Technical modification of testicular sperm extraction expedites testicular sperm retrieval

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Objective: To determine the predictive value and the quality of supernatant sperm (SS) achieved by a simple laboratory technical modification after testicular sperm extraction (TESE).
Design: A retrospective analysis.
Setting: An IVF unit in a university medical center.
Patient(s): Azoospermic patients undergoing TESE between January 2001 and December 2006.
Intervention(s): Before the mechanical shredding, the testicular specimen in toto was placed in medium. The medium was spun and the pellet resuspended and transferred for SS detection. Then a wet preparation of the testicular tissue was shredded roughly and inspected for tissue sperm (TS) as described.
Main Outcome Measure(s): Detection of SS versus TS, fertilization and pregnancy rates (PR) after intracytoplasmic sperm injection (ICSI) with SS versus TS.
Result(s): The SS was detected in all specimens where TS was eventually found, independent of their testicular pathology. When the supernatant was spermatozoa-negative, no spermatozoa were detected in the tissue. For embryos derived from ICSI the fertilization rate of SS was significantly higher than TS (52% vs. 44%), whereas the PR was comparable.
Conclusion(s): The SS serves as an excellent predictor of TESE outcome and as a superior source for fertilization. This modified technique enables faster decision of TESE outcome and an easier switch to donor sperm when available. (Fertil Steril® 2009;91:281–4. ©2009 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, technique, testicular sperm extraction, sperm retrieval

Since the introduction of testicular sperm extraction (TESE) in 1993 (1, 2), intracytoplasmic sperm injection (ICSI) with testicular sperm has become a routine procedure for patients with azoospermia, both obstructive azoospermia with normal spermatogenesis and nonobstructive azoospermia with testicular failure. In obstructive azoospermia, sperm can be retrieved in almost 100% of the cases (3), whereas in patients with nonobstructive azoospermia, the possibility of finding sperm is only about 50% (3, 4). Since the first report of testicular biopsy as a method to detect testicular sperm (5) direct inspection of spermatozoa inside the seminiferous tubules has been the only way to obtain spermatozoa for ICSI. In both obstructive azoospermia and nonobstructive azoospermia the laboratory handling of the testicular tissue after TESE includes tedious shredding and further mincing to disrupt the seminiferous tubules (6). The laboratory work process is usually time consuming, laborious, and stressful to both the patient and the personnel. Here we present a technical modification of the laboratory work process, whereby the medium in which the testicular tissue is initially suspended is analyzed for supernatant sperm independently from the medium in which the tissue is manually shredded to obtain tissue sperm. We sought to investigate the predictive value of supernatant sperm to TESE outcome. We hypothesize that this modification could expedite sperm detection.

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MATERIALS AND METHODS

Study Design

This was a retrospective analysis of a technical modification of testicular tissue handling after TESE at an embryology laboratory of a university-based IVF unit.

Population

All patients were diagnosed as azoospermic on the basis of at least two semen analyses, including a centrifugation step at high speed. They all had a clinical workup including a physical examination, hormonal assessment (FSH, LH,

TABLE 1

The causes of azoospermia.

Causes	Sperm-positive biopsies (n = 78)	Sperm-negative biopsies (n = 68)	*P* value
Cryptorchidism	10 (13%)	7 (10%)	NS
After chemotherapy or radiotherapy	0	2 (3%)	NS
Varicocele	6 (8%)	6 (9%)	NS
After inguinal hernioplasy	4 (5%)	4 (6%)	NS
Hypogonadotrophic hypogonadism	2 (2.5%)	1 (1.5%)	NS
Genetic defect	0	7 (10%)	.02
Aspermia	1 (1.25%)	0	NS
Unexplained	55 (70%)	41 (60%)	NS

Note: P value insignificant at >.05.
NS = not significant.

Haimov-Kochman. Laboratory modification for TESE. Fertil Steril 2009.

and T), testicular ultrasound, and karyotype analysis. A total of 146 azoospermic men had a sperm recovery trial by TESE between January 1, 2001 and December 31, 2006. Repeated TESE for sperm collection after a successful procedure was carried out in six patients. The patients were diagnosed as nonobstructive azoospermic based on histopathology of maturation arrest with or without focal spermatogenesis, germ cell aplasia (Sertoli cell-only syndrome) with or without focal spermatogenesis, and tubular sclerosis/atrophy (7). In the case of a mixed histologic pathology the most prominent pattern was used for classification. Azoospermic patients with histology of normal spermatogenesis were classified as having obstructive azoospermia. Hypospermatogenesis indicates a state of complete but reduced spermatogenic activity and was considered a separate population.

Intervention

Open excisional testicular biopsies were taken under general anesthesia during a preliminary surgery with a view to cryopreservation. An incision of approximately 1 cm was made through the skin and underlying layers. After performing a small (4 mm) incision in the tunica albuginea, gentle pressure was applied to the testicular mass, and a small specimen of the protruding testicular mass was removed using a pair of curved scissors. The testicular tissue was placed in a Falcon dish (Fal351007; Becton Dickinson, San Jose, CA) containing HEPES medium (IR-90126; Irvine Scientific, Santa Ana, CA) with 4% synthetic serum supplement (SSS) (IR-99193; Irvine Scientific) (first media), and handed to the adjacent laboratory. During surgery, a single randomly taken biopsy of each testis was sent for histologic examination. For histopathology, the specimens were fixed in 10% buffered formalin and stained with hematoxylin and eosin (H & E). Given the possible focality of histologic patterns, this approach might introduce misclassification in some patients. The incisions in the tunica albuginea and the overlaying skin were sutured with VICRYL 4-0 (Ethicon; Johnson & Johnson, Dilbeek, Belgium).

Technical Modification

In the laboratory, after 10 minutes, the specimens were transferred into new Falcon dishes containing 5 mL of HEPES medium with 4% SSS (later media) and the first media were decanted into new tubes. Tubes were spun for 5 minutes at 1,800 × *g* (Eppendorf centrifuge 5702; Wesseling-Berzdorf, Germany) at room temperature, and the pellet was resuspended in 20 μL of HEPES medium. Plates with 5 μL of HEPES medium drops were prepared and covered with oil (IR9305; Irvine Scientific). A volume of 1–5 μL of the suspension, was transferred into the oil-covered drops and incubated for at least 30 minutes. Supernatant spermatozoa were counted in the drops using an inverted microscope (magnification, ×200) (Nikon Diaphot 200, Tokyo, Japan). Then a wet preparation of testicular tissue was shredded roughly in the Petri dish, using two tuberculin needles, on the warmed stage of a stereomicroscope at ×40 magnification. During this procedure, the seminiferous tubules were unraveled and broken. The tissue was then further minced until tissue pieces were approximately 1 mm^3, or free tubuli pieces of a few millimeters in length were obtained. Then the suspension was spun as previously. The supernatant was discarded and the pellet resuspended in minimal volume and a sample of 1–5 μL was transferred into oil-covered drops and examined under an inverted microscope (magnification, ×200) for the presence of tissue spermatozoa. A successful biopsy was defined as the finding of at least one spermatozoon in the biopsy specimen. The testicular cell suspension, as well as the supernatant sperm suspension, was frozen (Sperm Freezing Medium; IR-90128; Irvine Scientific) for later use if either supernatant sperm or tissue sperm was observed, or saved for histopathologic confirmation if no spermatozoa were found.

Statistical Analysis

We used χ^2 to compare categorical variables and *t*-test for comparison of means of fertilization and pregnancy rates (PR) of cycles where fertilization was done with supernatant sperm versus tissue sperm.