

## Review Article

# CRYOPRESERVATION OF SPERM: INDICATIONS, METHODS AND RESULTS

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### ABSTRACT

**Purpose:** We review the history of the cryopreservation of human sperm. The current methods of cryopreservation and indications are highlighted, and the success rates of the various uses of cryopreserved sperm are reviewed. Potential adverse sequelae of human cryopreservation are also discussed.

**Materials and Methods:** Indications for cryopreservation include several applications, namely homologous and donor insemination, cryopreservation prior to surgical infertility treatment, intraoperative cryopreservation, postoperative cryopreservation, cryopreservation prior to treatment for malignancies and nonmalignant diseases, as well as premortem and postmortem cryopreservation.

**Results:** With the use of intracytoplasmic sperm injection, open microsurgical epididymal sperm aspiration and testicular sperm extraction with cryopreserved sperm in men with obstructive azoospermia yields pregnancy rates similar to those using fresh sperm. Intraoperative cryopreservation is recommended for complex reconstructive cases with lower patency rates, including vasopididymostomy and vasoepididymostomy to the efferent ductule. In addition, sperm banking is strongly recommended for all patients with malignant disease who may wish to have children, since a small but significant percent of these men later use their banked semen. Although there is concern about the transfer of genetically damaged sperm after cryopreservation, no proven increase in genetic or phenotypic abnormalities in offspring has been identified.

**Conclusions:** Sperm cryopreservation has revolutionized the field of assisted reproduction. Cryopreserving sperm avoids the need for additional surgery in couples undergoing repeated in vitro fertilization/intracytoplasmic sperm injection cycles. Moreover, it provides hope for men undergoing chemotherapy, radiation or radical surgery who once had no chance for future fertility.

**KEY WORDS:** testis; sperm; infertility, male; cryopreservation; sperm preservation

Interest in the storage and cryopreservation of sperm dates back to 1776, when Lazzaro Spallanzani, an Italian priest and physiologist, first reported that sperm became motionless when cooled by snow.<sup>1,2</sup> However, it was not until 1866 that Montegazza suggested that “a man dying on the battlefield may beget a legal heir with his semen frozen and stored at home.”<sup>1</sup>

In the late 1930s and early 1940s many observers found that sperm could survive freezing to temperatures below  $-321^{\circ}\text{F}$  ( $-160^{\circ}\text{C}$ ). However, survival was limited in the absence of a cryoprotectant such as glycerol.<sup>3</sup> When cryoprotectants became known, studies of sperm cryopreservation accelerated in the fields of animal and veterinary medicine. Conception rates from thawed sperm averaged 65% in cows. This rate has remained unchanged for the last 30 years. The major advancements in animal and veterinary cryopreservation have occurred in technique rather than in an improved understanding of cryophysiology.

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In 1953 the first human pregnancy with frozen sperm was reported.<sup>1,4</sup> At that time a successful, practical technique for cryopreservation of human spermatozoa was introduced by the demonstration that the sperm, after being frozen and stored in dry ice ( $-78^{\circ}\text{C}$ ), were capable of fertilization and the subsequent induced development of normal progeny.<sup>5</sup> However, due to the moral and legal controversy surrounding the use of artificial insemination, it was not until the 11th International Congress of Genetics in 1963 that this breakthrough was reported and interest in human sperm banking began.

The introduction in 1963 of a method for freezing human semen in liquid nitrogen vapor and its storage at  $-196^{\circ}\text{C}$  was followed by reports of normal births with its use.<sup>5</sup> The basic principles of technique refined since 1953 have proved suitable for the establishment of clinical cryobanks, which have resulted in normal, healthy offspring in various parts of the world. Greater appreciation of the applications of cryobanking developed with the wider use of artificial insemination of donor and husband/partner semen in the early 1970s.<sup>5</sup>

