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Male Infertility

ORIGINAL ARTICLE

A comparison of the relative efficiency of ICSI and extended culture with epididymal sperm versus testicular sperm in patients with obstructive azoospermia

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This is a retrospective cohort study comparing blastocyst transfer outcomes following intracytoplasmic sperm injection utilizing epididymal versus testicular sperm for men with obstructive azoospermia. All cases at a single center between 2012 and 2016 were included. Operative approach was selected at the surgeon's discretion and included microepididymal sperm aspiration or testicular sperm extraction. Blastocyst culture was exclusively utilized prior to transfer. The primary outcome was live birth rate. Secondary outcomes included fertilization rate, blastulation rate, euploidy rate, and implantation rate. A mixed effects model was performed. Seventy-six microepididymal sperm aspiration cases and 93 testicular sperm extraction cases were analyzed. The live birth rate was equivalent (48.6% *vs* 50.5%, *P* = 0.77). However, on mixed effects model, epididymal sperm resulted in a greater likelihood of fertilization (adjusted OR: 1.37, 95% CI: 1.05–1.81, *P* = 0.02) and produced a higher blastulation rate (adjusted OR: 1.41, 95% CI: 1.1–1.85, *P* = 0.01). As a result, the epididymal sperm group had more supernumerary blastocysts available (4.3 *vs* 3, *P* < 0.05). The euploidy rate was no different. Pregnancy rates were no different through the first transfer cycle. However, intracytoplasmic sperm injection following microepididymal sperm aspiration resulted in a greater number of usable blastocysts per patient. Thus, the true benefit of epididymal sperm may only be demonstrated via a comparison of cumulative pregnancy rates after multiple transfers from one cohort.

INTRODUCTION

The utilization of surgery as a means to obtain functional spermatozoa for use in assisted reproduction has revolutionized the care of men with obstructive azoospermia (OA). By combining operative techniques with micromanipulation in the embryology laboratory, men with OA can now be successfully treated and achieve high pregnancy rates in most infertility practices.

However, there is still a lack of consensus regarding the optimal surgical technique for sperm retrieval in men with OA. Publications from the American Society for Reproductive Medicine reflect this controversy, stating that "the best technique for sperm aspiration for men with obstructive azoospermia has not been determined".[1] The epididymal approach has long been intriguing, given the theory that sperm retrieved more distally show relatively higher developmental maturity and exhibit greater motility. Early studies comparing

microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE) encouraged the preference toward MESA, demonstrating higher fertilization and pregnancy rates with epididymal sperm.[2] The superiority of MESA sperm was also recently supported in a large cohort of surgical sperm retrievals for men with OA.[3]

Nevertheless, as more data have accumulated, the superiority of epididymal sperm has been called into question. A meta-analysis of ten studies reported no difference in fertilization or pregnancy rates between epididymal and testicular sperm in OA cases.[4] Furthermore, some reports have suggested that sperm retrieved from the epididymis are more likely to display elevated rates of DNA fragmentation and mitochondrial DNA deletions.[5] Recent clinical data have supported this notion, providing evidence that testicular retrieval of sperm may be associated with improved outcomes in men with a history of ejaculated samples demonstrating high levels of DNA fragmentation.[6]

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Indeed, even in OA men without prior evidence of elevated DNA fragmentation, testicular sperm has been reported to have better DNA integrity and produce improved clinical outcomes.[7]

One limitation of the current literature assessing testicular and epididymal sperm is the paucity of data on embryologic development parameters in programs utilizing extended embryo culture. Of the available studies, only observational data are available regarding blastulation of embryos derived from testicular and epididymal sperm in men with OA.[8] Comparative data would prove valuable for many reasons. First, given activation of the embryonic genome around day 3, data on successful conversion to blastocyst may provide some insight into the male contribution to postcompaction embryonic development. Second, an increasing number of *in vitro* fertilization (IVF) programs are utilizing extended culture for multiple reasons:

1. As an embryo selection tool to facilitate single embryo transfer[9]
2. To utilize trophectoderm biopsy and genetic testing for aneuploidy screening or single gene disorders[10]
3. To allow active management of embryo-endometrial synchrony.[11]

With this in mind, this study sought to compare the laboratory and pregnancy outcomes between embryos derived from a testicular versus an epididymal approach in a program exclusively utilizing extended culture.

