) and pregnancy rates (40% per couple, 29% per cycle) with ICSI using cryopreserved sperm obtained from MESA. This approach eases the burden of partner scheduling on both the couple and the clinicians involved without compromising fertilization or pregnancy rates [74].

Studies have shown identical pregnancy rates with IVF and ICSI using freshly aspirated sperm compared to frozen epididymal sperm [58]. With a single MESA procedure, a mean total of 82 million sperm per patient were cryopreserved and divided into a mean of 4.7 vials. No statistically significant difference in oocyte fertilization rate or number of embryos transferred was noted between groups. Of 108 patients using freshly aspirated sperm, 72 (66.7%) achieved clinical pregnancy. Of 33 patients in the group using cryopreserved sperm, 20 (60.6%) achieved clinical pregnancy ($P = 0.47$) [58].

Devroey et al. evaluated the use of cryopreserved epididymal sperm in seven patients who did not become pregnant following MESA and ICSI [75]. A subsequent ICSI was performed using previously cryopreserved epididymal spermatozoa. From 68 intact oocytes injected with frozen–thawed epididymal spermatozoa, a fertilization rate of 45% and cleavage rate of 82% were obtained. It was concluded that cryopreservation of sperm during MESA avoids further scrotal surgery [75] and results in equal fertilization and cleavage rates between fresh and frozen samples.

TESE/ICSI

To determine the feasibility of using frozen–thawed testicular spermatozoa for ICSI, Kupker et al. evaluated 175 men with non-obstructive azoospermia who underwent testicular biopsy for cryopreservation of tissue to be used in consecutive ICSI treatment cycles [66]. Their female partners underwent controlled ovarian hyperstimulation for conventional IVF treatment. In 77% of the patients, spermatozoa could be harvested from the testis by an open testicular biopsy technique and used for ICSI after freezing and thawing of testicular tissue. In all patients, viable spermatozoa could be visualized after the tissue samples were thawed. 135 ICSI treatment cycles were performed, with a fertilization rate of 45% and a clinical pregnancy rate of 30% per oocyte retrieved [66]. These clinical pregnancy rates are comparable to pregnancy rates with freshly ejaculated sperm (28%) [66]. Similarly, when comparing the results of ICSI cycles with either fresh or cryopreserved testicular spermatozoa, Friedler et al. found no statistically significant differences in fertilization rates, embryo cleavage rates, implantation rates, and clinical pregnancy rates [76].

Prins et al. **recommend routine testicular sperm extraction and cryopreservation of sperm at the time of diagnostic testicular biopsy.** A total of 73 men with obstructive and 42 with non-obstructive azoospermia underwent testicular sperm extraction at diagnostic biopsy. Sperm was retrieved and cryopreserved in all cases of obstruction and in 15 non-obstructive azoospermia cases. Among 17 couples a total of 20 cycles of IVF/ICSI were performed. Fertilization, cleavage, and pregnancy rates were determined in cases of obstruction and non-obstruction. Sperm count was decreased and morphology was impaired in the testicular biopsies of men with non-obstructive versus obstructive azoospermia, and motility was low or absent in all testicular sperm extraction specimens. Importantly, pre-freeze (63%) and post-thaw (31%) viability was the same in both patient groups. After IVF/ICSI using frozen and thawed testicular sperm, the fertilization, cleavage, implantation, and clinical pregnancy rates were excellent: 60, 86, 16, and 50%, respectively. Using cryopreserved sperm no differences in outcome of any IVF/ICSI procedures in patients with obstructive versus non-obstructive azoospermia were observed [77].

The outcome of ICSI using cryopreserved–thawed testicular spermatozoa of men with non-mosaic Klinefelter syndrome, an important cause of non-obstructive azoospermia, is also comparable with that following the use of fresh spermatozoa [78]. Mature testicular spermatozoa were found in five out of 12 (42%) patients who underwent testicular sperm extraction, and ICSI was performed while excess tissue was cryopreserved. The outcome of ICSI using fresh or cryopreserved–thawed testicular spermatozoa was compared. No statistically significant difference was found in the two pronuclear fertilization rate (66 versus 58%), embryo cleavage rate (98 versus 90%), and embryo implantation rate (33.3 versus 21.4%) for fresh or cryopreserved sperm, respectively [78].