During the last 30 years several improvements in sperm cryobanking have occurred. Storage in liquid nitrogen ( $-384^{\circ}\text{F}/-196^{\circ}\text{C}$ ) has become the standard and extenders

containing cryoprotectants have been added to the medium. These extenders have several functions, including 1) optimizing osmotic pressure and pH, 2) providing an energy source to prevent undesirable use of intracellular sperm phospholipid, 3) preventing bacterial contamination by including an antibiotic and 4) allowing for semen dilution while offsetting the deleterious effect on survival produced by high dilution.<sup>1</sup> Solutions used for sperm preservation have become standardized and commercially available. This has ensured consistency in post-thaw survival. Also, programmable controlled rate freezers, which are constructed to maintain temperature precisely and uniformly throughout the chamber, can be used to freeze samples reproducibly.

The indications for sperm cryobanking have been greatly expanded by recent breakthroughs in assisted reproduction, in which immotile but viable sperm can be used successfully for oocyte fertilization through intracytoplasmic sperm injection (ICSI).<sup>6</sup> This procedure has enabled men who have few sperm in ejaculated semen or even men with only rare sperm retrieved from the testes to be able to fertilize partner oocytes.

#### METHODS

The most commonly reported detrimental effect of cryopreservation on human spermatozoa is a marked decrease in motility.<sup>7</sup> It 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, and understanding the profound protectant effect of this medium has led to the development of numerous protective agents.<sup>8</sup>

Cryoprotectants such as glycerol or propanediol can be added to decrease freezing damage by lowering the salt concentrations and increasing the unfrozen water fraction, thereby reducing osmotic stress.<sup>7</sup> Osmotic stress attributable to differences in the relative permeability of cryoprotectants appears to be an important factor in cryodamage.<sup>9</sup> Glycerol and egg yolk are the accepted cryoprotectants used to maintain cell membrane integrity during cooling and thawing. The percentage of sperm survival increases as higher concentrations of glycerol are used with a constant cooling rate.<sup>10</sup>

Techniques of human sperm cryopreservation are diverse. Semen samples are collected by masturbation after 2 or 3 days of abstinence and liquefied at room temperature. Semen analysis is performed prior to processing for cryopreservation.<sup>11</sup> Test yolk buffer with 20% egg yolk and 12% glycerol is added in a 1:1 ratio to semen as a cryoprotectant at room temperature to prevent hyperosmolar stress.<sup>12</sup> Current techniques commonly use plastic straws or vials. Semen is diluted with egg yolk in citrate or physiological 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 at  $-80^{\circ}\text{C}$ ) for 15 minutes. The samples are then plunged into liquid nitrogen ( $-196^{\circ}\text{C}$ ) and stored until required.<sup>7</sup>

Routinely 1 cryovial is set aside and thawed the next day to assess post-thaw motility without thawing the whole sample. This method indicates how well sperm in the other remaining vials will survive when thawed later. For thawing the cryovial is brought to room temperature or to  $37^{\circ}\text{C}$ . It is further processed for use by diluting it with a suitable buffer (eg human tubal fluid-HEPES) and then centrifuging it to a pellet slowly ( $300 \times$  gravity) to remove the cryoprotectant.<sup>13</sup> When cryopreserved epididymal sperm are to be used for ICSI, the pellet is then resuspended in a small aliquot of medium from which motile or twitching sperm are isolated for the ICSI procedure.

Similar procedures are used for testicular tissue, except tissue must first be macerated and minced prior to cryopreservation.<sup>14</sup> Testicular sperm may be alive but nonmotile at testicular sperm extraction (TESE) and at post-cryopreservation thawing. Artificial stimulants such as pentoxifylline and 2-deoxyadenosine can significantly improve the motion characteristics of thawed cryopreserved sperm.<sup>15</sup> Alternatively hypo-osmotic swelling tests can be used to select live sperm without destroying their viability.<sup>16</sup>

