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ORIGINAL ARTICLE *Andrology*

Live birth rates after MESA or TESE in men with obstructive azoospermia: is there a difference?

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STUDY QUESTION: How do live birth rates compare after intracytoplasmic sperm injection (ICSI) for men with obstructive azoospermia when using sperm derived from testicular sperm extraction (TESE) versus microsurgical epididymal sperm aspiration (MESA)?

SUMMARY ANSWER: Our study suggests that proximal epididymal sperm (from MESA) result in higher live birth rates as compared with testicular sperm (from TESE) in couples where the man has obstructive azoospermia due to congenital bilateral absence of the vas deferens (CBAVD) or vasectomy.

WHAT IS KNOWN ALREADY: For couples with obstructive azoospermia, MESA (epididymal sperm) and TESE (testicular sperm) have generally been assumed to be equivalent for use in ICSI. But this assumption has never been confirmed, and this view has important clinical and basic scientific consequences.

STUDY DESIGN, SIZE, DURATION: This was a retrospective study of a consecutive cohort of 374 men with obstructive azoospermia and normal spermatogenesis, who underwent IVF and ICSI using either epididymal sperm or testicular sperm in the period 2000–2009.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study included men undergoing MESA or TESE at St. Luke's Hospital for obstructive azoospermia due to CBAVD or vasectomy.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 280 couples underwent MESA and 94 underwent TESE with ICSI. The live birth rate was 39% after MESA-ICSI and 24% after TESE-ICSI. The MESA-ICSI cycles also resulted in a significantly higher implantation rate and significantly higher clinical and ongoing pregnancy rates than the TESE-ICSI cycles. There was no significant difference in results between fresh or frozen sperm for both MESA and TESE. When adjusted for the available confounders, the odds ratio for live birth was significantly in favour of MESA-ICSI versus TESE-ICSI (OR 1.82; 95% CI 1.05–3.67). The only significant confounders were female age and ovarian reserve.

LIMITATIONS, REASONS FOR CAUTION: This is a retrospective cohort study and not a randomized clinical trial.

WIDER IMPLICATIONS OF THE FINDINGS: Our study suggests that some aspect of sperm maturation after the sperm leaves the testicle to enter the epididymis is required for the most optimal results, even when ICSI is used for fertilization.

STUDY FUNDING/COMPETING INTEREST(S): No funding was used and there are no competing interests.

Key words: obstructive azoospermia / MESA / TESE / infertility / assisted reproduction

Introduction

Approximately one in 200 men in any population are azoospermic (Hull *et al.*, 1985). Varying surgical techniques are available to extract spermatozoa from these men. These surgically retrieved spermatozoa can subsequently be used to fertilize the oocytes of their female partners by means of intracytoplasmic sperm injection (ICSI) giving these men the possibility of becoming the biological parent of a child (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1993; Silber *et al.*, 1994, 1995, 1996; Devroey *et al.*, 1995, 1996; Beharka *et al.*, 2006; Diemer *et al.*, 2011). Azoospermia is either the consequence of an obstruction, in the case of obstructive azoospermia (OA), or due to a severe spermatogenic defect whereby there is not enough quantity of sperm production to 'spill over' into the ejaculate, in the case of non-obstructive azoospermia

(NOA) (Silber and Rodriguez-Rigau, 1981; Silber et al., 1990a,b; Silber, 2000a,b). NOA may be caused by genetic mutations, chromosomal aberrations, cryptorchidism, chemotherapy or mumps orchitis, or may be idiopathic, but is seen histologically as Sertoli cells only, maturation arrest, or seminiferous tubule pathology with absence or near absence of mature spermatids. OA may be caused most commonly by congenital bilateral absence of the vas deferens (CBVAD), or by vasectomy or post-infectious epididymal obstruction. First-line therapy in men with OA often is the reconstruction of the seminal tract, to enable spontaneous conception without assisted reproductive techniques (Silber and Grotjan, 2004). However, this is not always possible, for example, with CBVAD (Silber et al., 1990b; Patrizio et al., 1993). Surgical sperm retrieval therefore offers a treatment for patients with obstructive azoospermia in cases where microsurgical reconstruction is not an option or has already failed. Among surgical techniques that have been explored over the years, microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE) have always been the standard methods used for sperm retrieval (Silber et al., 1994, 1996; Tournaye et al., 1994; Devroey et al., 1996; Beharka et al., 2006; Diemer et al., 2011).