Palermo GD et al. evaluated the results of ICSI using testicular spermatozoa in men with both non-obstructive and obstructive azoospermia. 57.0% of 533 eggs were fertilized in non-obstructive cases compared to 80.5% of 118 eggs (P = 0.0001) in obstructive azoospermia. The clinical pregnancy rate was 49.1% (26/53) for non-obstructive cases and 57.1% (8/14) for testicular spermatozoa obtained in obstructive azoospermia, including three pregnancies with frozen-thawed testicular spermatozoa [64]. At this point in time, it is still uncertain whether frozen testicular sperm in men with NOA survives freezing and fertilizes better than semen from men with NOA. **For men with NOA, we recommend simultaneous micro-TESE and oocyte retrieval with ICSI using fresh sperm since, with NOA, the number of sperm retrieved may only be a handful and freeze–thaw may yield no viable sperm.**

Intraoperative cryopreservation

One study evaluated 100 consecutive men who had sperm cryopreserved intraoperatively at the time of surgical exploration for non-obstructive azoospermia. Ten of these couples used their cryopreserved sperm for IVF/ICSI, resulting in 10 deliveries. Eight of these involved epididymal aspiration from men with congenital bilateral absence of the vas deferens (CBAVD), and two were from men who had unsuccessful complex reconstructions with persistent post-operative azoospermia. Both of these men had failed reconstructions: one a V-E to the efferent ductule, and the other a combined V-V/V-E [67]. **Intraoperative cryopreservation is therefore recommended at the time of complex reconstructive cases which have lower patency rates, including vasoepididymostomy, especially when bilateral, and vasoepididymostomy to the efferent ductule** [67]**. Intraoperative cryopreservation during uncomplicated vasovasostomies is often not necessary due to the very high success rate of vaso-vasostomy** [79].

Electroejaculation

Electroejaculation has become an accepted form of semen procurement in men suffering from anejaculation, although sperm in these ejaculates often exhibit low motility. In such cases, ICSI is offered to improve the possibility of successful pregnancy. In a study of 25 men suffering from psychogenic anejaculation, 37 sessions of electroejaculation in combination with ICSI were performed. **The fertilization and pregnancy rates with cryopreserved sperm from electroejaculation were found to be at least as good as those of freshly obtained sperm.** When motile sperm is found in the thawed ejaculate, additional electroejaculation can be avoided [80].

Cryopreservation methodology

The most commonly reported detrimental effect of cryopreservation on human spermatozoa is a marked reduction in motility [81]. This occurs despite many advances in cryopreservation methodology. The primary cause of cellular damage during cryopreservation is the formation of intracellular ice. However, cell survival depends on the nature of the suspending medium. Understanding the profound protectant effect of the suspending medium has led to the development of numerous protective agents [82].

Media used in cryopreservation

Cryoprotectants such as glycerol or propanediol can be added to cells to reduce freezing damage by lowering the salt concentrations and increasing the unfrozen water fraction, thereby reducing osmotic stress [81]. Osmotic stress attributed to differences in the relative permeability of cryoprotectants appears to be an important factor in cryodamage [83]. Glycerol and egg yolk are the accepted cryoprotectants used to maintain cell membrane integrity during cooling and thawing. The percentage of stem cell survival increases as higher concentrations of glycerol are used with a constant cooling rate [22].

Several studies have evaluated various cryoprotectants for optimal protection of sperm cells during the freeze–thaw process [84]. Gilmore et al. [85] tested several cryoprotectants to determine which would result in the least amount of volume excursion during its addition and removal. They found that there was no significant difference in sperm motility between the slow addition of the various cryoprotectants. However, abrupt addition of the cryoprotectant resulted in significant impairment in motility. In addition, as the concentration of the cryoprotectant was increased there was a decrease in motility both for slow and abrupt addition of the cryoprotectant. They concluded that the optimal cryoprotectant would be one that can permeate the cell in the shortest period of time causing the least amount of volume excursion during its addition and removal. Also, rate of addition of the cryoprotectant as well as the concentration is important in preservation of sperm motility during the freeze-thaw process.

Studies have also evaluated the effect of media used for cryopreservation on sperm chromatin integrity. Hammadeh et al. [86] conclude that TES-yolk buffer (TYB) is superior to Human Sperm Preservation Medium (HSPM) for human sperm cryopreservation and offers better preservation of chromatin integrity and morphology of human spermatozoa.

Protocols used for cryopreservation

There is no one method used for cryopreservation of human sperm. Usually, semen samples are collected by masturbation after 2–3 days of abstinence and liquefied at room temperature. Semen analysis is performed prior to processing for cryopreservation [87]. The test yolk buffer contains 20% heat-inactivated egg yolk. 12% glycerol is added slowly in a 1:1 ratio to semen as a cryoprotectant at room temperature in order to prevent hyperosmolar stress [88]. Current techniques commonly use plastic straws or vials and the dilution of semen with an egg yolk in citrate or a physiologic salt extender, often with antibiotics. Specimens are vortexed and divided equally between vials or straws for long-term storage. Aliquots are suspended in liquid nitrogen vapor 10 cm above the level of liquid nitrogen (–80°C)

for 15 minutes. The samples are then plunged into liquid nitrogen (–196°C) and stored until required [81]. As the samples are suspended vertically on a cane held in liquid nitrogen vapor, each vial is subjected to a different freezing temperature since the temperature gradient in the vapor freezer varies. Alternatively, programmable controlled-rate freezers, which are constructed to precisely and uniformly maintain temperature throughout the chamber, can be used to reproducibly freeze samples.

