

The Difficult MESA: Findings From Tubuli Recti Sperm Aspiration

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Submitted April 9, 2003; accepted April 12, 2003

Purpose: To investigate sperm quality aspirated from the tubuli recti compared to that obtained from microsurgical epididymal sperm aspiration (MESA).

Methods: Sixteen patients with congenital bilateral absence of the vas deferens (CBAVD) underwent MESA. Six MESA procedures were difficult, and therefore sperm were retrieved from the tubuli recti ductules. Intraoperative sperm parameters, recovery after freeze-thaw, and ICSI outcomes were analyzed and compared between tubuli recti and MESA sperm.

Results: Mean initial sperm concentration was similar in both groups (18 vs. 16 million sperm/mL). Initial sperm motility was significantly higher in the tubuli recti group (35%) than the MESA group (25%). However, post thaw motility was higher with MESA compared to tubuli recti sperm (8.7 vs. 1.5%). ICSI fertilization rates after sperm freeze-thaw were 66% for tubuli recti sperm and 71% for MESA sperm.

Conclusions: Tubuli recti sperm may provide an attractive alternative to testis sperm extraction. Poor sperm recovery after freeze-thaw should be expected.

KEY WORDS: Azoospermia; epididymal sperm; infertility; MESA; CBAVD.

INTRODUCTION

In cases of obstructive azoospermia in which microsurgical reconstruction is not an option, several methods for recovering epididymal or testicular spermatozoa have been described. Sperm can be obtained by microepididymal sperm aspiration (MESA), percutaneous sperm aspiration (PESA), testis sperm extraction (TESE), or testis sperm aspiration (TESA) (1–5). Epididymal sperm (MESA, PESA) is the preferred source of sperm in obstructive azoospermia, because such sperm tend to be more mature, and are

obtainable in higher, bankable numbers relative to testis sperm (6). However, there are instances in which epididymal sperm retrieval can be difficult, and testicular sperm is used instead. These scenarios may be encountered in patients who have undergone multiple prior procedures (reconstruction or aspiration) and those with congenital absence of the vas deferens (CBAVD) harboring small, vestigial remnants of a caput epididymis without sperm.

Although testicular sperm is an alternative in difficult MESA cases, embryologists prefer sperm in fluid rather than in tissue for intracytoplasmic sperm injection (ICSI). Furthermore, testis sperm that has been frozen and thawed often exhibits poor motility, necessitating repeat procedures on the male partner for each in vitro fertilization (IVF) cycle (7).

During difficult MESA cases, we sought to acquire sperm from the tubuli recti (efferent ductules) that drain the rete testis before proceeding to testis sperm extraction (8). We report and compare the andrological findings and ICSI outcomes from two cohorts

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of patients: those who underwent routine epididymal sperm extraction and those in whom tubuli recti sperm were obtained because of failed MESA. With this data, we hope to better define the quality and cryobiological behavior of tubuli recti sperm for assisted reproductive techniques (ART) procedures.

MATERIALS AND METHODS

Sixteen patients with congenital bilateral absence of the vas deferens (CBAVD) underwent MESA for sperm retrieval in conjunction with ICSI at a single institution. In all cases, retrieved sperm was frozen for future in vitro fertilization and intracytoplasmic sperm injection (IVF-ICSI) cycles. Formal evaluation of each patient included a history and physical examination, semen analysis including centrifuged pellet analysis, and measurement of follicle stimulating hormone (FSH) and testosterone levels. One patient had prior testicular biopsy showing normal spermatogenesis; 15 others had no prior surgical procedures. These patients were divided into two cohorts, based on the andrological findings during the MESA procedure.

In 10 patients, MESA was performed as described below, and adequate motile sperm were obtained from the corpus or caput epididymis. In six patients however, sufficient motile sperm were not available with routine MESA: four patients had intraoperative caput epididymal sperm motility <5%, and two had bilateral caput epididymal remnants without functional tubules containing sperm. These six patients underwent tubuli recti sperm aspiration as described below.

