Fresh MESA improved embryo fertilization, cleavage, blastula formation and implantation rates after failed TESA in couples with obstructive azoospermia.

Martin D. Keltz · Elisheva M. Rovner · Eric Gonzalez · David Weiner

Received: 13 October 2010 / Accepted: 22 December 2010 / Published online: 7 January 2011
© Springer Science+Business Media, LLC 2011

Abstract

Purpose To determine whether our use of fresh MESA cycles improved outcomes in patients with obstructive azoospermia who had failed IVF with TESA.

Methods A prospective observational trial of couples undergoing IVF for obstructive azoospermia was performed at an academic IVF center.

Results When TESA resulted in poor embryo cleavage, implantation and ongoing pregnancy rates, subsequent fresh MESA cycles in these same couples, demonstrated dramatic improvement in cleavage, blastulation, implantation and live birth rates.

Conclusions In patients undergoing IVF-TESA-ICSI with obstructive azoospermia resulting in poor cleavage rates, blastulation rates and cycle failure, a repeat cycle with MESA may result in marked improvement in outcome.

Keywords Blastulation · Cleavage · MESA · Obstructive azoospermia · TESA

Testicular aspiration (TESA) has largely replaced microsurgical epididymal aspiration (MESA) for in vitro fertilization (IVF) in obstructive azoospermia as it is a less invasive and better tolerated procedure that offers acceptable fertilization

rates when combined with intracytoplasmic sperm injection (ICSI) [1–7]. TESA however, may be associated with lower fertilization and embryo cleavage rates when compared to IVF-ICSI with mature ejaculated sperm [8–11]. A series of IVF-ICSI cases performed for obstructive azoospermia resulted in poor embryo cleavage, very low blastulation rates and cycle failure following TESA with adequate motile and viable sperm. These cycles were followed by a cycle of IVF-ICSI employing microsurgical epididymal aspiration (MESA) with high cleavage rates, blastulation rates and implantation and live birth rates. The following presents our review of TESA failure in obstructive azoospermia and its amelioration in the subsequent cycle employing MESA.

This study is a prospective cohort analysis of couples undergoing IVF-TESA-ICSI for obstructive azoospermia at a university hospital based IVF center between January 2007 and December 2009. Four consecutive couples that displayed poor embryo cleavage with IVF-TESA-ICSI chose to undergo a repeat IVF cycle with fresh MESA. All couples underwent IVF cycles employing a standard long GnRHa protocol with normal ovarian reserve and normal response. After retrieval all mature oocytes were fertilized with ICSI employing either fresh or frozen TESA sperm. All sperm in these cases were viable, motile and morphologically normal.

In each of the first cycles, testicular aspiration was performed with local anesthesia and a 3 mL syringe and 18-gauge 11/2 in. needle to obtain testicular tissue. A 25-gauge needle was used to separate sperm from the tissue, followed by a protein media wash and centrifugation for sperm isolation. After incubation of fresh or thawed frozen TESA, motile sperm or rarely HOS tested, non-motile sperm were used for ICSI in our standard protocol.

Capsule In patients with obstructive azoospermia with poor cleavage rates, blastulation rates and cycle failure after IVF-TESA-ICSI, repeat cycle with MESA may result in improved outcomes.

M. D. Keltz · E. M. Rovner · E. Gonzalez · D. Weiner
St Luke's Roosevelt Hospital,
New York, NY, USA

E. M. Rovner (✉)
1400 Pelham Parkway South,
Bronx, NY 10461, USA
e-mail: erovner@gmail.com

In each of the second MESA cycles, the husband underwent open microsurgical epididymal sperm aspiration under general anesthesia. After scrotal incision, an operative microscope was used to identify a dilated epididymal tubule that was sharply incised and aspirated with an 18-gauge angiocath. Once adequate motile sperm was confirmed with inverse microscopy, the sperm underwent our standard density gradient processing to isolate motile sperm followed by our standard ICSI protocol.

