Development of a New and Efficient Laboratory Method for Processing Testicular Sperm¹

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Purpose: Testicular biopsy specimens contain large amounts of debris that makes sperm pick-up for ICSI more difficult than with epididymal aspirates. We sought to develop improved processing techniques for testicular sperm extraction (TESE).

Methods: Retrievals were with azoospermic male partner scheduled to undergo percutaneous epididymal sperm aspiration (PESA) and TESE. The study group consisted of 9 retrievals with a new TESE technique (TESE-N). The control group was 21 retrievals with PESA and 3 retrievals with a previous TESE technique (TESE-P).

Results: TESE-N eliminated almost all debris, which made ICSI sperm pick-up more rapid. TESE-N, PESA, and TESE-P fertilization (77, 75, and 72%) and ongoing/delivered pregnancy rates per retrieval (67, 76, and 67%) were similar.

Conclusions: Our new technique provides for easy removal of debris from TESE specimens and fertilization and pregnancy rates equal to epididymal sperm's. Eliminating debris from TESE specimens allows for rapid sperm pick-up for ICSI, making the procedure more efficient for embryology staff.

KEY WORDS: Assisted reproductive technologies; IVF; male-factor infertility; testicular biopsy; testicular sperm extraction.

INTRODUCTION

Testicular and epididymal sperm have been used successfully for treating men with azoospermia (1–10). For males with normal spermatogenesis, testicular sperm extraction (TESE) requires less surgical expertise to obtain good quality specimens as compared with microepididymal sperm aspiration (MESA) (4,7,9). Hence, TESE specimens can be provided by several staff urologists. This allows for

much greater flexibility in scheduling sperm harvest procedures in conjunction with the female partner's oocyte retrieval. Additionally, for males with normal spermatogenesis, TESE can be performed under local anesthesia without an operating microscope and generally is associated with less postoperative discomfort and a shorter recovery time than MESA (4,7,9).

Despite these advantages, TESE has been used less frequently than MESA in the treatment of obstructive azoospermia because fertilization and ongoing pregnancy rates have tended to be higher in a few studies with epididymal sperm (7). The reasons for this difference are unknown, but it may be partially due to the methods used in past studies for preparing testicular sperm for ICSI. When working with testicular tissue it is much more difficult to isolate a clean preparation of motile sperm because of the presence of numerous cell types in

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testicular tissue (3,6). The routine method of testicular tissue processing, i.e. pellet centrifugation, results in high concentrations of cell contaminants in the final specimen for ICSI. Because of this, considerably more time is required for isolating motile sperm for ICSI when working with testicular specimens compared with epididymal specimens, where the only significant cell contaminant is the red blood cell (3,6).

When using testicular sperm processed in the standard fashion, i.e. by pellet centrifugation, sperm selected for ICSI must incubate in PVP for longer intervals than what is typically required for epididymal or ejaculated sperm because of the greater difficulty isolating motile testicular sperm from the surrounding extracellular contaminants (3,6). Incubation of sperm in PVP was recently found to cause submicroscopic alterations in sperm (11,12). PVP has a detrimental action on acrosomal, mitochondrial, and plasma membranes of sperm and induces deterioration of the chromatin, axonemal tubules, fibrous sheath, and accessory fibers (11,12). Prolonged incubation in PVP could cause extensive damage to sperm and lead to decreased fertilization and live-birth rates. Therefore, it is important that sperm be incubated in PVP for the shortest possible interval before injection into the oocyte. If testicular sperm are to be considered an equal option to epididymal sperm, then clearly improved processing procedures are needed to permit sperm pick-up with the same relative speed as with epididymal specimens.

Reactive oxygen species (ROS) are highly damaging to human sperm, causing disruption in motility, oocyte membrane fusion, and chromatin damage (13). Levels of ROS may be high in testicular tissue because of the extensive dissection of tissue to release sperm from the seminiferous tubules and the high concentrations of extracellular material in the emulsified specimens. Density gradient centrifugation (DGC) has been used successfully with ejaculated sperm to reduce sperm exposure to ROS (14). To our knowledge, the usefulness of DGC in processing testicular sperm suspensions has not been formally studied in patients undergoing IVF.