In MESA, the epididymis is isolated via an operative scrotal incision under local anaesthesia and, with the use of an operating microscope, fluid is aspirated from the dilated epididymal tubule and examined for the presence and quality of sperm. Only the proximal-most sperm are used for MESA because the distal sperm are more senescent and therefore have the most DNA sperm fragmentation (Silber et al., 1988; Silber, 2000a,b) (Fig. 1). In TESE, testicular tissue is removed, placed in culture media, and cut into tiny pieces, as sperm are liberated from within the seminiferous tubules and are then extracted from the surrounding testicular tissue. Although many testicular sperm are non-motile or barely motile, motile sperm are preferably used for ICSI.

Whereas TESE is the only option for men with NOA, both MESA and TESE can be used in men with OA. The benefit of MESA over TESE is that more spermatozoa can be obtained, and if the most proximal tubules are identified, highly motile sperm can be obtained. On the other hand, MESA is more time consuming and surgically challenging than TESE. Strikingly, there are no data in the literature to demonstrate whether TESE or MESA in men with OA result in different success rates in terms of chances of pregnancy, and the decision whether to perform either MESA or TESE is usually made arbitrarily. Furthermore such knowledge would be helpful in lending direction to future research in epididymal physiology.

We therefore performed a retrospective cohort study comparing pregnancy outcomes in couples in which the man suffered from obstructive azoospermia and where spermatozoa were retrieved via either MESA or TESE.

Methods

Patients

In this cohort study, we consecutively included the first (to avoid previous failure bias) ICSI cycle in couples where the man had undergone MESA- or TESE-ICSI for obstructive azoospermia caused either by congenital bilateral absence of the vas or by previous vasectomy between 2000 and 2009 at the Infertility Center of St. Louis (USA). All MESA and TESE procedures were performed by a single surgeon (S.S.). MESA was our primary treatment strategy in men with OA and TESE was only performed in those men in whom MESA was not feasible, i.e. if the entire epididymis was absent or scarred from previous surgery, or if MESA did not result in any sperm due to epididymal blockage. All MESA and TESE cases where fresh sperm were used were due to CBAVD. All MESA and TESE cases involving frozen sperm used sperm samples that were cryopreserved at the time of a vasectomy reversal that had eventually failed. Thus, the results for fresh cases reflect CBAVD, and the results for frozen cases reflect vasectomy reversal.