Routinely, one cryovial is set aside and thawed the next day to assess post-thaw motility. This indicates how well the spermatozoa in the other remaining vials will survive when thawed later. In order to thaw a cryovial, it is brought to room temperature or to 37°C. It is further processed for use by diluting it with a suitable buffer (e.g., HTF-HEPES), then centrifuging it slowly (300 g) to form a pellet and remove the cryoprotectant [89]. When sperm are to be used for ICSI, the pellet is then resuspended in a small aliquot of medium from which the motile or twitching epididymal sperm are isolated for the ICSI procedure. Analogous procedures are used for testicular tissue; however, tissue must first be macerated and minced [66].

There are two basic methods used in freezing sperm. The first, a rapid freeze cycle is most often employed by placing the vials or straws containing the specimens in the liquid nitrogen vapor present above the liquid nitrogen level in a dewar. The second, a slow-freeze cycle, uses a programmed freezer which is designed to provide freezing of straws or vials at a defined rate.

The rate of both cooling and warming has a profound effect on cell survival. Controlled-rate freezing is currently the best technique for determining and achieving optimal cooling and thaw rates for a specific specimen. Not only are human spermatozoa resistant to freezing–thawing, they also survive various methods of freezing–thawing and storage [14,90]. It is critical for each cryobank to determine the optimal rate of freezing and thawing for its preparation of specimens.

In 1997, a method was developed to allow for the cryopreservation of very low numbers of sperm [91]. This is achieved by injecting them into cell-free human, mouse, or hamster zona pellucidae before the addition of cryoprotectant. This technique is still experimental, and its efficacy in humans has not been established. Another important contribution of cryobanking with regards to donor insemination programs is the quarantine of sperm for longer than 6 months with the capacity to screen donors for human immunodeficiency virus (HIV) and other known sexually transmitted diseases [92]. Testing is performed before and 6 months after collection, while holding semen in frozen-storage, in quarantine, for release only after re-testing of the donor.

Other methods of sperm preservation

Vitrification

Vitrification is an ultra rapid cooling technique based on direct contact between the vitrification solution containing the cryoprotectant agents and the liquid nitrogen (LN2) [93]. Vitrification can be defined as the solidification of a solution at low temperature, not by ice crystallization but by extreme elevation in viscosity during cooling resulting in a glassy, vitrified state [94]. During vitrification, in contrast to the "slow-rate" freezing protocols presently used for routine cryopreservation, the entire solution remains unchanged and the water does not precipitate, so ice crystals are not formed [95]. A theoretical advantage of vitrification would be a faster freeze cycle and an improved recovery of viable spermatozoa.

Vitrification of water inside cells can be achieved by increasing the speed of temperature conduction and/or by increasing the concentration of cryoprotectant. This process is most efficient when using a small volume (0.1 mL) of high-concentration cryoprotectant. However, achieving high cooling rates requires the use of high concentrations of the cryoprotectant solution, which depresses ice crystal formation. A technical issue that needs to be addressed is that concentration of the cryoprotectant solution required can lead to either osmotic or chemical toxicity. In contrast to the routine slow-freeze cryopreservation in which ice crystal formation is the major concern with vitrification, osmotic shock on thawing is the major cause of damage to spermatozoa [96]. To address this issue some authors advocate cryoprotectant-free vitrification [97]. Also, with vitrification, DNA integrity and motility [97,98] as well as acrosomal status and mitochondrial activity [99] appear comparable to slow freezing.

Freeze-dry

Freeze-drying of sperm offers great potential for the future of sperm banking. A protocol proposed by Sanchez-Partida et al. [100] begins by diluting the specimen with a 0.3 M trehalose solution. The specimen is then frozen as pellets on dry ice and stored in liquid nitrogen for 24 hours prior to freeze-drying. The pellets are then transferred to borosilicate glass vials and allowed to dry for 24 hours. Argon gas is then injected and the vial is sealed. The pellets are rehydrated prior to use. Neither liquid nitrogen nor dry ice is needed for the storage and shipment of preserved spermatozoa.