The purpose of this study was to develop a method of processing testicular tissue that would provide a clean preparation of motile sperm relatively free of extracellular debris. Fertilization and pregnancy rates obtained with this new method (TESE-N) were compared with those obtained from PESA and a previous TESE technique (TESE-P).

MATERIALS AND METHODS

Patients with azoospermia who underwent IVF in our program from November 1997 through August 2001 were included in this study. The TESE-N protocol was used for patients treated from January 2000 through August 2001.

Patient stimulation protocols, oocyte retrieval, ICSI, pronuclear zygote freezing, and laboratory culture conditions were performed as previously described (15,16). When the female partner age was less than 39, only the number of pronuclear zygotes required for embryo transfer, or one extra zygote, were held in culture for transfer on Day 2 or Day 3. All other zygotes were cryopreserved at the pronuclear stage (16). For women 39 years and over, all pronuclear zygotes were retained in culture and the best three or four embryos were selected and these underwent assisted hatching 1–2 h prior to embryo transfer. Extra nontransferred embryos were cultured for 2 additional days and cryopreserved if they developed into normal morphology blastocysts.

Prior to the sperm aspiration procedures, all but three patients were diagnosed as having obstructive azoospermia. One patient with nonobstructive azoospermia had anejaculation subsequent to retroperitoneal lymph node dissection and one had complete retrograde ejaculation subsequent to bilateral ureteral implants. Both were offered electroejaculation and bladder-washing treatments, but opted to undergo PESA and TESE instead. The third case was azoospermic post removal of one testicle following cancer with a normal testicular biopsy on the remaining testicle.

Patients were scheduled for PESA and TESE using local anesthesia and IV sedation (Propofol, Versed, Fentanyl) in an outpatient setting. Percutaneous Epididymal Sperm Aspiration was attempted in all patients and was performed by one of four staff urologist with a butterfly catheter as previously described (4,7). The catheter was passed to the laboratory where it was rinsed with Sperm Wash Media (SWM; Irvine Scientific, Santa Ana, CA) and evaluated for the presence of sperm. Sperm were left in 100- μ L drops until ICSI or washed with SWM and placed in a 45- μ L elongated drop for sperm swim-out. Sperm were picked up from the edge of the swim-out drop and rinsed twice through polyvinylpyrrolidone (PVP; Irvine Scientific, Santa Ana, CA) prior to injection. Sperm pick-up was initiated 20–60 min prior to injection of the first oocyte. Longer periods were allowed for sperm pick-up for greater numbers of oocytes since more

sperm had to be harvested with increasing numbers of oocytes.

A small testicular biopsy was taken directly following PESA for the purpose of sperm cryopreservation for use in future IVF cycles. TESE was done using an open excisional technique (7). The open excisional technique allows samples to be taken from different sites while maintaining full control of bleeding and minimizes the risk of testicular hematoma (17).

If no motile sperm, or insufficient motile sperm for ICSI, were obtained from the PESA specimen, a small piece of testicular tissue (approximately 3-mm square ranging from 50 to 100 mg) was processed for ICSI. A technician in the IVF laboratory, directly adjacent to the procedure room, performed a quick dissection of a few seminiferous tubules from the TESE specimen and provided immediate feedback to the urologist as to whether sperm were found. A few slightly twitching sperm (1–2 per 350X field) were usually seen immediately upon evaluation. No further biopsies were taken after the lab confirmed the presence of sperm in the TESE sample.

The testicular tissue was processed by dissection with 27-gauge needles in 2-mL SWM. For the previously used TESE processing protocol (TESE-P), the dissected testicular tissue was centrifuged twice at 300g for 10 min. A small quantity (5–10 μ L) of the pelleted sample was placed in the center of a 45- μ L elongated drop for sperm swim-out. Pentoxifylline (0.1% v/v; Sigma, St. Louis, MO) was used as a motility stimulant if there were insufficient motile sperm found for ICSI. Sperm pick-up was initiated 90–120 min prior to injection of the first oocyte. A longer time was required for sperm pick-up with TESE-P because of the large amount of debris in the specimens and the fact that few, if any, sperm were able to swim to the edge of the microdrop because they were trapped and coated within extracellular debris. More extensive washing of the sperm was also required in the PVP to remove debris bound to the sperm surface.