The cooling and warming rates have a profound effect on cell survival. Controlled rate freezing is currently the best technique for determining and achieving optimal cooling and thawing rates for a specific specimen. Human sperm are not only resistant to freezing-thawing, but they also survive various methods of freezing-thawing and storage.<sup>1,17</sup> What is critical is that each cryobank determines the optimal rate of freezing and thawing for its preparation of specimens. Conventional slow-rate freezing requires approximately 1.5 hours.<sup>18</sup> Recently the technique of ultra-rapid freezing of a small number of sperm using cryoloops has been developed, decreasing freezing time to only 5 minutes.<sup>18,19</sup>

After it is frozen, donor sperm is placed in quarantine for at least 6 months. This allows donor screening for HIV and other known sexually transmitted diseases.<sup>20</sup> Sperm donor testing is performed before and 6 months after collection, while holding semen in frozen storage in quarantine for release only after donor re-testing. The banking of husband sperm does not require quarantine unless there is concern about contaminating other samples.

In 1997 a method was developed to enable the cryopreservation of very low numbers of sperm.<sup>21</sup> It 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 to our knowledge its efficacy in humans has not been established.

#### INDICATIONS

*Homologous and donor insemination.* Successful cryobanking of human sperm expands the uses of assisted reproduction to include several applications. Cryopreservation is a widely practiced procedure for storing donor sperm prior to intrauterine insemination (IUI) or in vitro fertilization (IVF).<sup>22</sup> In fact, due to quarantine criteria donor semen must be cryopreserved prior to insemination. IUI with husband sperm is usually performed with a fresh semen specimen. However, cryopreserved semen can be used for multiple timed artificial inseminations of husband/partner or donor semen to coincide better with irregular female cycles or special conditions of the female tract, with the same or a naturally optimal number of normal-appearing, progressively motile spermatozoa. Storage, pooling and concentration of many oligospermic samples from one partner can increase the number of progressively motile cells inseminated at one time. Also, preservation can be done prior to IUI or IVF in the event of inability to produce a specimen or unexpected azoospermia on the day of the procedure.

*Preoperative cryopreservation.* Sperm can be cryopreserved before surgical infertility treatment, such as varicocele ligation in men with severe oligospermia, to protect against possible postoperative azoospermia in the event of testicular artery injury or ligation. Sperm can also be cryopreserved at diagnostic transrectal ultrasound with seminal vesicle aspiration prior to planned transurethral resection of the ejaculatory ducts (TURED). It provides insurance in case azoospermia ensues postoperatively. Although it is not performed as commonly as initially anticipated, sperm can also be cryopreserved preceding vasectomy. This technique provides the opportunity for possible successful assisted reproduction in the future if circumstances change regarding the desire for progeny.

*Intraoperative cryopreservation.* Cryopreservation is performed intraoperatively at the time of primary procedures performed for unreconstructable obstructive azoospermia (OA), such as congenital bilateral absence of the vas deferens (CBAVD). They include microsurgical epididymal sperm aspiration (MESA),<sup>23</sup> percutaneous epididymal sperm aspiration, electroejaculation (EEJ) procedures and TURED. Sperm can also be cryopreserved when found during complex surgical reconstructive procedures, including vasovasostomy (V-V) and vasoepididymostomy (V-E),<sup>24</sup> should the patient remains azoospermic postoperatively.

Cryopreservation of testicular spermatozoa can be performed at diagnostic testis biopsy or TESE in men with severe spermatogenic failure but with focal spermatogenesis or in men with unreconstructable OA. This procedure may obviate the need for repeat 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.<sup>14,25</sup>

*Postoperative cryopreservation.* Postoperative cryopreservation of ejaculated semen can be performed after successful V-E, V-V, TURED and varicolectomy in previously azoospermic men. It can provide insurance against late stricture and re-obstruction after initially having sperm in the ejaculate in the early postoperative period.

*Cryopreservation prior to treatment for malignancies.* Cryopreservation should be discussed and offered prior to therapy for malignant diseases. Cytotoxic chemotherapy, radiotherapy and some kinds of surgical treatment may lead to testicular failure, ejaculatory dysfunction or unreconstructable obstruction. Freezing sperm before the initiation of treatment provides patients with future fertility potential. Cryopreservation before medical treatment for malignant disease is now a common practice since alkylating and other chemotherapeutic agents cause acute azoospermia in 90% to 100% of treated men. Often these patients have few spermatozoa in the ejaculate and relatively few straws/vials can be made. Nevertheless, the vials can be preserved, thawed and refrozen several times.<sup>26</sup> When a specimen is of low volume with few sperm, aliquots can even be scraped from a nearly empty vial and used for IVF/ICSI.