Epididymal Sperm Aspiration (Mini-MESA)

Epididymal sperm aspiration was performed by a single surgeon (P.J.T) as previously described (6). Under local anesthesia, a small scrotal incision was made and the corpus and caput epididymis exposed. Using microsurgical technique, the epididymal tunic was incised and individual epididymal tubules were isolated and entered with micro scissors. Epididymal fluid was aspirated into small syringes (1 mL) and subsequently expelled into sterile 5 mL test tubes (Becton Dickinson, Franklin Lakes, NJ) containing Earle's medium (Gibco BRL, Grand Island, NY) supplemented with 4 mM sodium bicarbonate, 21 mM HEPES (Calbiochem-Novabiochem Corp., La Jolla, CA), 0.47 mM pyruvate, and 10% v/v synthetic serum substitute (Irving Scientific, Santa Ana, CA) and maintained at 37°C. During the procedure, 10 μ L of

extracted fluid was examined under (400 \times) bright-field microscopy for sperm.

Epididymal aspiration progressed from distal (corpus) to proximal (caput) epididymis until motile spermatozoa were observed. Patients with intraoperative caput epididymal sperm motility <5% or bilateral epididymal remnants without functional tubules were further considered for tubuli recti sperm aspiration.

Tubuli Recti Sperm Aspiration

The tubuli recti (testis efferent ductules) were exposed, as previously described, by incising the tunic overlying the junction between testis and the caput epididymis and sharply dissecting the caput epididymis off the testis (8). After this dissection, small tubules connecting the rete testis and the caput epididymis were readily visualized with intraoperative microscopy. The tubuli recti were sharply transected and clear fluid aspirated with a 24-gauge angiocatheter (Becton Dickinson Vascular Access, Sandy, UT) on a 1.0 mL syringe. Aspirated fluid was expelled into a sterile 5-mL test tube similar to the MESA procedure. Ten micro liters of fluid were examined under bright-field microscopy for motile spermatozoa. Once sufficient motile sperm were obtained, the tubuli recti were lightly fulgurated with bipolar cautery and the epididymal tunic reapproximated with 9-0 nylon sutures. No attempt was made to reconstruct the tubules. Sperm aspirates were formally analyzed and processed in the andrology laboratory.

Analysis and Cryopreservation

Aspirate volume, sperm concentration, percent motility, and forward progression were assessed for fresh and frozen-thawed sperm samples according to WHO established methods (9), after dilution and preparation using a micro cell slide (Conception Technologies, San Diego, CA). The total motile sperm count was calculated using the formula: (Volume) \times (Sperm Concentration) \times (Motile Fraction).

Sperm were washed twice (centrifuged: 170 g, 10 min) in phosphate buffered saline (Gibco BRL, Grand Island, NY) and diluted to obtain a concentration of 10⁵ motile sperm/vial prior to cryopreservation. Sperm were cryopreserved in a 1:1 dilution of sperm suspension to test yolk buffer (Irving Scientific, Santa Ana, CA) in 1.0-mL vials by the slow freeze method (10) in a Planar Biomed controlled rate freezer (T.S. Scientific, Perkasi, PA).

Table I. IVF-ICSI Outcome After MESA in Men With CBAVD

Patient	Sperm concentration (million/mL)	Sperm initial motility (%)	Sperm post thaw motility (%)	# Oocytes injected (%)	# Fertilized oocytes	2 PN fertilization (%)
1	58	33	1	33	22	66
2	0.8	40	30	15	11	73
3	10	20	13	8	8	100
4	4	15	10	6	4	66
5	1	20	<1	23	13	56
6	6.8	20	1	14	11	78
7	28	25	14	27	19	70
8	10	30	4	12	6	50
9	22	28	9	9	7	77
10	17	20	5	51	37	72
Mean	15.7	25	8.8	19.8	13.8	71

Upon thawing for IVF-ICSI cycles, vials were incubated at 37°C for 10 min and then gradually reintroduced into isotonic medium by adding an equal volume of Earle's medium over 5 min. The diluted specimen was centrifuged (170 × g, 10 min) and the entire procedure was repeated. The final pellet was resuspended in 0.5 mL of Earle's buffered medium for assessment of sperm parameters and for IVF-ICSI.