Freeze-drying has been used successfully with bovine [101], pig [102], rabbit [103], and rat [104] sperm. In addition, recent studies have demonstrated the feasibility of using freeze-dried spermatozoa in Rhesus macaque, mice, and humans [105,106]. However, many species do not require the paternal contribution of the sperm centrosome for natural fertilization and embryonic development as human spermatozoa do. Freeze-drying protocols often damage the acrosome and mitochondria which are necessary for sperm function [103,107]. The viability of the acrosome and mitochondria after the freeze–thaw cycle will therefore need to be evaluated prior to documenting the success of a freeze-drying protocol. Kusakabe et al. have also demonstrated the maintenance of chromosomal integrity after freeze drying [106].

Freeze-drying spermatozoa offers several significant advantages. Protocols for cryopreservation would be simplified and storage costs would be reduced significantly and potentially eliminated if the sample is given to the patient to

"store" himself. However, the potential ethical issues of having an essentially imperishable source of genetic material must be addressed before clinical use.

Tracking of cryopreserved specimens

An essential, yet often overlooked requirement of gamete storage is tracking of the cryopreserved specimens. Oftentimes, even a small sperm bank has thousands of specimens that need to be tracked throughout the process of collection, processing, storage, and distribution or destruction. With recent regulations promulgated by both State and Federal authorities, the tracking of these specimens as well as all environmental factors (e.g., storage temperature) and reagents (e.g., freeze media) that have come in contact with the specimens must be logged and validated for use. Handwritten notebooks and spreadsheets are most often used but are cumbersome, susceptible to damage or destruction, and make it a time-intensive process to produce the required reports.

Electronic databases have been advocated as a way to track cryopreserved specimens [108] with data entry being facilitated by the use of bar code scanners. Through the use of relational databases, inventory management and report generation is easily handled.

Clinical considerations

We are often asked questions regarding the effect of cryopreservation as well as the effects of gonadotoxic treatments on sperm quality. In particular our patients are concerned about the potential effects of the cryopreservation process as well as the disease and treatment for the disease on offspring that might result from use of cryopreserved sperm. In this section we will address some of the more common questions and provide reasonable suggestions.

What are the effects of cryopreservation on sperm, fertilization, and embryo development?

The cryopreservation process, in and of itself, appears to have a detrimental effect on spermatozoa. Donnelly et al. demonstrated that cryopreservation of sperm resulted in a decrease of 45% in the average velocity of sperm movement [81]. **A greater decrease in progressive movement has been observed in the semen of infertile men compared to fertile donors** [81,109]. Although **artificial stimulants such as pentoxyfylline and 2-deoxyadenosine can significantly improve sperm motion characteristics of thawed cryopreserved sperm** [110], there is a substantial decrease in sperm quality after cryopreservation. However, despite the fact that freezing–thawing is associated with a variable loss of sperm viability and motility, cryopreservation of spermatozoa from men with poor sperm quality does not negatively affect fertilization and pregnancy rates after ICSI [63], as long as motile spermatozoa are injected [111]. In fact, Kuczynski et al. demonstrated no difference in pregnancy rates after ICSI between freshly ejaculated groups (23.7%) and the frozen–thawed group (35.2%) [63]. In addition, Zorn et al. [112] found that a higher proportion of blastocysts developed after the use of frozen–thawed testicular spermatozoa in comparison to fresh testicular spermatozoa (P = 0.034). It also appears that once the oocyte is fertilized, implantation and pregnancy rates are similar between fresh and cryopreserved spermatozoa in both oligozoospermic and normozoospermic patients [113,114].

Concerns exist regarding the possibility of microbiologic cross-contamination of specimens with bacteria or viruses when stored in liquid nitrogen at –196°C. However, several measures are taken to reduce this perceived risk. All donors and many client depositors are screened for HIV, hepatitis B and C, syphilis, and other sexually transmitted diseases prior to cryopreservation of their semen. Another approach involves the use of quarantine tanks to hold samples until screening results are obtained. In addition, adequate sealing of vials is obviously crucial in preventing entry or egress of micro-organisms [115]. Storage in the vapor phase of liquid nitrogen is also an option to decrease the possibility of contamination between specimens. However, this requires close monitoring of storage temperatures to ensure that the minimum –130°C required for long-term storage of male gametes is maintained.

Cryopreserved sperm with damaged DNA can result from a patient's underlying disease and corresponding treatments, as well as the process of cryopreservation. In fact, genetic damage has been identified with the freezing/refreezing process [116]. Donnelly et al. identified a significant 20% decrease in DNA integrity in cryopreserved spermatozoa [81]. One study found the DNA of semen and prepared spermatozoa from fertile men to be unaffected by cryopreservation, whereas spermatozoa from infertile men were significantly damaged by freeze-thawing [81]. In addition, cryopreservation has been shown to have a detrimental effect on morphology of semen and prepared samples from both fertile and infertile men.