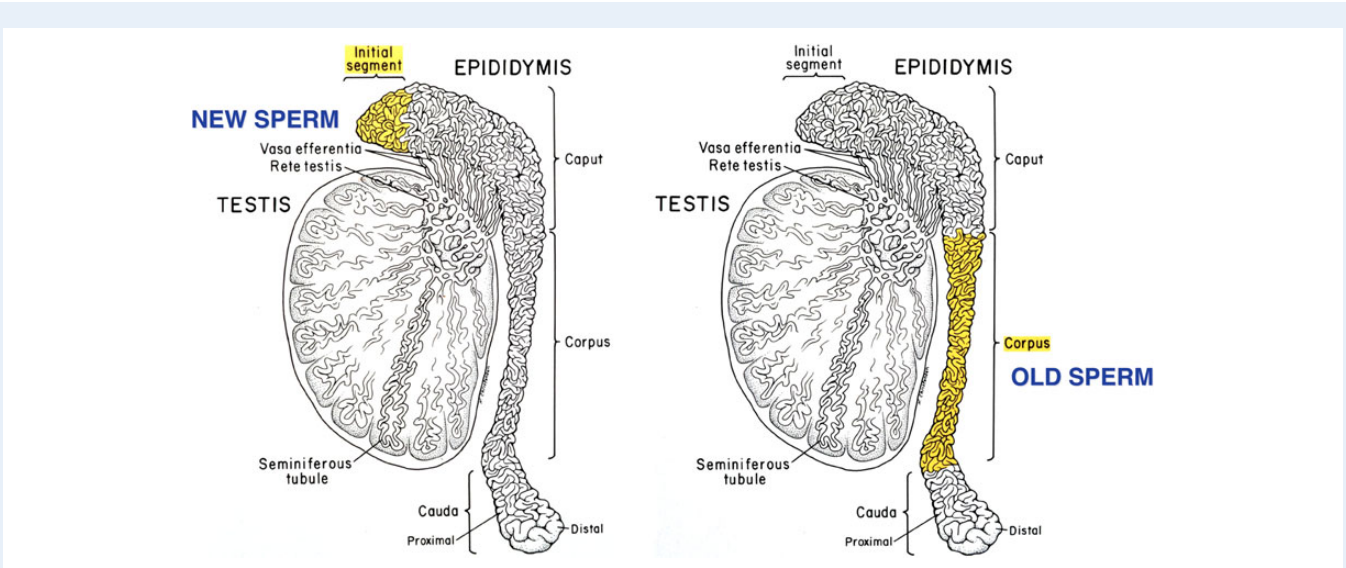

Figure 1 Schematic diagram of the human testis. In obstructive azoospermia (OA), distal epididymal sperm show senescent changes, increased DNA fragmentation, and are non-motile while sperm retrieved from the most proximal part of the epididymis show no senescent changes and are motile. Recently generated sperm are found at the most proximal part of the epididymis (left), while deteriorated sperm are found in the corpus and distal end of the epididymis in men with OA (right). This figure has been reproduced, with permission, from Silber and Barbey (2012).

MESA

For CBAVD cases, under 10× to 40× magnification with an operating microscope, a 1-cm incision was made with microscissors into the epididymal tunic to expose the tubules in the most proximal portion of the congenitally blind-ending or obstructed epididymis. Spermatozoa were aspirated with a micropipette (0.7 mm/22 mm; Cook Urological, Spencer, IN, USA) on a tuberculin syringe directly from the opening in the epididymal tubule. The specimens were immediately diluted in HEPES-buffered medium, and a tiny portion was examined for motility and quality of progression. If sperm motility was absent or poor, another aspiration was made even more proximally (Fig. 1). Once the area of motile sperm was found, an aliquot of epididymal fluid was used for ICSI, and the remainder was cryopreserved. The same procedure was performed for cases of vasectomy reversal at the time of the reversal, and all the retrieved sperm were frozen. If there were no epididymal sperm in the vasectomy reversal cases, then TESE was performed and again all the sperm were frozen.

Microsurgical TESE

Under the operating microscope, a longitudinal incision was made in the tunica albuginea exposing the anatomic lobules of the testis. Tissue from only the peripheral loops of the seminiferous tubules was sampled. This avoids damage to the testis and yet samples every corner from which sperm might be present. The sampled tissue was analysed in the laboratory for the presence of spermatozoa. If spermatozoa were found, these were used immediately for ICSI or cryopreserved for future use depending on the case. If spermatozoa were directly used for ICSI, any remaining spermatozoa or tissue after the procedure were frozen and stored for possible use in a second or third ICSI cycle. Although motility is always either very weak or nonexistent in testicular sperm, only motile, non-senescent sperm were used for ICSI. The tunica albuginea was closed with 9-0 nylon interrupted sutures, after meticulous hemostasis with micro-bipolar forceps. This prevents any increase in intratesticular pressure, resulting in minimal pain and no subsequent atrophy.

ICSI

The ICSI procedures were all performed using the long GnRH protocol, with transvaginal ultrasound-guided oocyte retrieval, and a minimum of a 2-h incubation of oocytes before cumulous stripping and sperm injection. For both MESA and TESE, the most motile sperm were injected, although for TESE cases, there was, as expected, minimal motility. Embryos were always transferred on Day 3 at cleavage stage, and a similar number of embryos was transferred in both MESA and TESE cycles.