For the new TESE processing protocol (TESE-N), the dissected tissue was transferred to a tube and allowed to incubate for 30 min at 37°C. The tissue was pipetted several times with a 5-in. prewashed glass pipet to emulsify the specimen and allow for release of sperm from the cut seminiferous tubules. After pipetting, the large tissue pieces were allowed to settle at the bottom of the tube. As soon as the large pieces sedimented at the bottom (10–30 s), the supernatant solution was removed. The tissue fragments in the bottom of the tube were discarded. Removal of large tissue fragments was important to enhance motile sperm

recovery. If these fragments were not removed, they accumulated at the Isolate–sperm suspension interface and created a barrier that sperm could not pass through.

The supernatant solutions were divided into two tubes. Pentoxifylline was added to one of the two tubes and both tubes were then incubated at 37°C for 10 min. Following this interval, the testicular suspensions were again pipetted and 1.0 mL of testicular sperm suspension was layered on 1.5 mL of 90% Isolate (Irvine Scientific, Santa Ana, CA). After centrifuging for 30 min at 1000g, the Isolate supernatant was removed down to 0.5 mL. This supernatant solution was discarded. The bottom 0.5 mL of Isolate–sperm suspension was washed twice with SWM at 400g for 10 min. The pentoxifylline tube was washed with 0.1% pentoxifylline in SWM. The other tube was washed with pure SWM. Two swim-out drops were prepared in the ICSI dish. One swim-out drop was prepared with SWM with 0.1% pentoxifylline and the other with SWM only. A small quantity (2–5 μ L) of washed sperm was placed into each of the two swim-out drops. The pentoxifylline washed sperm were placed into the swim-out drop prepared with pentoxifylline, and the nonpentoxifylline sperm into the drop without pentoxifylline. Sperm were incubated 1–2 h in the swim-out drops at 37°C under a humidity chamber before initiating sperm pick-up for ICSI. The drop with the best motility and least amount of red blood cells (RBC) was used for sperm pick-up for ICSI. Sperm were picked up from the edge of the swim-out drop and rinsed twice through PVP prior to injection. Sperm pick-up was initiated 20–60 min prior to injection of the first oocyte. With TESE-N approximately two or more sperm were harvested from the swim-out drop for each oocyte to be injected. This allowed for further final selection of sperm in the PVP drop based on sperm morphological features.

It was not feasible to objectively quantify sperm concentration, motility, or morphology in the TESE specimens because of the extremely low sperm concentrations in these types of specimens. Even with the TESE-N processing method, which greatly eliminates debris and increases the ability of sperm to swim to the edge of the drop, it is not usually possible to objectively count the motile sperm using a sperm-counting chamber. For this reason, a comparison of these sperm parameters among TESE-N, PESA, and TESE-P was not performed in this study.

Statistical significance was assessed using the chi-square test for fertilization rate, Fisher Exact test for ongoing pregnancies per retrieval, and ANOVA for

Table I. Fertilization and Pregnancy Outcomes for Patients Receiving TESE-N, PESA, and TESE-P

Sperm type	Number of oocyte retrieval cycles	2PN fertilization rate	Fresh clinical pregnancies per transfer	Frozen clinical pregnancies per thaw cycle	Ongoing or delivered pregnancies per retrieval
TESE-N	9	95/124 (77%)	6/8	2/4	6/9 (67%)
PESA	21	217/288 (75%)	14/19	5/10	16/21 (76%)
TESE-P	3	18/25 (72%)	1/3	1/2	2/3 (67%)

embryological data. The margins of error for the comparisons were obtained by calculating the 95% confidence intervals for the differences between group proportions.