When is it safe to use ejaculated sperm for conception after gonadotoxic therapy for conception or for cryopreservation?

One of the most frequently asked questions asked by our patients is how long after gonadotoxic therapy should they wait before it is safe to use ejaculated sperm for conception. This is also a concern for patients that were unable to give a specimen prior to chemotherapy. They often seek to store sperm after beginning their therapy. We are therefore asked to guide these patients as to when, and whether, it would be appropriate to cryopreserve sperm.

Damage to sperm DNA, while having the potential to occur spontaneously, is strongly correlated with mutagenic events [81,117]. Despite this, sperm with damaged genetic material are still capable of fertilization. Mutations and defects may not become evident until the embryo has divided or the fetus developed [81,118]. Strand breaks in DNA lead to chromosomal damage, and most sperm-derived genetic abnormalities

occur through chromosomal breakage rather than through chromosomal rearrangement as in the oocyte [81]. The highest susceptibility to transmissible mutagenesis induced by chemotherapeutic agents has been shown in post-meiotic cells. The high sensitivity of post-meiotic cells is likely related to the reduced capacity for DNA repair in late spermatids and spermatozoa as compared to early spermatids and the other spermatic cell types [119]. It is, therefore, crucial to ensure that sperm are frozen in such a way as to offer maximum protection to DNA to prevent possible conveyance of damage to offspring [81].

Although collection and cryopreservation of semen is feasible during chemotherapy until azoospermia ensues [24], some recommend that patients complete their semen cryopreservation prior to the initiation of chemotherapy [120]. Moreover, patients are instructed to practice reliable contraception from the time of initiation of treatment until 12 to 18 months after completion of treatment. This is based on data from experimental animals that indicate high levels of mutagenic effects in offspring from matings during or soon after treatment of the male with chemotherapy or radiation [120].

Impairment in spermatogenesis results from the cytotoxic effects of chemotherapy or radiation upon the rapidly dividing spermatogenic epithelium. These treatments are also mutagenic. Several assays including the Tunel, Comet, DNA oxidation, nuclear protein composition, sperm nuclear maturity test, and chromatin structure assays (SCSA) measure DNA damage. These assays have confirmed that damage is present in sperm from both fertile and infertile men [121]. The association with infertility is suspected but not proven. What is of concern is that these assays detect increased [32] and persistent sperm DNA damage [122] after in vivo treatment with genotoxic agents. However, studies of environmental agents with mutagenic properties have not been found to increase the rate of genetic disorders in humans [123] and more importantly studies on the offspring of cancer patients have not supported an association between paternal cytotoxic cancer therapy and genetic disease in their children. There were no differences in the rates of cytogenetic diseases, single gene defects, or simple malformations in the offspring when compared with sibling controls [124]. Genetic disease occurred in 3.4% of 2198 offspring of survivors, compared with 3.1% of 4544 offspring of controls ($P = 0.3$; not significant).

These findings have been confirmed in several other large population studies. The Childhood Cancer Survivor Study database, a multi-institutional retrospective cohort study started in 1994, was used to review 4214 live births from childhood cancer survivors. They found no significant differences between childhood cancer survivors and their siblings. The total rate of cytogenetic abnormalities, single gene defects, and malformations was 3.7% in the survivor offspring and 4.1% in the siblings [12]. When cancers with a known single gene inheritance were excluded, no increase in offspring childhood cancer was seen. Another study using the Danish Cancer Registry of childhood cancer survivors also compared the pregnancy outcomes of childhood cancer survivors to their unaffected siblings [125]. The cancer survivor group had 2630 live births from 4676 childhood cancer survivors. There was no difference in the rate of chromosomal abnormalities between the groups. The primary limitation in these studies is that the individual treatment regimens had not been evaluated separately. Therefore, subtle long-term differences between treatment types would not be detectable by grouping all survivors together [119]. Nonetheless, better surveillance of genetic disease in the offspring of men surviving cytotoxic therapies is needed to provide more robust risk assessment.

Single-cell gel electrophoresis (SCGE) and the Comet assay have the ability to detect damage at the single-cell level in stallions. Such detection of sublethal and/or uncompensable fertility factors in semen, such as DNA fragmentation, could be useful for detecting differences in semen for cooling or cryopreservation potential and could provide a tool for monitoring and preserving fertility [126].

In addition to the worrisome possibility of transmitting genetically damaged spermatozoa, concern also exists about cryopreserving malignant cells during TESE in men with diseases such as leukemia or lymphoma. Thus far the offspring born from cryopreservation have no proven increase in genetic abnormalities compared to the general population. Future studies that control for maternal age are needed to assess the long-term outcome of pregnancies after assisted reproduction using cryopreserved sperm.