Data collection

For the purpose of avoiding 'previous failure' bias, only the first ICSI cycle for each case was included in the analysis. Data on pregnancy outcome were collected prospectively, and for all couples the pregnancy outcome was known. Live birth was the primary outcome. Secondary outcomes were clinical pregnancy, ongoing pregnancy, miscarriage, implantation rate, number of embryos and number of sperm cells retrieved.

Miscarriage was defined as a clinical pregnancy (with registered heartbeat) that miscarried before 12 weeks of gestation. Ongoing pregnancy was defined as a viable pregnancy of at least 12 weeks gestation. Implantation rate was calculated as number of embryonic sacs/number of embryos transferred.

Further data that were collected were female age at time of ICSI, number of oocytes retrieved, and whether fresh or frozen sperm were used for ICSI.

No data were available on testicular volume, markers for epididymal function (e.g. alpha glucosidase) or FSH values for the included men.

Statistical analysis

Differences between MESA and TESE groups were tested for significance using analysis of variance for continuous variable and chi-square test statistics for categorical data.

Our primary analysis focused on differences between MESA and TESE in live birth, correcting for heterogeneity in clinical variables between the groups. We used multivariable logistic regression models that included female age and number of oocytes (to control for the female factor) as well as the use of fresh or frozen sperm for ICSI. Data were analysed using SPSS 19.

Results

Between 2000 and 2009, a total of 374 first ICSI cycles were performed in couples in which the man had obstructive azoospermia. In 280 (75%) of these cases, MESA had been used to retrieve spermatozoa and in 94 cases (25%), TESE had been performed.

There were no significant differences between MESA and TESE cases in terms of age of the female partner, number of oocytes retrieved, number of embryos, or number embryos transferred (Table I). As expected due to the nature or the procedure, more sperm was found using MESA than using TESE ($P < 0.001$). Similarly, frozen sperm accounted for a significantly higher proportion of cycles in the MESA group compared with the TESE group (60 versus 15%, $P < 0.001$), for the obvious reason that when vasectomy reversal is performed, we preferred freezing epididymal to testicular sperm.

The live birth rate after MESA-ICSI was significantly higher than after TESE-ICSI (39 versus 24%, $P = 0.011$). The clinical and ongoing pregnancy rates after MESA-ICSI (47 and 39%, respectively) were also significantly higher than after TESE-ICSI (30 and 24%, respectively). The implantation rate per embryo transferred was 22% after MESA-ICSI and 15% after TESE-ICSI ($P = 0.035$).

The regression analysis to assess the association between MESA or TESE and live birth is shown in Table II. In a univariable logistic analysis, we found female age, whether MESA or TESE, was performed, and the number of oocytes, to be significantly associated with live birth. The unadjusted odds ratio for live birth was 2.0 (95% CI 1.16–3.34) for MESA versus TESE. In multivariable analysis, MESA still resulted in a significantly higher live birth rate than did TESE. Adjusted for the available confounders, the odds ratio for live birth rate was 1.82 (95% CI 1.05–3.67) after MESA versus TESE. There was no indication of an interaction between use of fresh or frozen sperm and live birth rates following MESA or TESE. That is, there was no difference in the results related to whether fresh sperm or frozen sperm were used, or whether the obstruction was caused by CBVAD or vasectomy. Thus this difference between ICSI with testis sperm or with epididymal sperm was robust despite any other variables.

Discussion

This study may have clinical as well as basic science implications. In first ICSI cycles of couples with obstructive azoospermia, the use of epididymal spermatozoa resulted in a significantly higher live birth rate than did the use of testis spermatozoa. The chance of reaching a live birth was 39% following MESA-ICSI and 24% following TESE-ICSI. Adjusted for the available confounders, the odds ratio for ongoing pregnancy rate was 1.82 (95% CI 1.05–3.67) for MESA versus TESE.

