

Cryosurvival of testicular spermatozoa from obstructive azoospermic patients: The Cleveland Clinic Experience

We analyzed the testicular spermatozoa of 23 infertile men after cryopreservation to evaluate their cryosurvival and to scrutinize the effectiveness of freezing protocol used in our institution. An excellent postthaw motility was observed indicating an effective cryosurvival protocol. (*Fertil Steril*® 2006;86:1789–91. ©2006 by American Society for Reproductive Medicine.)

An important approach in management of patients with obstructive azoospermia (OA) is retrieval of sperm for various assisted reproductive technology (ART) procedures through microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA), and testicular sperm extraction (TESE). In general spermatozoa from epididymis are considered more mature than the testicular spermatozoa and provide a higher pregnancy rate (PR) (1). Often it is not possible to retrieve spermatozoa from the epididymis. In some cases there might be a complete absence of epididymis. Testicular sperm extraction is the modality of choice in the management of nonreconstructable obstruction of the excurrent duct system, when epididymal sperm aspiration is not available or unsuccessful.

It is possible to extract a large number of spermatozoa from the epididymis or testicular tissues of patients with azoospermia of obstructive etiology. In fact, pregnancy outcome in OA using these spermatozoa is more than nonobstructive azoospermia (2). Only a small portion of these spermatozoa are needed for IVF/intracytoplasmic sperm injection (ICSI) techniques and the remaining tissue can be divided into several aliquots and cryopreserved for subsequent use. After carrying out a TESE procedure the testicular tissue can be divided into several aliquots and cryopreserved. This would favor multiple ICSI cycles (3), thereby avoiding further surgical biopsies to retrieve spermatozoa in the future. However, the information regarding the cryosurvival of surgically retrieved testicular spermatozoa is not well documented. The purpose of our study was to evaluate the cryosurvival of testicular spermatozoa after the freeze–thaw cycle and to assess the effectiveness of freeze–thaw protocol used in our laboratory.

MATERIALS AND METHODS

Patients

The study was approved by our Institutional Review Board. We included 23 cases of OA who underwent testicular biopsy and subsequent cryopreservation of retrieved tissue at our institution between April 2002 and May 2004. Written consent was obtained from all the patients involved in the study. Semen analyses of normal ejaculate were first performed twice by our laboratory as per World Health Organization guidelines to confirm azoospermia.

All patients presented with primary or secondary infertility after obstruction due to previous surgery, infection, or inflammation. Patients were excluded if they had infections, cryptorchidism, or positive antisperm antibodies in the serum. Patients diagnosed with systemic diseases or, if on chronic medications affecting fertility, were excluded from the study. Couples with female factor infertility, such as endometriosis, tubal blockage, or anovulation, were assessed and excluded. Before surgery, a male infertility specialist (AJT) subjected all patients to a physical examination and workup.

Testicular Sperm Extraction

Testicular sperm extraction was performed surgically under general anesthesia. Testes were exposed through a single midline median raphe incision. To accurately identify the testis and to avoid any injury to the epididymis the entire testis and epididymis were delivered completely and tunica albuginea was examined at a magnification of $\times 8$ – $\times 15$.

An avascular region was chosen for incision of the tunica albuginea to avoid injury to testicular vessels. Different sites were chosen for harvesting testicular tissue.

The extracted contents were scanned for the presence of spermatozoa. If no spermatozoa were observed, a contralateral biopsy was performed. The testicular biopsy specimen was immediately placed in the sterile human tubal fluid (HTF) medium after collection.

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Tissue Freezing

Tissue samples were cryopreserved in properly labeled 1.0-mL cryovials containing 0.5 mL of protein-free HTF medium (4). Seminiferous tubules were frozen as whole sections. Of spermatozoa freezing medium 0.5 mL was added dropwise to the cryovial. The samples were allowed to equilibrate for 30 minutes at room temperature. The cryovials were then placed at -20°C in a standard freezer for 30 minutes and then stored into liquid nitrogen at -196°C .

Thawing

The cryovials containing the sperm samples were removed from the liquid nitrogen tank and allowed to thaw at room temperature for 5 minutes. The samples were incubated for 20 minutes at 37°C in a 5% carbon dioxide incubator. After thawing, a $5\text{-}\mu\text{L}$ aliquot was subjected to semen analysis by both manual and computer-aided sperm analysis (CASA) methods. Sperm concentration, motility, and motion kinetics were evaluated and recorded.

Statistical Analysis

Data were analyzed using the unpaired *t*-test. A *P* value of less than .05 is considered significant. The InStat (Graphpad software Inc., San Diego, CA) statistical software was used for statistical analysis.