Therefore, **all men undergoing potential gonadotoxic therapy should be counseled regarding the effect of their treatment on fertility and offered cryopreservation of sperm before their treatment.** As discussed earlier in this chapter, due to the detrimental effects of chemotherapy on spermatogenesis together with the 2- to 3-month cycle of spermatogenesis, **patients are often advised to delay attempting conception for four cycles of spermatogenesis, or approximately 1 year, after their last treatment of chemotherapy.** Couples should also be made aware that cryopreserved sperm may theoretically reduce the risk of genetic abnormalities that might be induced in the stem germ cell or spermatogonia cells by treatment. However, couples should be counseled that **there is no evidence of an increased risk for genetic disease in the offspring of men undergoing gonadotoxic therapy** and also that a waiting period prior to using ejaculated sperm for conception is based only on theoretical concerns.

What is the length of time cryopreserved sperm can be stored?

Sperm specimens are most often stored in liquid nitrogen (−196°C/−320.8°F) or in nitrogen vapor phase (below −130°C/−202°F). Empirical studies over the past 60 years have shown that many biologic systems, including sperm, can potentially survive storage for essentially unlimited periods of time at ultracold temperatures. That is, if they can survive the passage to and from the storage temperature. The major damage to sperm during cryopreservation appears to be related to the

freeze-thaw cycle although potential genetic changes during storage also need to be considered.

Temperature affects the "phase state" of the membranes, thereby altering their physical properties [127,128]. Extracellular ice crystallization occurring during the freeze process results in a phase change. The formation of the solid phase during freezing results in a large increase in the concentration of other solutes in the remaining liquid phase. The sperm cell (interior, interface, and exterior) faced with these changes must respond within a finite time dictated by the freeze protocol and cryoprotectant [129]. If a successful response occurs the sperm cell survives the process and if not, cell death occurs. Thawing of the specimen reverses the process and again requires a successful response from the cell to ensure survival [130,131]. It is therefore the freeze-thaw process, not the period of time frozen, which has the potential to affect sperm quality adversely.

When sperm are frozen using validated protocols, and temperature changes are negligible, there should be relatively little change in sperm quality. However, quantification of this is difficult to obtain. Survival of sperm after cryopreservation is evaluated by measurement of sperm motility and sperm viability both before cryopreservation and on an aliquot of the specimen thawed after an interval of cryopreservation (most often 24 to 48 hours). When using fresh unwashed human sperm freezing protocols (vapor or programmed freeze cycle) the motile sperm recovery rates (([post-thaw motile count $\times 10^6$/mL]/ [fresh motile count $\times 10^6$/mL]) $\times 100$) for normozoospermic samples is reported to be between 40% and 50% using glycerol as a cryoprotectant [132]. However, oftentimes the patients we see for cryopreservation have impaired semen quality and do not tolerate the freeze-thaw process as well. In addition, the specimen is analyzed using light microscopy and therefore subject to the vagaries in specimen collection and handling prior to evaluation. In addition, the protocol and cryoprotectant used for freezing as well as the protocol used for thawing all contribute to sperm survival. Also, specimens are often manipulated during storage which can subject them to variability in temperature. Variability in temperature can also occur even without specimen manipulation. This is a particular concern when specimens are stored in nitrogen vapor [132] where temperatures change significantly and immediately upon accessing specimens in the storage refrigerator. All these factors make it problematic to differentiate changes in specimen quality due to cryostorage from those related to specimen processing or the specimen itself.

Also concerning are time-dependent changes in the genetic integrity of stored sperm. As discussed previously, alterations in DNA appear to occur during the freeze-thaw process. Work by Jiang and coworkers on mouse sperm is encouraging. They reported that neither protein nor DNA profiles or sperm fertilization capability were altered by cryopreservation [133]. This also appears to hold for freeze-dried and dehydrated human sperm which were able to develop into a morphologically normal pronuclei after 12 months of storage at 4°C [134]. The genetic integrity of the dehydrated sperm nuclei still needs to be determined.

Clinically, cryopreservation of mammalian sperm is very successful. Mice sperm frozen in the early 1970s and periodically thawed have been successfully used to produce healthy offspring. Studies in humans show that specimens cryopreserved for 28 years retain good post-thaw motility recovery and normal levels of binding to the human zona pellucida as well as normal levels of zona-induced acrosome reaction [135]. The ultimate time interval that sperm can be stored and retain normal fertilization potential might never be known. Therefore, **the practical length of time that sperm can be stored and used for conception is at least equal to the reproductive lifetime of the man** [136].

Is cryopreserved sperm as good as sperm retrieved at the time of the insemination procedure?