Table I Couples' characteristics and treatment outcomes in the MESA and TESE ICSI-ET cycles due to obstructive azoospermia.

	MESA N = 280	TESE N = 94	P-value
Female age, mean (95% CI)	33.3 (32.7–33.9)	33.8 (32.7–34.8)	0.45
Spermatozoa retrieved, *N* (%)			
Less than 0.1×10^6	213 (76%)	88 (94%)	<0.001
0.1×10^6 up to 1×10^6	35 (13%)	6 (6%)	
More than 1×10^6	32 (11%)	0	
Fresh or frozen sperm, *N* (%)			
Fresh sperm	112 (40%)	80 (85%)	<0.001
Frozen sperm	168 (60%)	14 (15%)	
Oocytes, mean (95% CI)	13.9 (12.9–14.9)	13.0 (11.3–14.6)	0.32
Embryos, mean (95% CI)	6.9 (6.4–7.4)	6.3 (5.3–7.3)	0.26
Number of embryos transferred, mean (95% CI)	3.4 (3.2–3.6)	3.5 (3.1–3.8)	0.68
Implantation rate, mean (95% CI)	0.22 (0.19–0.26)	0.15 (0.10–0.20)	0.037
Clinical pregnancy, *N* (%)	132 (47%)	29 (30%)	0.005
Miscarriage, *N* (%)	23 (8%)	6 (6%)	0.57
Live birth, *N* (%)	109 (39%)	23 (24%)	0.011
Multiple pregnancy, *N* (%)	33 (30%)	10 (43%)	0.21

Table II Univariable and multivariable logistic regression to determine the association between the sperm retrieval method and ongoing pregnancy, controlled for female age, oocytes and sperm count.

	Univariable logistic analysis			Multivariable logistic analysis		
Screening parameters	$OR_{unadjusted}$	95% CI	P-value	$OR_{adjusted}$	95% CI	P-value
MESA versus TESE	2.00	1.22–3.29	0.01	1.82	1.05–3.67	0.01
Female age	0.92	0.88–0.97	<0.001	0.93	0.90–0.97	0.007
Number of oocytes	1.07	1.04–1.10	<0.001	1.06	1.03–1.09	<0.001
Fresh versus frozen sperm	0.73	0.48–1.11	0.14	0.72	0.43–1.22	0.22

Note. There was no indication for interaction between MESA/TESE and whether fresh or frozen sperm was used.

This is the first study showing that MESA-ICSI is more effective than TESE-ICSI in terms of chance for live birth while accounting for the most important potential confounders in a unique cohort of 374 couples with obstructive azoospermia (OA) and normal spermatogenesis. The data were collected from a single clinic and a single surgeon. This makes the data very robust as there will be only minor heterogeneity in the procedures performed. There was no difference between fresh or frozen sperm and thus no difference between CBVAD and vasectomy as causes of OA. Since the development of ICSI technology, pregnancy rates of partners of CBAVD patients have been comparable to that of the general ICSI population (Viville *et al.*, 2000; Silber, 2010). This allows pregnancy rates to be compared based on the location from where the sperm was collected since ICSI eliminates a variance in pregnancy rates based on different aetiology of OA.

When ICSI and sperm retrieval for azoospermia was first introduced, the high success rate with testicular sperm led to an assumption that with ICSI, sperm origin was of no significance (Silber *et al.*, 1994, 1995, 1996; Devroey *et al.*, 1995, 1996; Beharka *et al.*, 2006). This assumption unfortunately led to less research on epididymal physiology. Yet many prior decades of studies had revealed the importance of that transition in sperm from the testis to epididymis (Bedford, 1963, 1965, 1978a, b, 1994, 2004; Calvin and Bedford, 1971; Horan and Bedford, 1972; Bedford *et al.*, 1973; de Larminat *et al.*, 1978; Garberi *et al.*, 1982; Gonzalez Echeverria *et al.*, 1982; Tezon and Blaquier, 1983; Schoysman and Bedford, 1986; Phillips and Bedford, 1987; Holland and Orgebin-Crist, 1988; Silber *et al.*, 1990a,b; Ong *et al.*, 2000; Robaire *et al.*, 2006). We and other originally thought that this transition from testis to epididymis was irrelevant in the era of ICSI (Devroey *et al.*, 1996). But the current study suggests otherwise as pregnancy rates and live birth rates were higher when epididymal sperm instead of testicular sperm was used.