Oocyte Stimulation, Oocyte Retrieval, and ICSI

Women underwent pituitary desensitization with a gonadotropin hormone-releasing agonist and ovarian stimulation with human menopausal gonadotropins (Pergonal or Fertinex; Serono, Norwell, MA) as previously described (6). Retrieved oocytes were handled in the laboratory according to standard techniques and cultured in medium with 5% CO₂ at 37°C. For ICSI, morphologically normal and motile spermatozoa were recovered using a "swim-out" technique (6). Only the oocytes with the presence of the first polar body were injected. Following ICSI, oocytes were transferred into culture medium (P1, Irvine Scientific, Santa Ana, CA) under oil (Sigma) and incubated for 16 h. Normal fertilization was confirmed when two pronuclei (2PN) were found within the oocyte plasma 16–19 h after ICSI.

Statistical Analyses

Using descriptive measures of variance, measured sperm parameters from aspirated fluid were compared between the two cohorts of patients. Unpaired *t* tests were used to assess the significance of any differences in sperm quality between cohorts. In addition, paired *t* tests were used to assess differences in initial and post thaw sperm motility within each cohort.

RESULTS

Routine MESA

Ten of 16 patients with CBAVD had sufficient sperm from routine MESA procedures. Sperm parameters before and after cryopreservation and oocyte fertilization rates are outlined in Table I. Sperm concentration ranged from as low as 0.8 million/mL to 28 million/mL, with a mean of 15.7 million/mL. The mean motility of fresh MESA sperm was 25% (range: 15–40%) and decreased to 8.8% (range: 1–30%) after freeze–thaw. In this cohort of patients, the average female age was 30.9 years (range: 24–39). The mean normal oocyte fertilization rate with MESA sperm was 71% (range: 50–100%).

Tubuli Recti Sperm Aspiration

Six of 16 patients required tubuli recti sperm aspiration. In each of those cases, routine MESA was attempted first. The andrological findings from attempted MESA were compared to those of tubuli recti sperm aspiration in each patient and are summarized in Table II. Compared to caput epididymal sperm, tubuli recti sperm were found in more

Table II. Comparison of Sperm Quality in Patients With Attempted MESA and Tubuli Recti Sperm Aspiration

Patient	Caput MESA		Tubuli recti aspiration	
	Concentration	Motility	Concentration (Million/mL)	Motility (%)
1	16,000/mL	1%	40	25
2	40,000/mL	0%	2.2	40
3	6 million/mL	1%	15	25
4	19 million/mL	4%	38	40
5	None	None	3.9	40
6	None	None	10	40

Table III. ICSI-IVF Outcome After Tubuli Recti Sperm Aspiration

Patient	Sperm concentration in million/mL	Sperm initial motility (%)	Sperm post thaw motility (%)	# Oocytes injected	# Fertilized oocytes	2 PN fertilization
1	40	25	2	12	6	50%
2	2.2	40	1	N/A ^a	N/A ^a	N/A ^a
3	15	25	2	13	9	69%
4	38	40	2	19	13	68%
5	3.9	40	1	18	13	72%
6	10	40	1	15	11	73%
Mean	18.2	35	1.2	15.4	10.4	66.4%

^aN/A = couple has not proceeded to ICSI as yet.

abundant numbers and exhibited higher motility. Two patients with very low numbers on routine MESA sperm (16,000 sperm/mL and 40,000 sperm/mL) revealed 10–1000× more sperm within the tubuli recti (40 and 2.2 million sperm/mL) with much higher motility (25–40%). Furthermore, two patients with no sperm during MESA, revealed 3.9–10 million sperm/mL with 40% motility from tubuli recti sperm aspiration. Importantly, similar to testis sperm, tubuli recti sperm initially exhibited only “twitching” motility, gaining forward progression after 1 h of incubation at 37°C.