Although improved chemotherapeutic treatment regimens have resulted in a high degree of fertility recovery,<sup>27</sup> the incidence of azoospermia in patients after treatment is still high. In fact, only 20% to 50% of these men eventually recover spermatogenesis.<sup>28</sup> Most patients who survive malignancy discontinue sperm banking because either they regain fertility or have improved semen quality. Since it is difficult to predict which category of patients with cancer will survive or become sterile after treatment, sperm banking is strongly recommended for all patients with malignant disease who may wish to have children, even if they eventually decide that the specimens are not needed.<sup>29</sup>

The collection and cryopreservation of semen is even feasible during chemotherapy, at least until azoospermia ensues,<sup>28</sup> although the effects of chemotherapeutic agents on sperm are not known. It is recommended that patients should complete semen cryopreservation prior to the initiation of chemotherapy, when possible.<sup>30</sup> Moreover, patients are instructed to practice reliable contraception from the time of initiation of treatment until 12 to 18 months after the completion of treatment. This recommendation 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.<sup>30</sup>

*Cryopreservation prior to treatment for nonmalignant diseases.* Cryopreservation of sperm is also recommended for men with nonmalignant, systemic disease or corresponding treatment regimens that may cause infertility.<sup>31</sup> These include men with diseases such as autoimmune disorders, kidney disorders, diabetes, ulcerative colitis and heart trans-

plants. Many of these patients require immunosuppressive or cytotoxic therapy. Prolonged treatment with sulphasalazine has been shown universally to depress semen quality and cause reversible infertility,<sup>32</sup> whereas azathioprine does not decrease semen quality in men treated for inflammatory bowel disease.<sup>33</sup> Cryopreservation is only indicated if the anticipated treatment regimen is known to negatively affect spermatogenesis. Although the pretreatment quality of semen in these men is inferior to that of healthy donors,<sup>31</sup> the presence of any viable sperm in the frozen-thawed specimen is sufficient for IVF/ICSI.

*Premortem and postmortem cryopreservation.* The retention of fertilizing capacity of a partner or donor in his temporary or permanent absence is possible with the use of cryopreservation.<sup>5</sup> Sperm can be cryopreserved prior to entry into military service or anticipation of toxin exposure. Pregnancy with ICSI using sperm from a deceased partner<sup>34</sup> has also been achieved. The postmortem use of frozen sperm to attempt to achieve pregnancy has been available for patients with cancer since the commencement of sperm cryopreservation, but only recently has assisted reproduction technology provided a realistic chance of success for those in need. 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.<sup>35</sup>

A set of guidelines addressing postmortem sperm retrieval was recently developed at our institution.<sup>36</sup> 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.<sup>36</sup>

At the time of cryobanking prior to treatment for malignancy or other medical illnesses, a legal document should be signed that clarifies who is allowed access to the sperm should the husband die of disease. Usually the wife becomes the only responsible party and she 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.

## RESULTS

Cryopreservation has established effects on sperm. Donnelly et al reported that sperm cryopreservation resulted in a 45% decrease in the average velocity of sperm movement.<sup>7</sup> A greater decrease in progressive movement was observed in the sperm of infertile men compared to fertile donors.<sup>7,37</sup> Although artificial stimulants such as pentoxifylline and 2-deoxyadenosine can significantly improve the motion characteristics of thawed cryopreserved sperm,<sup>15</sup> there is a substantial decrease in sperm quality after cryopreservation. Although it has been shown that freezing-thawing is associated with a variable loss of sperm viability and motility, cryopreservation of sperm from men with poor sperm quality does not negatively affect fertilization and pregnancy rates after ICSI,<sup>22</sup> as long as viable sperm are injected.<sup>37</sup> In fact, Kuczynski et al noted no difference in pregnancy rates after ICSI between freshly ejaculated and frozen-thawed groups (23.7% and 35.2%, respectively).<sup>22</sup>