RESULTS

Sufficient sperm were harvested from the PESA procedure for injection of all oocytes with epididymal sperm in 64% (21/33) of the patients in this study. Twelve patients had no, or insufficient, motile sperm for ICSI harvested with PESA. The TESE specimen was used in these 12 cases for ICSI (nine TESE-N and three TESE-P). Patient oocytes were injected with either PESA or TESE sperm, but never a combination of the two types in one retrieval cycle.

The main outcome measures of interest in this study were fertilization and ongoing pregnancy rates per retrieval. These results are shown in Table I. Fertilization rates (77, 75, and 72% for TESE-N, PESA, and TESE-P, respectively) and delivered or ongoing pregnancy rate per retrieval (pregnancies from the transfer of fresh and frozen embryos from one oocyte retrieval cycle) were similar among the three groups (67, 76, and 67% for TESE-N, PESA, and TESE-P, respectively). One patient in the TESE-N group and two patients in the PESA group had all their pronuclear zygotes cryopreserved because they were at risk for ovarian hyperstimulation syndrome.

Male infertility diagnosis is shown in Table II. Female partner demographic data and embryological data for the three groups are shown in Table III. There were no significant differences found among the aver-

age ages of the female partners or embryological data for patients receiving TESE-N, PESA, and TESE-P.

Two cases of mild epididymitis occurred following the sperm aspiration procedures. Both resolved with antibiotic treatment. There were no other postoperative complications.

DISCUSSION

The goals of sperm retrieval have been described as 1) obtaining the best quality sperm possible; 2) retrieving an adequate number of sperm for both immediate use and cryopreservation; and 3) minimizing damage to the reproductive tract so as not to jeopardize future attempts at sperm retrieval (4,7). We believe the approach described herein, PESA followed by TESE, fulfills all of these requirements, plus allows for flexibility in scheduling sperm harvest procedures.

The use of testicular sperm may have several advantages over epididymal sperm in treating men with azoospermia in association with IVF. Microepididymal sperm aspiration (MESA) requires subspecialty microsurgical training and therefore, in most practices, can only be performed by one of several staff urologists. The availability of only one urologist to cover all sperm harvest procedures for an IVF unit causes scheduling dilemmas since sperm harvest needs to occur on the same morning as the female partner's oocyte retrieval. The use of frozen epididymal sperm has been considered as an option by some programs. However, since up to 10–20% of patients' epididymal samples may not survive cryopreservation (7) this option has limitations since backup sperm harvest must still be available on the day of oocyte retrieval.

In our program, PESA has been used preferentially over MESA because it can be performed under local or IV sedation without the need for surgical scrotal exploration, can be repeated easily at a low cost, results in minimal postoperative discomfort, and does not require an operating microscope (4,7,9). Although PESA does not require expertise in microsurgery (4,7), we have found that it does require more

Table II. Male Partner Diagnosis

	TESE-N	PESA	TESE-P
Failed vasectomy reversal	2 (22%)	4 (19%)	1 (33%)
Vasectomy	2 (22%)	9 (43%)	1 (33%)
CBAVD ^a	3 (33%)	6 (29%)	0 (0%)
Nonobstructive with normal spermatogenesis	2 (22%)	2 (9.5%)	1 (33%)

^a Congenital bilateral absence of the vas deferens.

Table III. Female Partner and Embryological Data for Fresh and Frozen Embryo Transfer Cycles

	TESE-N	PESA	TESE-P
Age (years)	33.7 ± 4.55	34.1 ± 3.91	33.0 ± 4.12
Primary infertility (%)	9 (100)	10 (48)	1 (33)
Secondary infertility (%)	0 (0)	11 (52)	2 (67)
Fresh embryo transfers, <i>n</i>	8	19	3
E ₂ day of hCG, pg/mL	1596 ± 544	2047 ± 1143	1761 ± 1047
Number of oocytes retrieved	16.6 ± 8.55	17.5 ± 11.60	11.7 ± 9.87
Number of oocytes inseminated	13.8 ± 8.63	13.7 ± 9.66	8.3 ± 6.81
Number of 2PN zygotes	10.6 ± 5.68	10.3 ± 7.96	6.0 ± 5.00
Average number of embryos transferred	2.75 ± 0.69	2.74 ± 0.99	2.33 ± 1.15
Implantation rate (%)	7/22 (32)	23/52 (44)	1/7 (14)
Frozen embryo transfers, <i>n</i>	4	10	2
Average number of embryos transferred	2.0 ± 0.8	2.9 ± 1.1	2.5 ± 0.7
Implantation rate (%)	2/8 (25)	9/29 (31)	2/5 (40)
Implantation rate for fresh and frozen embryos (%)	9/30 (30)	32/81 (39)	3/12 (25)