Tubuli recti sperm parameters before and after cryopreservation are outlined in Table III. Sperm motility decreased from a range of 25–40% prefreeze to 1–2% post thaw. Furthermore, frozen–thawed tubuli recti sperm exhibited relatively poor forward progression, with mainly “twitching” motility. Interestingly, the poor recovery of motility reflects a cryobiological behavior that more closely resembles testicular rather than epididymal sperm in our experience (7).

Regarding ICSI outcome, five of six couples proceeded to IVF-ICSI with frozen–thawed sperm from tubuli recti sperm aspiration. The average female age was 32.8 years (range: 28–39 years). Sperm parameters, number of oocytes, and fertilization rates are summarized in Table III. The average fertilization rate with frozen–thawed tubuli recti sperm was 66.4% (range: 50–73%).

Sperm Quality and Pregnancy Outcome: MESA and Tubuli Recti Sperm

Regarding sperm quality, there was no significant difference in retrieved sperm concentration between cohorts. However, fresh sperm motility was significantly higher in the tubuli recti group, compared the MESA group (35 vs 25%, $p = 0.024$). Interestingly, post thaw motility was much lower in the tubuli recti group compared to the MESA group (1.2 vs 8.7% $p = 0.058$). When analyzed differently, the motility of fresh tubuli recti sperm fell 96% after cryopreser-

vation, whereas epididymal sperm motility decreased 64% after freeze–thaw, revealing a statistically significant difference ($p = 0.001$).

Regarding pregnancy outcome, five of 10 couples (50%) from the MESA cohort achieved a clinical pregnancy (fetal heartbeat on ultrasound) with frozen–thawed sperm after a single IVF-ICSI cycle (average female age: 30.9 years). From the tubuli recti group, five of six patients had a consecutive IVF-ICSI cycle and two (40%) conceived with frozen–thawed tubuli recti sperm (average female age: 32.8 years).

DISCUSSION

With CBAVD and unreconstructable obstructive azoospermia, microepididymal sperm aspiration is now performed routinely to retrieve sperm for ICSI. Indeed, in well-chosen patients, epididymal spermatozoa can be retrieved in >90% of attempts, and this includes a generous quantity for cryopreservation (6). It is also clear that motile, frozen–thawed epididymal sperm perform equally as well as freshly retrieved sperm in ICSI cycles characterized by obstructive azoospermia (11). Given this, the ability to freeze large amounts of high quality epididymal sperm can reduce the need for further surgical procedures in the male partner for subsequent ICSI procedures.

However, when one fails to find sperm in the epididymis, the standard approach is to proceed to testis sperm extraction or aspiration. Although normal fertilization and pregnancy rates are roughly equivalent to that reported with epididymal sperm, cryopreservation is less successful with testicular than epididymal sperm (12,13). Indeed, testicular sperm motility falls by at least 90% after freezing and thawing, whereas the falloff in epididymal sperm motility is generally less dramatic (68%) (7). Furthermore, embryologists prefer sperm in fluid rather than sperm in tissue for ICSI cycles because of handling ease.

In this study we suggest that the testis tubuli recti is a relatively untapped anatomical source of high

quality sperm. Physiologically, this makes sense since as sperm production continues in the obstructed male reproductive tract, motile sperm is more likely to be found proximally, toward the caput rather than cauda epididymis (8). Therefore it is not unexpected that tubuli recti sperm are more viable than sperm downstream in the obstructed epididymis. In our study, fresh tubuli recti sperm show similar concentration and motility to other epididymal sperm. However, although the motility of fresh tubuli recti sperm is excellent, a dramatic decrease in sperm motility is observed on freeze–thaw. In fact, cryobiologically, our findings suggest that tubuli recti sperm “act” more like testicular sperm than epididymal sperm after freeze–thaw. This is an important point for embryologists and andrologists who may handle these aspirated specimens.