*IUI.* It is well established that IUI with frozen-thawed sperm results in lower pregnancy rates compared with insemination with fresh spermatozoa.<sup>38</sup> In a study of the efficacy of fresh versus cryopreserved semen for the treatment of



male factor infertility by donor insemination, Richter et al found that fresh semen was more than 3 times as likely to induce pregnancy as frozen semen.<sup>39</sup> In any 1 cycle 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 achieving pregnancy per cycle of exposure) was 18.9% with fresh semen. Cryopreserved semen was used in 1,200 cycles and 60 pregnancies occurred for a fecundity rate of 5.0%.<sup>39</sup>

**MESA/ICSI.** In select cases of unreconstructable OA, such as CBAVD, elective open MESA with cryopreservation yields a pregnancy rate identical to that of fresh sperm.<sup>23</sup> Abundant high quality sperm can be cryopreserved at a single procedure for all future attempts at IVF/ICSI.<sup>23</sup> Although only a small number of motile spermatozoa are required for successful fertilization with ICSI,<sup>6</sup> using MESA to obtain abundant, high quality sperm yields superior results. Oates et al also achieved an excellent fertilization rate (37% per oocyte) and pregnancy rate (40% per couple and 29% per cycle) with ICSI using cryopreserved sperm obtained by MESA.<sup>40</sup> This approach eases the burden of partner scheduling on the couple and clinicians involved without compromising the fertilization or pregnancy rate.<sup>40</sup>

Janzen et al reported identical pregnancy rates with IVF and ICSI using freshly aspirated sperm compared with frozen epididymal sperm.<sup>23</sup> With a single MESA procedure a mean total of 82 million sperm were cryopreserved per patient and divided into a mean of 4.7 vials. No statistically significant difference in the oocyte fertilization rate or number of embryos transferred was noted between the groups. Of the 108 patients who provided freshly aspirated sperm 72 (66.7%) achieved clinical pregnancy. Of the 33 patients in the group who provided cryopreserved sperm 20 (60.6%) achieved clinical pregnancy ( $p = 0.47$ ).

Devroey et al evaluated the use of cryopreserved epididymal sperm in 7 patients who did not become pregnant following MESA and ICSI.<sup>41</sup> Subsequently 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 attained. They concluded that sperm cryopreservation during MESA avoids further scrotal surgery 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 nonobstructive azoospermia (NOA) who underwent testicular biopsy for the cryopreservation of tissue to be used in consecutive ICSI treatment cycles.<sup>14</sup> Their female partners underwent controlled ovarian hyperstimulation for conventional IVF treatment. In 77% of the patients sperm 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 sperm could be visualized after the tissue samples were thawed. A total of 135 ICSI treatment cycles were performed with a fertilization rate of 45% and a clinical pregnancy rate of 30% per oocyte retrieval. These clinical pregnancy rates were comparable to pregnancy rates with freshly ejaculated sperm (28%). Similarly, when comparing the results of ICSI cycles with those of fresh or cryopreserved testicular spermatozoa in men with NOA, Friedler et al found no statistically significant differences in the fertilization, embryo cleavage, implantation or clinical pregnancy rate.<sup>42</sup>

Prins et al recommend routine TESE and sperm cryopreservation at the time of diagnostic testicular biopsy.<sup>43</sup> A total of 73 men with obstructive and 42 with NOA underwent testicular sperm extraction at diagnostic biopsy. Sperm was retrieved and cryopreserved in all cases of obstruction and in 15 of the 42 NOA cases (36%). In 17 couples a total of 20

cycles of IVF/ICSI were performed. Fertilization, cleavage and pregnancy rates were determined in cases of obstruction and nonobstruction. Sperm count and morphology were lower in the testicular biopsies of men with NOA vs OA and motility was low or absent in all TESE specimens. Importantly pre-freeze (63%) and post-thaw (31%) viability was the same in the 2 patient groups. After IVF/ICSI using frozen and thawed testicular sperm from men with NOA and OA, the combined fertilization and clinical pregnancy rates were excellent (60% and 50%, respectively). Using cryopreserved sperm no differences in the outcome of any IVF/ICSI procedure in patients with OA vs NOA were observed.