The present study however has limitations. We failed to differentiate male age and the date since vasectomy if performed. The effects of these potentially confounding or modifying factors on our findings therefore remain unknown. However, all frozen 'first' cycles were for cases of

attempted but failed vasectomy reversal, in which the males were always older, yet there was no difference in results between 'fresh' and 'frozen' sperm. Therefore male age is not likely to be a significant confounder. That the time since vasectomy does not seem to be of great importance was also observed by another study (Silber and Grotjan, 2004). In this study the age of the male was not associated with the chance of pregnancy rate (Silber and Grotjan, 2004).

Another limitation of the present study is that TESE was done in those men in whom MESA was not feasible or when MESA did not result in any sperm due to epididymal blockage. Although not likely, this subgroup of azoospermic men could in theory have a different fertility potential compared with azoospermic men without blockage or in whom MESA is feasible.

A further limitation is that no data were available on testicular volume or on the levels of FSH or markers for epididymal function for the included men. These factors may have had effects on success rates after MESA or TESE. Besides its apparent advantage in terms of more live births, MESA for OA has several additional advantages above TESE. MESA usually results in a higher yield of spermatozoa than TESE such that excess cells can be frozen and stored for later use for a next ICSI cycle (Beharka *et al.*, 2006; Diemer *et al.*, 2011). Therefore, as TESE may sometimes need to be repeated multiple times in case of treatment failure, a single MESA procedure will be sufficient for all treatments necessary. The disadvantage of MESA is mainly that it is more time consuming and surgically challenging than TESE.

PESA (percutaneous epididymal sperm aspiration) is an alternative to MESA for centres with no microsurgical expertise. It may be more difficult to find the proximal-most, least senescent sperm with PESA than with MESA, but if successful, then PESA would most likely give similar results as MESA, but smaller numbers of sperm to freeze. In both PESA and MESA, if only distal senescent epididymal sperm are used, this would give very poor results (Silber *et al.*, 1994; Tournaye *et al.*, 1994).

In obstructive azoospermia due to vasectomy, vasectomy reversal is an effective alternative to MESA or TESE. In a decision scenario analysis, vasectomy reversal was more cost-effective than percutaneous TESE and MESA for treatment of obstructive azoospermia (Lee *et al.*, 2008). MESA and ICSI is the preferred treatment option in obstructive azoospermia only where such reconstruction is not available or has failed. Our study suggests that only in those cases where virtually no epididymal spermatozoa are present, TESE should be performed for sperm retrieval for OA.

Based on our observations, although perfectly capable of fertilizing oocytes via ICSI (i.e. the same number of embryos were found after TESE-ICSI as after MESA-ICSI), testicular sperm apparently do not support optimal embryo development as well as epididymal sperm do. This may be because epididymal sperm maturation contributes to proper embryo development for a large variety of reasons which need to be studied: i.e. the contribution of paternal RNAs to early embryo development, effects on the mitotic spindle of the resulting embryo, or disulphide bonding or ultra-structural changes already described in the literature previously alluded to. Indeed, we have previously found that embryos generated via TESE-ICSI had extremely high levels of chaotic chromosomal aneuploidies (Schatten *et al.*, 1986; Schatten, 1994; Silber *et al.*, 2003).

In conclusion, it appears that in cases of obstructive azoospermia with normal spermatogenesis, epididymal sperm may be more effective than testicular sperm even with the utilization of ICSI techniques.

Authors' roles

S.J.S. provided the initial idea for the study; M.v.W. performed the statistical analysis; S.R., N.B. and S.J.S. wrote the draft manuscript; and all authors critically reviewed the data, discussed the outcomes and edited the manuscript.

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Conflict of interest

None declared.

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