It is well established that intrauterine insemination (IUI) with frozen-thawed spermatozoa results in lower pregnancy rates compared with insemination with fresh spermatozoa [137]. In a study of the efficacies of fresh versus cryopreserved semen in the treatment of male factor infertility by artificial insemination by donor (AID), Richter et al. found fresh semen to be more than three times as likely to induce pregnancy as frozen semen. In any one cycle, either fresh or frozen semen was used and served for direct comparison by using the patient as her own control. In 676 cycles fresh semen was used and 128 pregnancies were achieved. **Fecundity, the chance of getting pregnant per cycle of exposure, was 18.9% with fresh semen. Cryopreserved semen was used** in 1200 cycles and 60 pregnancies occurred, **for a fecundity of 5.0%** [138].

However, **when used for in vitro fertilization with intracytoplasmic sperm injection** (IVF/ICSI) **cryopreserved sperm appears equal to sperm retrieved at the time of the IVF/ICSI** procedure. In a study by Wald et al. [139], the fertilization rate for the TESE group alone (304 cycles) was 60.0% for cryopreserved sperm and 55.1% for fresh sperm, while the pregnancy rate for the TESE group was 27.3% for cryopreserved sperm and 27% for fresh sperm. These results were similar to earlier studies [76,113,140,141,142,143] which also compared fertilization and clinical pregnancy rates between cryopreserved and fresh surgically retrieved sperm.

In summary, for IUI, the results with fresh semen are better than the results for frozen sperm. For IVF/ICSI the results appear equal for fresh when compared to cryopreserved sperm except for testicular sperm retrieved from men with non-obstructive azoospermia.

How often is cryopreserved sperm used?

In a single academic hospital in Sydney, Australia, 930 men sought semen cryostorage prior to undergoing treatments likely to cause infertility [144]. Of these men, 833 (90%) had spermatozoa cryostored over a 22-year period. Among 692 (74%) men surviving their illness, sperm samples were discarded for

193 (21% of all applicants, 28% of survivors) and cryostored spermatozoa were used for 64 men (7% of all applicants, 9% of survivors) in 85 treatment cycles. Cycles commenced at a median of 36 months post-storage (range 12–180 months) with nearly 90% of usage starting within 10 years of storage and none after 15 years. A total of 141 (15%) of men had died and of these, 120 (85% of those dying) had their spermatozoa discarded; requests to prolong cryostorage were received from relatives of 21 men (2% of all applicants, 15% of deceased) of which three cases had spermatozoa transferred for use with no pregnancies reported [144].

Hallak et al. [145] retrospectively reviewed a database of 342 cancer patients in which 52 patients discontinued sperm storage. Reasons for discontinuing storage included patient death (n = 21); fertility but no plans for more children (n = 23); good sperm quality (n = 8); and no plans to have children (n = 4). Cost of cryopreservation and specimen storage was not cited as a reason. They concluded that most patients decided to discontinue sperm banking because either they regained fertility or had improved semen quality.

This data demonstrates the importance of sperm cryopreservation for men undergoing chemotherapy. ***Sperm banking should be strongly recommended for all patients with malignant diseases who may wish to have children, even if they eventually decide that the specimens are not needed.***

Conclusions

In addition to revolutionizing the field of assisted reproduction, cryopreservation of sperm has provided the mechanism to preserve future fertility in men undergoing gonadotoxic therapies. Recent developments in the protocols used for cryopreservation, including vitrification have resulted in significant improvement in post-thaw sperm survival. The indications for cryopreservation have also expanded greatly with the development of ICSI. Although intrauterine insemination with frozen–thawed spermatozoa results in lower pregnancy rates compared with insemination with fresh spermatozoa, the fertilization and pregnancy rates with ICSI using cryopreserved sperm parallel those with freshly obtained sperm. In addition, cryopreserving sperm often avoids the need for additional surgery and optimizes results for couples undergoing repeated IVF/ICSI cycles.

