Post-thaw recovery of rare or very low concentrations of cryopreserved human sperm

Martin Kathrins, M.D.,[a] Nikita Abhyankar, M.D.,[b] Ohad Shoshany, M.D.,[b] Juergen Liebermann, Ph.D.,[c] Meike Uhler, M.D.,[c] Gail Prins, Ph.D.,[b] and Craig Niederberger, M.D.[b]

[a] Division of Urology, Brigham and Women's Hospital, Boston, Massachusetts; [b] Department of Urology, University of Illinois, Chicago, Illinois; and [c] Fertility Centers of Illinois, Chicago, Illinois

Objective: To analyze cases in which no sperm could be identified after thawing among cryopreserved samples of rare or very low concentrations of sperm.
Design: Retrospective, single-institution, cross-sectional.
Setting: Male infertility clinic.
Patient(s): We identified couples that underwent intracytoplasmic sperm injection (ICSI) with the use of either ejaculated or testicular cryopreserved-thawed sperm. Inclusion criteria were men with <100,000 total ejaculated sperm or men with azoospermia due to spermatogenic dysfunction who underwent microsurgical testicular sperm extraction with similarly low pre-cryopreservation sperm counts. Pre-cryopreservation specimens were categorized as "rare sperm only" (Group 1) or <100,000 total sperm (group 2). "Rare sperm only" applied to cases in which only one to three sperm were identified in a search of >20 high-power fields.
Intervention(s): None.
Main Outcome Measure(s): Cases in which no sperm were able to be found post-thaw (i.e., complete cellular loss) for use at the time of a programmed IVF cycle.
Result(s): We analyzed 55 men (83 ICSI cycles). There were five ICSI cycles (6.0%) among five different couples in which no sperm could be identified post-thaw. Of these, four cases were from group 1 (8.5%) and one from group 2 (2.8%). Complete cellular loss occurred in 5.8% of testicular sperm samples and 7.1% of ejaculated sperm samples. There were no statistical associations between the ability to locate sperm post-thaw and the pre-cryopreservation parameters or sperm source.
Conclusion(s): Failure to retrieve any sperm after thawing of rare or very low concentrations of cryopreserved sperm is an infrequent event and largely limited to those patients with rare quantities of sperm. (Fertil Steril® 2017;107:1300–4. ©2017 by American Society for Reproductive Medicine.)
Key Words: Cryptozoospermia, cryopreservation, intracytoplasmic sperm injection, testicular sperm extraction, azoospermia

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Cryopreserved sperm were first used to achieve pregnancy in the 1950s (1). Since that time, cryopreservation technologies have become integral to the management of male-factor infertility, particularly among men with severe oligozoospermia or azoospermia due to spermatogenic dysfunction (ASD). Indeed, men with severe oligozoospermia may be encouraged to prophylactically cryopreserve ejaculated sperm in anticipation of in vitro fertilization (IVF) owing to the risk of transient azoospermia at the time of ovulation induction (2, 3). Furthermore, men who had previously cryopreserved ejaculated sperm reported positive psychologic effects (3). Therefore, the optimization of sperm cryopreservation techniques is of the utmost importance to the management of the infertile man.

Surgical sperm retrieval rates for men with ASD remain low (4). Therefore, cryopreservation of surgically extracted sperm in this setting represents a path to avoid unnecessary ovulation induction for the female partner should sperm retrieval fail. However, technologic limitations of cryopreservation present special problems for men with very low sperm counts. Conventional

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Reprint requests: Martin Kathrins, M.D., Urology, Brigham and Women's Hospital, 45 Francis St., ASB-II, Boston, MA 02115 (E-mail: mkathrins@bwh.harvard.edu).

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techniques of cryopreservation often represent a significant barrier to men with severe male-factor infertility owing to perceived diminished post-thaw sperm recovery rates (5). To address these concerns, newer techniques involving cryopreservation of very low sperm quantities have been used, including alginic acid capsules, hamster oocycte zona pellucida injection, and single-cell vitrification (6, 7). Yet widespread adoption of these methods has not occurred, perhaps owing to a lack of technical expertise and associated costs.

The most detrimental outcome of traditional cryopreservation of very low sperm numbers is the complete absence of identifiable sperm after thawing, i.e., complete cellular loss. Such a situation in the setting of a programmed IVF cycle would necessitate either the use of back-up donor sperm for fertilization or the cancellation of the cycle, depending on the couple's choice. Herein, we sought to investigate the real-world incidence and predictive factors behind such cases in which no previously cryopreserved sperm could be identified after thawing for ICSI utilization.