training to successfully obtain good quality sperm in a high percentage of cases as compared with TESE. In contrast to MESA and PESA, TESE is a procedure routinely performed by most urologists. The use of PESA and TESE in combination provides the option to use epididymal sperm in a high percentage of cases (64% in this study), while allowing for backup testicular sperm for the oocyte retrieval cycle and for cryopreservation in future cycles. Most important, this approach results in high rates of fertilization and pregnancy, as demonstrated in this study.

Despite the advantages of using testicular sperm, epididymal sperm is still preferred by some IVF programs because fertilization and live-birth rates have tended to be somewhat higher with epididymal sperm in a few earlier reports (7). In these studies, diminished results with testicular sperm may have been caused by inadequate methods of sperm processing prior to ICSI.

Testicular sperm are usually immotile or only show twitching movements upon extraction (2,3,17,18). Some centers have used immotile testicular sperm for ICSI, assuming that a high percentage of such sperm were viable. Injection of immotile ejaculated sperm resulted in decreased fertilization and pregnancy rates (19). Injection of immotile testicular sperm may have partially contributed to the lower rates of fertilization and pregnancy in some earlier studies. In more recent studies, attempts have been made to induce motility in extracted testicular sperm via incubation in culture media, by adding motility stimulating agents such as pentoxifylline or by incubating in culture media with recombinant FSH (5,20). In this study, our new method of processing testicular tissue (TESE-N) allowed for all oocytes to be injected with motile sperm.

Density gradient centrifugation has been suggested as being pointless to attempt with testicular sperm from men with obstructive azoospermia because of the small number and weak motility of testicular sperm (10). However, we found plenty of highly motile sperm using DGC with testicular tissue and believe it to be an important processing step for TESE specimens to obtain optimal fertilization and live-birth rates. Density gradient centrifugation has been reported to improve samples by enriching the percentage of morphologically normal forms by 138% and sperm nuclear integrity by 450% (21), and to decrease the percentage of immature forms (22,23).

The highest percentage of mature, morphologically normal sperm are found in the pellet fraction (90% Isolate layer) following DGC (22). Sperm aneuploidy (23), DNA damage, and ROS production (22) have been shown to increase with increasing rates of abnormal and immature forms. Increased sperm DNA damage, ROS levels, and aneuploidy appear to be associated with a lower chance of delivery with IVF (13,22,24,25). Since DNA damage, sperm aneuploidy and ROS production are highest in abnormal/immature forms (22,23), the reduction of sperm with these features in the final sample to be used for ICSI with DGC may increase fertilization and live-birth rates.

Testicular biopsy samples contain large amounts of extracellular contaminants. The removal of this extracellular material with DGC may serve several important functions in preparing a high quality specimen for ICSI. Reactive oxygen species are likely to be elevated in testicular samples as a result of the aggressive dissection and mincing of tissue required to release sperm from the seminiferous tubules.

Additionally, ROS appear to be released at higher levels from immature sperm (22). The detrimental effects of ROS on human sperm motility, oocyte membrane fusion, and DNA integrity have been well described (13,14,22). Sources of ROS in sperm samples include white blood cells, dead or abnormal sperm, immature sperm, and transition metals in culture media. Levels of ROS were shown to be 5 times higher following pellet centrifugation as compared with DGC of ejaculated sperm (13,14). The removal of extracellular material and immature sperm with DGC may decrease levels of ROS and better preserve the function and motility of viable testicular sperm for ICSI.