Palermo et al evaluated the results of ICSI using testicular spermatozoa in men with NOA and OA.<sup>44</sup> In nonobstructive cases 57.0% of 533 eggs were fertilized compared with 80.5% of 118 for OA ( $p = 0.0001$ ). The clinical pregnancy rate was 49.1% (26 of 53 cases) for NOA and 57.1% (8 of 14) for testicular spermatozoa obtained in OA cases, including 3 pregnancies established with frozen-thawed testicular spermatozoa.<sup>44</sup> At this point it is still uncertain whether frozen testicular sperm in men with OA survive freezing and fertilize better than sperm from men with NOA. However, the much greater numbers of high quality motile sperm obtained from men with OA always provides a better post-thaw yield of motile sperm from which to choose.

Sperm cryopreservation at the time of TESE in men with NOA is not recommended without synchronous egg retrieval. In a comparison of success rates with microdissection TESE in men with NOA according to testicular histology, 31 of 39 (79%) with hypospermatogenesis on diagnostic biopsy had successful sperm retrieval compared with 9 of 19 (47%) with maturation arrest and 5 of 21 (24%) with a pure Sertoli-cell-only pattern.<sup>45</sup> Given the variable rates of success in sperm retrieval with TESE in cases of NOA, cryopreservation should only be used when a timed TESE/ICSI cycle does not result in pregnancy. Elective TESE procedures should not be scheduled well in advance of egg retrieval since retrieval rates are variable, the sperm may fail to survive a freeze-thaw cycle and the wife's eggs may not be stimulated.

**Intraoperative cryopreservation.** We evaluated 158 consecutive men who had sperm cryopreserved intraoperatively at surgical exploration for OA. A total of 35 pregnancies resulted from sperm cryopreserved intraoperatively, of which 24 involved epididymal aspiration (20 in men with CBAVD) and 10 were achieved by men who underwent unsuccessful difficult reconstruction with persistent postoperative azoospermia.<sup>24</sup> Therefore, intraoperative cryopreservation is offered in complex reconstructive cases with a lower patency rate, including V-E and V-E to the efferent ductule.<sup>24</sup> Although intraoperative cryopreservation at vasectomy reversal may not be cost-effective,<sup>46</sup> it avoids the need for additional invasive procedures. Intraoperative cryopreservation during uncomplicated V-V is neither cost-effective<sup>46</sup> nor necessary due to the high success rate of V-V.<sup>47</sup>

**EEJ.** EEJ has become an accepted form of semen procurement in men with anejaculation due to neurogenic or psychogenic causes. Sperm in the ejaculate of men with spinal cord injury often has low motility and poor quality.<sup>48</sup> In fact, Brackett et al noted that men with spinal cord injury had significantly lower motility (13.7% vs 61.3%) and a significantly lower percent of normal forms (47.2% vs 72.4%) compared with controls, despite a similar total sperm count.<sup>49</sup> EEJ with stepwise application of assisted reproductive technology is effective for treating anejaculatory infertility in these men.<sup>48</sup>

Ohl et al recommended IUI with the least expensive monitoring protocol for most couples after EEJ.<sup>48</sup> However, it is cost-effective to bypass IUI and proceed directly to IVF when men require anesthesia for EEJ or have a total inseminated motile sperm count of less than 4 million.<sup>48</sup> In these men sperm cryopreservation at EEJ can decrease the need for

repeat procedures when motile sperm is found in the thawed ejaculate.<sup>50</sup> In fact, in a study of 25 men with psychogenic anejaculation who underwent EEJ combined with ICSI, Hovav et al found that the fertilization and pregnancy rates with cryopreserved sperm were at least as good as those of freshly obtained sperm.<sup>51</sup>