MATERIALS AND METHODS

A retrospective analysis was performed of a prospectively maintained single-institution male infertility clinic at the University of Illinois, Chicago. Sperm specimens were prepared and analyzed at two different andrology laboratories—the University of Illinois and the Fertility Centers of Illinois, Chicago. Institutional Review Board approval was obtained at the locations of both andrology laboratories before data accrual. Clinic visits ranged from January 2010 until March 2016. We identified men with ASD who underwent successful microsurgical testicular sperm extraction (micro-TESE) in which the initial testicular sperm yield was $<$100,000 total sperm. We also identified men with ejaculated cryptozoospermia or severe oligozoospermia, defined as rare sperm seen after centrifugation or $<$100,000 total sperm per ejaculate, respectively. All patients identified underwent sperm cryopreservation and subsequent thaw at the time of planned oocyte extraction for IVF. Exclusion criteria included men who had obstructive azoospermia and men who participated in IVF cycles that used fresh testicular or ejaculated sperm.

Processing of semen and testicular tissue and cryopreservation were performed at one of two dedicated andrology laboratories (University of Illinois or Fertility Centers of Illinois/River North IVF Center). All procedures were performed under a laminar flow hood with the use of sterile technique. Micro-TESE was performed under general anesthesia in a manner described previously (8). The tissue was transported off-site from the operating room for processing by the andrology laboratory.

Testicular Tissue Processing

The tissue was transferred into a sterile centrifuge tube containing 3–4 mL human tubal fluid (HTF)–HEPES medium at room temperature and transported to the andrology laboratory for processing. At the University of Illinois, the biopsy specimen was placed in a 35-mm Petri dish with HTF-HEPES supplemented with 0.3% bovine serum albumin at 37°C. Under a dissecting microscope, the seminiferous tubules were gently teased apart with the use of 21-gauge needles and the contents were gently squeezed into the surrounding medium. The tubules were transferred to a 15-mL conical tube containing 1 mL fresh medium, and the cell suspension was transferred to a separate centrifuge tube. Each tube was incubated at 37°C for 15–30 minutes, and the supernate of the tube containing tubules was combined with the cell suspension. The suspension was centrifuged at 500*g* for 5 minutes and the pellet resuspended in 1 mL Ham F-10 with 0.3% bovine serum albumin. The cells were counted and the suspension diluted or concentrated to 0.5–1.0 $\times$ 10^6 sperm/mL. When the count was lower, the cells were suspended in 200 μL medium. At Fertility Centers of Illinois, the biopsy specimen was placed in a 35-mm Petri dish with modified HTF-HEPES diluted with 10% human serum albumin (In-Vitro Care or Sage). Sperm were separated from testicular tissue by means of macerating the tissue between two sterile glass slides. The crushed tissue was suspended in serum-free medium (SFM)/10% sodium polyanethol sulfate (SPS) and checked thoroughly for the presence of sperm cells. If no spermatozoa were observed, the suspension was incubated at 37°C and with a CO_2 concentration of 5%–7% and checked again at intervals. When sperm were observed, the suspension was centrifuged for 10 minutes at 300*g*, the supernate was removed and replaced with 2 mL SFM/10% SPS, and the suspension was centrifuged again for 5 minutes. The supernate was then removed and carefully replaced with 0.10 mL SFM/10% SPS.

Processing of Cryptozoospermic or Severely Oligozoospermic Semen Samples

Both centers used similar protocols. The specimen was allowed to liquefy for up to 30 minutes before processing. When no sperm were found on initial check (with the use of a Mackler counting chamber; Sefi Medical Industries; or Leja counting slide, 20-micron analysis chamber), the specimen was centrifuged at 500g for 10 minutes. The supernate was then removed, leaving 0.1 mL of specimen. A 5-μL sample was assessed (several times if needed), and if any sperm were seen, the cell suspension was slowly diluted 1:1 with TEST-citrate-yolk buffer with 12% glycerol so that the final concentration of glycerol was 6%. Attempts were made to prepare multiple aliquots of sperm when possible.

Cryopreservation

The sperm suspension was cryopreserved as previously described (9). In brief, samples were slow cooled at -0.5°C per minute to 4°C and packaged in 1-mL cryopreservation vials. The vials were frozen at -10°C per minute to -90°C with the use of static nitrogen vapor freeze and then plunged into liquid nitrogen for storage at -196°C. To analyze sperm quality after thawing, a 100-μL aliquot of sperm was separately frozen in each case. After 24 hours in liquid nitrogen, the vials were thawed at 37°C, and sperm count, motility, and viability were assessed within 30 minutes after thawing.