Density gradient centrifugation eliminated most of the nonsperm cells from the emulsified testicular tissue. This made sperm pick-up from the swim-out drop quicker. With TESE-P few, if any, motile sperm accumulated at the edge of the swim-out drop for easy pick-up because of the heavy contamination with extracellular debris. Motile sperm often had to be retrieved from the center of the drop and then washed extensively in PVP to remove debris that bound to the surface of the sperm. In contrast, with TESE-N many clean motile sperm accumulated at the edge of the swim-out drop, making sperm pick-up more rapid than with TESE-P. The time required for sperm pick-up with TESE-N was approximately half that required for TESE-P.

The reduced sperm pick-up time for TESE-N permitted sperm to be incubated for shorter periods in PVP compared with TESE-P, where sperm must be manually removed with the ICSI pipet from large amounts of extracellular debris (3). Shorter incubation times in PVP may enhance the quality of injected sperm as incubation in PVP leads to submicroscopic alterations in sperm, such as defects in the acrosomal, mitochondrial, and plasma membranes and deterioration of the chromatin, axonemal tubules, fibrous sheath, and accessory fibers (11,12).

Density gradient centrifugation removes bacteria and other contaminating debris from the sperm surface (13). The removal of debris from the sperm surface may be especially important when directly injecting sperm into the oocyte. Because such material would not normally enter the oocyte at the time of fertilization, care should be taken to avoid its injection during ICSI. Eliminating debris attached to the sperm plasma membrane may increase embryo quality and live-birth rates with TESE.

Recent studies suggest that it is beneficial to incubate testicular sperm for 48–72 h before ICSI

(2,18,26). In vitro incubation was found to increase the percentage of morphologically normal and motile sperm (2,18) and sperm with double-stranded DNA (26). However, implementing a 2–3 day culture period prior to ICSI is logistically difficult. In most IVF programs the decision to give hCG is not made until the afternoon 2 days prior to oocyte retrieval. This makes it difficult to schedule a fresh sperm harvest 2 days prior to ICSI. The use of frozen sperm does not fully obviate these logistical difficulties since it requires that lab personnel be on call seven days a week for immediate thaw of the frozen testicular sperm upon notification of hCG to achieve 2 days of culture. Three days of in vitro culture would not be feasible on a routine basis for fresh or frozen testicular sperm. In our study, fertilization rates were as high and pregnancy rates higher than in previous studies where testicular sperm were incubated for 2–3 days before ICSI (2). The new processing techniques reported in our study may provide the same, and possibly additional, benefits to those achieved by others with in vitro incubation of testicular sperm. Morphology and motility were greatly improved with DGC processing of testicular tissue and a short 2–4 h incubation at 37°C.

Among the sperm retrieval techniques, MESA has often been recommended over PESA or TESE so that sufficient sperm can be cryopreserved for use in future cycles and to avoid alterations in testicular function from damage during TESE or TESA (4,7). No alterations in testicular function occurred in the 33 cases of TESE reported in this study and in all cases sufficient sperm were cryopreserved from the original sperm harvest procedure to allow for multiple cycles of future IVF. Using the TESE-N processing procedures described herein, only a very small piece of testicular tissue needs to be removed to provide sufficient sperm for the fresh oocyte retrieval cycle and for cryopreservation for future cycles. When only small biopsies are taken the risk of alteration in testicular function with TESE appears to be low.

Further studies are needed to compare survival and fertilization rates between frozen-thawed epididymal and testicular sperm. Up to 10–20% of frozen epididymal samples contain inadequate viability after thawing for use with ICSI (7). In contrast to past reports that freezing of testicular sperm is difficult because low numbers of motile sperm are present (7), we have found that testicular sperm appear to survive freezing and thawing at high rates (unpublished observations). Testicular sperm may survive freezing and thawing at

higher rates than epididymal sperm because epididymal sperm are more aged and fragile since they have been stored in blocked epididymal tubules containing high concentrations of ROS and macrophages reabsorbing old, degenerated sperm (7). This may make epididymal sperm more susceptible to damage during freezing and thawing.