Systat 13 software and Chi square and Fisher's exact test were used for statistical evaluation.

Fresh MESA resulted in a similar, though slightly higher fertilization rate, but MESA resulted in far higher rates of embryo development past the 4-cell stage, blastulation rates, as well as a higher implantation rates (Table 1).

In their TESA cycle, the first couple required PGD for Cystic Fibrosis, and their cycle was cancelled when none of their 16 embryos developed past four cells. The second couple had five embryos, and 3 were transferred with only one progressing past six cells on day 5. The third couple had four embryos all arrested at the four cell stage and were transferred on day 4. The fourth couple had four embryos all arrested at the four cell stage all transferred without a pregnancy.

In their MESA cycle, the first couple had four blastocysts and two CF negative blastocysts were transferred with two cryopreserved. The second couple had five blastocysts with two transferred and three cryopreserved. The third couple had two blastocysts, which were transferred along with a morula and compaction embryo, with none cryopreserved. The fourth couple failed to have epididymal sperm obtained at their microsurgical procedure, and underwent fresh TESA IVF-ICSI. These couples failed to conceive in their five IVF-TESA-ICSI cycles, and three of the couples have a live birth from IVF-MESA-ICSI one with twins.

Previous concerns regarding testicular versus epididymal sperm centered on the maturity and ultimately the motility of the sperm. The advent of technology such as

ICSI allows for use of immature or immotile sperm thus, the overall experience of successful outcomes with testicular sperm. Recent data as well as our experience suggests that there may be other factors, which are activated or matured in the epididymis, which may in fact contribute to successful embryo development. Stalf et al., discuss the concept of immotile sperm as having potentially lost "vitality" in their retrospective study of ICSI cycles performed with ejaculated motile, ejaculate immotile and testicular motile and testicular immotile sperm. They found that pregnancy rates were higher with ejaculated compared to testicular sperm, and further that when subdivided into the four aforementioned groups the motile groups performed comparably and better than the immotile groups [12]. Similarly, Pasqualetto et al. compared the results of IVF cycles based on sperm source in a retrospective analysis of patients receiving treatment for azospermia. The study found higher pregnancy rates using epididymal sperm [13]. Goker et al. evaluated cycles based on sperm quality and source, comparing ejaculated mobile sperm to ejaculated immobile sperm to testicular sperm. He found that regardless of sperm quality the ejaculated sperm performed better than the testicular sperm with increased fertilization rates, rates of grade I embryos and pregnancy rates [14]. A recent published abstract of a randomized prospective trial, documented that percutaneous epididymal aspiration improves embryo development and pregnancy rates when compared to TESA, also suggesting a benefit when using further matured sperm [9].

In our experience, although fertilization rates were no better following MESA after failed TESA, the embryos underwent more advanced cleavage and markedly improved implantation. This may be due to reduced DNA fragmentation or oxidative stress in epididymal sperm. It may further be due to a maturation in gene expression present in sperm retrieved from the epididymus than from the testis allowing the early embryo to bypass cleavage arrest and improve implantation. Further studies, however, would be required to elucidate these differences.

While MESA was the preferred route for our urologist at the time of this study, PESA or percutaneous epididymal aspiration may allow for a less traumatic and equally effective option following or instead of TESA.

Given the small sample size, larger studies would be required to confirm our findings.

Thus, while TESA is more easily tolerated and performed, fresh MESA may improve embryo cleavage and blastulation rates, implantation rates and pregnancy rates in couples that have failed IVF-TESA-ICSI.