*Cryopreservation prior to chemotherapy.* At a single academic medical center in Sydney, Australia 930 men sought semen cryostorage prior to undergoing treatments likely to cause infertility.<sup>52</sup> Of these men 833 (90%) had sperm cryostored in a 22-year period. Of the 692 men (74%) who survived the illness sperm samples were discarded for 193 (21% of all applicants and 28% of survivors), and cryostored spermatozoa were used for 64 (7% of all applicants and 9% of survivors) in 85 treatment cycles. Cycles commenced at a median of 36 months after storage (range 12 to 180). Almost 90% of use started within 10 years of storage and none was used after 15 years. A total of 141 men (15%) had died, of whom 120 (85% of those who died) had the sperm discarded. Requests to prolong cryostorage were received from the relatives of 21 men (2% of all applicants and 15% of those deceased), including 3 for whom sperm was transferred for use with no pregnancies reported.<sup>52</sup> These data demonstrate the importance of sperm cryopreservation for a significant proportion of men undergoing chemotherapy.

#### POTENTIAL COMPLICATIONS OF CRYOPRESERVATION

Concern exists regarding the possibility of microbiological cross-contamination of specimens with bacteria or viruses when stored in liquid nitrogen at  $-196^{\circ}\text{C}$ . However, several measures are taken to decrease this potential risk. All donors and many client depositors are screened for sexually transmitted diseases prior to sperm cryopreservation. According to the American Society for Reproductive Medicine guidelines, sperm donor testing is performed for HIV, hepatitis B and C, syphilis, human T-lymphotropic virus, cytomegalovirus, chlamydia and gonorrhea. Samples are held in quarantine tanks for at least 6 months and donors are retested prior to specimen release. In addition, adequate vial sealing is obviously crucial to prevent the entry or egress of microorganisms.<sup>53</sup> Storage in the vapor phase of liquid nitrogen is also an option to decrease the possibility of contamination among specimens. However, it requires close monitoring of storage temperatures to ensure that the minimum  $-130^{\circ}\text{C}$  required for long-term sperm storage is maintained.

Cryopreserved sperm with damaged DNA can result from an underlying disease and corresponding treatments, as well as the cryopreservation process. In fact, genetic damage has been associated with the freezing-refreezing process.<sup>54</sup> Donnelly et al identified a significant decrease of 20% in DNA integrity in cryopreserved spermatozoa.<sup>7</sup> The DNA of sperm from fertile men was found to be unaffected by cryopreservation, whereas sperm from infertile men were significantly damaged by freezing-thawing. In addition, cryopreservation has been shown to have a detrimental effect on the morphology of sperm from fertile and infertile men.

Damage to sperm DNA strongly correlates with mutagenic events.<sup>7,55</sup> However, sperm with damaged genetic material are still capable of fertilization, and defects may not become evident until the embryo has divided or the fetus has developed.<sup>7,56</sup> 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.<sup>7</sup> Therefore, it is crucial to ensure that sperm are frozen in a way that provides maximum protection to DNA to prevent the possible conveyance of damage to offspring.<sup>7</sup>

Single-cell gel electrophoresis, or comet assay, has 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.<sup>57</sup>

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

#### CONCLUSIONS

Sperm cryopreservation has revolutionized the field of assisted reproduction. Methods of cryopreservation have improved so that post-thaw survival of sperm is optimized. The indications for cryopreservation have also expanded greatly with the development of ICSI. Although IUI with frozen-thawed spermatozoa results in a lower pregnancy rate than insemination with fresh spermatozoa, the fertilization and pregnancy rates of ICSI using cryopreserved sperm parallel those of freshly obtained sperm. Cryopreserving sperm avoids the need for additional surgery and optimizes results in couples undergoing repeat IVF/ICSI cycles. Moreover, it provides hope for men undergoing systemic chemotherapy, radiation or radical surgery, who once had no chance for future fertility.

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