RESULTS

The obstructive etiology of azoospermia was confirmed by the normal histology of tissue samples. There was a good postthaw recovery of spermatozoa in all 23 patients with OA who underwent testicular biopsy followed by cryopreservation at our center. The mean (\pm SD) postthaw percent motility was 15.65% (\pm 9.81%) as compared to

mean (\pm SD) prethaw motility of 20.13% (\pm 7.28%) showing about 25% reduction in motility (Table 1).

The two-tailed *P* value was not significant (.0857). The samples showed motility ranging from 3%–33% after the freeze–thaw process. Of these 23 patients only one had rare motile spermatozoa observed in his postthaw specimen. The etiology of OA (ejaculatory duct obstruction, congenital anomalies, surgical complications, and others) or the previous fertility status had no influence on motility outcomes in these patients.

DISCUSSION

Obstructive azoospermia is caused by several different etiologic processes. Ejaculatory duct obstruction, vasectomy, postinfection obstruction, and congenital bilateral absence of the vas deferens are some of the major causes of obstruction. Irrespective of the causes of obstruction our study did not find any significant difference in the postthaw motility based on etiology. Because the obstruction is mainly posttesticular in nature its effect on testicular spermatozoa is usually negligible. Similarly, the previous fertility status of the man had no correlation with the postthaw motility.

We are in the era where the treatment of infertility has taken a different dimension and reproductive endocrinologists look at minimal invasive procedures like testicular biopsy or aspiration, or MESA (5–7). Testicular sperm was used in patients with a nonreconstructable obstruction of the excurrent duct system, where epididymal sperm aspiration was unsuccessful. Different ART (IVF/ICSI) can be combined to use sperm extracted by TESE to fertilize ova of their partners and achieve pregnancy (6–8).

In case of failed pregnancy, cryo-TESE becomes a preferable option, as the sperm can be used for multiple ART cycles until pregnancy is established. This would avoid repetitive sperm retrieval procedures, thereby preserving testicular architecture and avoiding complications associated with frequent biopsies.

It has been reported in a study that cryopreserved testicular spermatozoa could yield a fertilization rate of 64%, and a PR of 35% (9). Other investigators have found that there was no difference in fertilization rates, embryo cleavage, or pregnancy when fresh or frozen–thawed testicular sperm was used from azoospermic patients. Moreover in patients with OA and non-OA there were no differences in the fertilization rate and PR by ICSI when fresh spermatozoa and frozen–thawed spermatozoa were used (10).

Cryopreservation mainly affects motility in comparison to other semen parameters (11). Percent motility can prove to be a reliable predictor of the outcome of pregnancy and IVF (12). In fact some studies have shown that sperm motility and survival is better with cryopreserved testicular tissue than fresh samples (13).

TABLE 1

Pre- and postcryopreservation motility data in cryo-TESE samples.

	Before cryopreservation	After cryopreservation
No of samples	23	23
Mean % motility	20.13	15.65 ^a
SD	7.28	9.81
Median	20.00	13.00
95% CI	16.98–23.28	11.41–19.89

^a *P* = .0857 was not significant when pre- and post-thaw motility were compared.

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According to the results of our study the mean prefreeze percentage motility and postthaw percentage motility did not differ significantly ($P = .0857$). Some of the studies done on semen samples reported reduced postthaw motility. O'Connell et al. (14) have demonstrated prefreeze and postthaw motility of 40.2% and 24.8% respectively, showing almost a 50% reduction postthaw ($P = .001$). Similarly, Keel et al. (15) reported a postthaw motility reduction of 37% from a prefreeze value of 64% ($P = .01$). Our freezing protocol, on the other hand, has given a good postthaw sperm recovery with only about 25% reduction in motility.

It is believed that the spermatozoa from the epididymis are more mature than the testicular spermatozoa. However, Griffiths et al. (16) have shown that in men with OA, cryopreserved testicular spermatozoa has ICSI outcomes comparable with epididymal spermatozoa. Although our study measured the percent motility in the same sample before and after the freeze-thaw process and not the pregnancy outcome, many other studies reinforce our finding that cryopreserved testicular spermatozoa are as efficacious as fresh spermatozoa with regard to their motility and fertilizing ability and therefore can be used successfully in ART (10, 16).

In conclusion, our study results are in conformity with other comparable studies that reported similar results on the preservation of postthaw motility. It also reiterates that testicular spermatozoa from patients with OA can achieve good cryosurvival allowing their use in future ART procedures. Our results emphasize the fact that cryopreservation is an effective tool in this modern era of ART. It would help to avoid repeated TESE, which are potentially damaging to the testicular architecture and also result in exorbitant costs associated with such surgical procedures. Our results further demonstrate that cryo-TESE is a useful procedure for the patients with nonreconstructable obstruction of the excurrent duct system (17).

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