Table 1 Outcomes following TESA and MESA (Elisheva change the order between four cell and blast)

Outcomes	TESA (%)	MESA(%)	p-value
Fertilization/M2	35/50 (70.0)	26/29 (90.0)	$p=.054$
Blastocysts/2PN	2/35 (5.7%)	11/26 (42.3%)	$p<0.001$
>4cell Embryos/2PN	4/35 (11.4)	22/26 (84.6)	$p<0.001$
Implantation/transfer	0/14 (0.0)	5/9 (55.6)	$p=0.004$
Live Birth Rate	0/5 (0.0)	3/3 (100%)	$P=0.029$

References

1. Watkins W, Nieto F, Bourne H, Wutthiphan B, Speirs A, Gordon Baker HW. Testicular and epididymal sperm in a microinjection program: methods of retrieval and results. *Fertil Steril*. 1997;67:527–35.
2. Fahmy I, Mansour R, Aboulghar M, Serour G, Kamal A, Tawab NA. Intracytoplasmic sperm injection using surgically retrieved epididymal and testicular spermatozoa in cases of obstructive and non-obstructive azoospermia. *Int J Androl*. 1997;20:37–44.
3. Pasqualotto FF, Rossi-Ferragut LM, Rocha CC, Iaconelli JA, Borges JE. Outcome of in vitro fertilization and intracytoplasmic injection of epididymal and testicular sperm obtained from patients with obstructive and nonobstructive azoospermia. *J Urol*. 2002;167:1753–6.
4. Bukulmez O, Yucel A, Yarali H, Bildirici I, Gurgan T. The origin of spermatozoa does not affect intracytoplasmic sperm injection outcome. *Eur J Obstet Gynecol Reprod Biol*. 2001;94:250–5.
5. Rhouma KB, Miled EB, Attallah K, Marrakchi H, Khouja H, Sakly M. Successful pregnancies after using immotile spermatozoa from ejaculate, epididymis and testis. *Eur J Obstet Gynecol Reprod Biol*. 2003;108:182–5.
6. Nicopoullos JD, Gilling-Smith C, Ramsay JW. Does the cause of obstructive azoospermia affect the outcome of intracytoplasmic sperm injection: a meta-analysis. *BJU International* 2004;93:1282–6.
7. Demirel LC, Evirgen O, Aydos K, Unlu C. The impact of the source of spermatozoa used for ICSI on pronuclear morphology. *Hum Reprod*. 2001;16:2327–32.
8. Van Steirteghem A, Nagy P, Joris H, Janssenswillen C, Staessen C, Verheyen G, et al. Results of intracytoplasmic sperm injection with ejaculated, fresh and frozen-thawed epididymal and testicular spermatozoa. *Hum Reprod*. 1998;13:134–42.
9. Ravizzini P, Carizza C, Abdelmassih V. O-202 Oral: PESA vs TESA in vasectomized men undergoing ICSI: what is the best sperm source? Prospective randomized comparative study. *Hum Reprod*. 2009;24:81–3.
10. Reis AP, Salgueiro LL, Abdelmassih V, Ravizzini P, Abdelmassih S, Abdelmassih R. Influence of semen source on early cleavage in ICSI cycles. *Fertil Steril*. 2007;88:S313.
11. Ghazzawi I, Sarraf M, Taher M, Khalifa F. Comparison of the fertilizing capability of spermatozoa from ejaculates, epididymal aspirates and testicular biopsies using intracytoplasmic sperm injection. *Hum Reprod*. 1998;13:348–52.
12. Stalf T, Mehnert C, Hajimohammad A, Manolopoulos K, Shen Y, Schuppe HC, et al. Influence of motility and vitality in intracytoplasmic sperm injection with ejaculated and testicular sperm. *Andrologia*. 2005;37:125–30.
13. Pasqualotto FF, Rossi LM, Guilherme P, Ortiz V, Iaconelli JA, Borges JE. Etiology-specific outcomes of intracytoplasmic sperm injection in azoospermic patients. *Fertil Steril*. 2005;83:606–11.
14. Goker ENT, Sendag F, Levi R, Sendag H, Tavmergen E. Comparison of the ICSI outcome of ejaculated sperm with normal, abnormal parameters and testicular sperm. *Eur J Obstet Gynecol Reprod Biol*. 2002;104:129–36.