Of course, there are limitations to this study. Although statistical comparison between two cohorts of patients could be made in this study, definitive conclusions about sperm quality from the tubuli recti is somewhat premature at this point. We hope that this study will stimulate interest in this anatomical sperm source. Also, it is not clear if the procedure can be repeated, or if scarring and permanent obstruction are the rule. Because of this, we would discourage this anatomical source for sperm retrieval, if microsurgical reconstructive procedures such as vasectomy reversal might be considered in the future. Finally, clinical pregnancy rates and birth outcomes with the use of fresh and frozen–thawed tubuli recti sperm need further assessment.

CONCLUSIONS

During difficult MESA procedures in men with congenital absence of the vas deferens, tubuli recti sperm may provide an excellent alternative to testis sperm extraction in selected cases. Importantly, although fresh tubuli recti sperm has similar characteristics than epididymal sperm, the cryobiological behavior of this sperm appears to have a “look” that is more testicular than epididymal in nature.

REFERENCES

1. Cha K-Y, Oum K-B, Kim H-J: Approaches for obtaining sperm in patients with male factor infertility. *Fertil Steril* 1997;67:985–995
2. Tournaye H: Surgical sperm recovery for intracytoplasmic sperm injection: Which method is to be preferred? *Hum Reprod* 1999;14(Suppl)1:71–81
3. Ubaldi F, Camus M, Tournaye H, Clasen K, Nagy E, Smits J, Van Steirteghen A, Devroey P: Results of microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE) in azoospermic men using intracytoplasmic sperm injection. *Andrologia* 1996;28(Suppl)1:71–75
4. Takihara H: The treatment of obstructive azoospermia in male infertility—Past, present, and future. *Urology* 1998;51 (Suppl) 5A:150–155
5. Dohle GR, Ramos L, Pieters MH, Braat DD, Weber RF: Surgical sperm retrieval and intracytoplasmic sperm injection as treatment of obstructive azoospermia. *Hum Reprod* 1998;13:620–623
6. Nudell DM, Conaghan J, Pedersen RA, Givens CR, Schriock ED, Turek PJ: The mini-micro-epididymal sperm aspiration for sperm retrieval: A study of urological outcomes. *Hum Reprod* 1998;13:1260–1265
7. Bachtell N, Conaghan J, Turek PJ: The relative viability of human spermatozoa from the testis, epididymis and vas deferens before and after cryopreservation. *Hum Reprod* 1998;14:101–104
8. Schlegel PN, Berkeley AS, Goldstein M, Cohen J, Alikani M, Adler A, Gilbert BR, Rosenwaks Z: Epididymal micropuncture with in vitro fertilization and oocyte micromanipulation for the treatment of unreconstructable obstructive azoospermia. *Fertil Steril* 1994;61:895–900
9. WHO: WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Cambridge, UK, Cambridge University Press, 1992.
10. Madadevan M, Trounson AO: Effect of cryoprotective media and dilution methods on the preservation of human spermatozoa. *Andrologia* 1983;15:355–366
11. Cayan S, Lee D, Conaghan J, Givens CA, Ryan IP, Schnock ED, Turek PJ: A comparison of intracytoplasmic sperm injection outcomes with fresh and cryopreserved epididymal sperm from the same couple. *Hum Reprod* 2001;16:495–499
12. Hovatta O, Moilanen J, von Smitten K, Reima I: Testicular needle biopsy, open biopsy, epididymal aspiration and intracytoplasmic sperm injection in obstructive azoospermia. *Hum Reprod* 1995;10:2595–2599
13. Schoysmann R, van Roosendaal E, Bollen N, Vandervorst M, Vanderzwalmen P, Standaert V, Berting G, Debauche C, Lefere C: Modern sperm retrieval techniques and their usefulness in oocyte fertilization. *BJU International* 2001;88:141–146