In our experience, frozen testicular sperm appear to survive cryopreservation at high rates, and when combined with the processing methods described herein (TESE-N), provide sufficient motile sperm for ICSI. In this study, frozen-thawed testicular sperm was used in four oocyte retrievals, with a fertilization rate of 83% (35/42) and ongoing/delivered pregnancy rate of 100%.

Further studies are needed to determine the optimal method of freezing testicular sperm. Freezing testicular sperm intact within the seminiferous tubules has been reported to provide good postthaw survival (27), but this method needs to be further examined and controlled comparisons made with sperm frozen in emulsified testicular tissue. Cryoprotective agents such as glycerol and TEST-yolk buffer may not penetrate the seminiferous tubules quickly enough to provide protection to sperm within the tubular environment. Additionally, the formation of ice crystals in the cells in which the testicular sperm are embedded could damage sperm during freezing and thawing. However, it is possible that freezing sperm within the tubular environment exerts some type of protective action against sperm cryodamage (27). We are currently conducting studies comparing survival of testicular sperm frozen in emulsified testicular tissue versus the intact within the seminiferous tubules. Of the four cases of frozen testicular sperm performed to date, emulsified sperm were used in two cases and tubule sperm in two cases with fertilization rates of 70% (16/23) and 100% (19/19), respectively.

The methods described in this study do not permit the complete removal of RBC from testicular sperm because both cell types sediment to the same density gradient fraction. A few groups have reported some elimination of RBC with lysate buffer (6,28). However, in our experience, the removal of RBC from TESE specimens with lysate buffer is only minimal. Additionally, the toxicity of lysate buffer requires further testing as it has been reported to decrease sperm motility and possibly fertilization rates (6). In our experience, when all of the other types of extracellular material are removed from testicular samples, RBC provide minimal interference with sperm pick-up be-

cause RBC settle at the middle of the swim-out drop while the motile sperm swim to the clean edge of the drop where they can be readily picked up with the ICSI pipet.

Additional processing methods may be required for maximizing motile sperm recovery from testicular tissue for cases of nonobstructive azoospermia. Enzymatic treatment of testicular tissue may be beneficial for such cases. Additionally, incubation of testicular sperm with recFSH for 24 h has been shown to significantly increase sperm motility (5). Fertilization rates, implantation rates, and clinical pregnancy rates were also significantly increased compared with incubation of sperm in a simple culture medium without FSH (5). It is presently unknown whether DGC can be used with TESE specimens from men with nonobstructive azoospermia because of the extremely low sperm numbers in these cases.

To date, testicular sperm have primarily been used for treating men with nonobstructive azoospermia. In light of the high fertilization and pregnancy results achieved with testicular sperm in this study and other studies (10), the use of TESE for severe cases of oligoasthenospermia and as a replacement for electro- and vibroejaculation for spinal injury patients should be explored. Sperm quality in such cases may be higher in testicular than epididymal or ejaculated sperm. Ejaculated sperm from men with severe oligoasthenospermia may be highly susceptible to aging and ROS damage and unable to withstand transit and storage in the epididymis or incubation in the disturbed epididymal milieu that may accompany severe male infertility (22). Sperm abnormalities may be reduced in testicular sperm as compared with ejaculated or epididymal sperm because of their more recent production, lesser period of in vivo incubation, and lesser contamination with white blood cells and necrotic sperm.

In conclusion, the processing techniques for testicular tissue described in this report provide for easy removal of contaminating cells and rapid sperm pick-up for ICSI similar to epididymal sperm. These processing techniques may decrease rates of abnormal sperm morphology and sperm aneuploidy in the final ICSI sample and thereby increase fertilization and live-birth rates. Since similar fertilization and pregnancy rates were achieved with this new method of testicular sperm processing and epididymal sperm, this new method of preparing testicular tissue may permit the routine use of TESE in all patients with obstructive azoospermia, thus allowing greater flexibility in

coordinating the sperm harvest procedure with the female partner's oocyte retrieval.

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