Pearls

- Over 50% of male cancer survivors in the reproductive age group desire to preserve their future fertility.
- Storage in liquid or vapor phase nitrogen (−384°F/−196°C) has become the standard since it is readily available, inert and can maintain cryopreserved sperm below the −150°C temperatures required. Cryoprotectants such as glycerol and dimethyl sulfoxide (DMSO) which protect sperm from damage from the freezing process have become standard.
- The ability for a single, viable, yet immotile sperm to be used successfully for fertilization of an oocyte through intracytoplasmic sperm injection (ICSI) has greatly expanded the indications for sperm cryopreservation.
- Cryopreservation of sperm should be done prior to therapy for malignant diseases.
- Alkylating agents cause azoospermia in 90–100% of treated adult males.
- Vials can be preserved, thawed, and refrozen several times.
- Aliquots can even be scraped from a vial and used for IVF/ICSI.
- Sperm banking should be strongly recommended for all patients with malignant diseases who may wish to have children.
- The likelihood of rapid recovery of spermatogenesis following gonadotoxic insult is related to the agent(s) used and the doses received.
- Cyclophosphamide and procarbazine have been known to result in prolonged azoospermia.
- Up to 50% of patients treated with cisplatin-based therapy recover from its effects in the first 2 years and 80% recover after 5 years.
- All agents in MOPP are gonadotoxic.
- The COPP also has a significant effect on spermatogenesis.
- ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine) is often used for treatment of Hodgkin's disease with males generally returning to near baseline roughly 18 months after the last chemotherapeutic treatment.
- Fractionated radiation doses to the testis greater than 35 cGy cause azoospermia.
- More than 200 cGy can result in irreversible azoospermia.
- Owing to the detrimental effects of chemotherapy on spermatogenesis together with the 2- to 3-month cycle of spermatogenesis, patients are often advised to delay attempting conception for 4 cycles, approximately 1 year, after their last treatment of chemotherapy.
- Antibiotics may have a negative effect on male fertility.
- Antiretroviral drugs can have a negative effect on fertility as well.
- In the treatment of Crohn's disease sulfasalazine therapy resulted in a significant decline in sperm count, and an increased occurrence of specific morphologic aberrancies.
- Anti-epileptics such as valproate, carbamazepine, and phenytoin may reduce sperm motility and testicular volume.
- St. John's Wart is shown to decrease sperm motility and viability within 1 week of treatment.
- Sperm can be cryopreserved before surgical infertility treatment, such as varicocele ligation in men with severe oligospermia.
- Sperm can also be cryopreserved preceding vasectomy.
- Intraoperatively, cryopreservation is indicated at the time of microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA), electroejaculation (EEJ) procedures, TURED,

vasovasostomy, vasoepididymostomy, and testis biopsy/testicular sperm extraction (TESE).

- Post-operative cryopreservation can be performed after successful vasoepididymostomy, vasovasostomy, TURED, and varicocelectomy in previously azoospermic men. This can provide insurance against a late stricture and re-obstruction.
- Pregnancy with ICSI using spermatozoa from a deceased partner has been achieved.
- Studies have shown identical pregnancy rates with IVF and ICSI using freshly aspirated sperm compared to frozen epididymal sperm.
- Recommend routine testicular sperm extraction and cryopreservation of sperm at the time of diagnostic testicular biopsy.
- For men with NOA, we recommend simultaneous micro-TESE and oocyte retrieval with ICSI using fresh sperm since, with NOA, the number of sperm retrieved may only be a handful and freeze-thaw may yield no viable sperm.
- Intraoperative cryopreservation is therefore recommended at the time of complex reconstructive cases which have lower patency rates, including vasoepididymostomy, especially when bilateral, and vasoepididymostomy to the efferent ductule. Intraoperative cryopreservation during uncomplicated vasovasostomies is often not necessary due to the very high success rate of vasovasostomy.
- The fertilization and pregnancy rates with cryopreserved sperm from electroejaculation were found to be at least as good as those of freshly obtained sperm.
- The most commonly reported detrimental effect of cryopreservation on human spermatozoa is a marked reduction in motility.
- The cryopreservation process, in and of itself, appears to have a detrimental effect on spermatozoa.
- A greater decrease in progressive movement has been observed in the semen of infertile men compared to fertile donors.
- Artificial stimulants such as pentoxyfylline and 2-deoxyadenosine can significantly improve sperm motion characteristics of thawed cryopreserved sperm.
- All men undergoing potential gonadotoxic therapy should be counseled regarding the effect of their treatment on fertility and offered cryopreservation of sperm before their treatment.
- Patients are often advised to delay attempting conception for four cycles of spermatogenesis, or approximately 1 year, after their last treatment of chemotherapy.
- There is no evidence of an increased risk for genetic disease in the offspring of men undergoing gonadotoxic therapy.
- The practical length of time that sperm can be stored and used for conception is at least equal to the reproductive lifetime of the man.
- Fecundity, the chance of getting pregnant per cycle of exposure, was 18.9% with fresh semen. Cryopreserved semen was used for a fecundity of 5.0%.
- When used for in vitro fertilization with intracytoplasmic sperm injection cryopreserved sperm appears equal to sperm retrieved at the time of the IVF/ICSI.
- In summary, for IUI, the results with fresh semen are better than the results for frozen sperm. For IVF/ICSI the results appear equal for fresh when compared to cryopreserved sperm except for testicular sperm retrieved from men with non-obstructive azoospermia.
- Sperm banking should be strongly recommended for all patients with malignant diseases who may wish to have children, even if they eventually decide that the specimens are not needed.

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