MATERIALS AND METHODS

All cases including surgical sperm retrieval for OA in a single center between 2012 and 2016 were evaluated. This study period was selected as all embryos in this center were cultured to the blastocyst stage during the study period. Cases were identified by review of a couple's chart upon presentation to the reproductive endocrinologist's office. If the nature of azoospermia (nonobstructive *vs* obstructive) was not clear from chart review, the case was not included in the analysis. Cases involving preimplantation genetic diagnosis for single gene or translocation defects and cases involving donor oocytes were excluded. This study was performed in accordance with Advarra institutional review board (IRB) approval and guidelines.

Only the first oocyte retrieval and transfer cycles were analyzed to avoid previous failure bias. Surgical procedures were performed by multiple surgeons during the study period, and operative approach was selected according to individual surgeon preference. Operative reports for each case were not available for review, and preferred method of retrieval was not able to be determined by each individual urologic surgeon. However, method of retrieval was recorded in each case and included in the IVF chart. In general, MESA was attempted initially and TESE was performed if no spermatozoa were retrieved by epididymal approach or scarring and fibrosis following vasectomy precluded epididymal retrieval. The majority of cases during the study period utilized cryopreserved samples. As a result, only cases utilizing cryopreserved samples were included in the analysis to promote homogeneity in the comparison.

IVF/ICSI and embryo transfer

Controlled ovarian hyperstimulation cycles were conducted utilizing a GnRH antagonist, long GnRH agonist, or GnRH microflare protocol. Oocyte retrieval was performed 36 h after inducing final oocyte maturation with GnRH agonist or human chorionic gonadotropin. Cumulus stripping was performed approximately 3 h after oocyte retrieval with hyaluronidase. Intracytoplasmic sperm injection (ICSI) was performed approximately 2 h later. Fertilization check was performed approximately 18 h later.

A sequential culture system was utilized for blastocyst culture. Laser-assisted hatching was performed on day 3 of development, and

all embryos were placed in extended culture. Assessment for embryo transfer or cryopreservation was made on day 5 and day 6. Only embryos achieving a 4CC grade or better by Modified Gardner scoring were considered eligible for transfer or vitrification.[12] Based on this scoring system, expansion grade 4 was consistent with an expanded blastocyst, a cavity larger than the embryo, and thinning of the shell. Inner cell mass (ICM) grade C was consistent with very few cells, and trophectoderm (TE) grade C was consistent with very few large cells. If patients utilized preimplantation genetic screening for aneuploidy, trophectoderm biopsies occurred on either day 5 or 6. Embryos were vitrified for one of the following reasons:

1. To minimize ovarian hyperstimulation risk
2. Promote embryo-endometrial synchrony
3. Or to await results of aneuploidy screening.

In the case of a fresh transfer, all surplus embryos were vitrified for future use. In the cases of frozen embryo transfer, endometrial preparations followed standard institution protocols. These consisted primarily of oral estrogen followed by progesterone in oil injections. However, alternative regimens, including natural transfer cycle protocols, are considered and utilized on an individual patient basis.

Data collection and analysis

The primary outcome of this study was live birth rate per cycle. For this outcome, only the first transfer was considered to avoid previous failure bias. Secondary outcomes included fertilization rate, blastulation rate (number of usable blastocysts per 2PN), aneuploidy rates, and sustained implantation rate (presence of fetal cardiac activity beyond 8-week gestation).

Additional data collected and analyzed included female and male age, number of oocytes collected, and fresh versus frozen transfer. Etiology of male obstruction (prior vasectomy, congenital bilateral absence of the vas deferens [CBAVD], or other inflammatory or postsurgical obstruction) was also obtained.

Categorical variables were compared using Chi-squared analysis. Continuous variables were compared using Student's *t*-test when distribution was normally distributed and Mann–Whitney U test when not normally distributed. The primary outcome of live birth rate (LBR) and secondary outcomes of fertilization rate and blastulation rate were compared between oocytes and embryos derived from testicular versus epididymal sperm utilizing a mixed effects model. This strategy was employed to account for the effect of female age, cohort size, and to account for correlation between oocytes and embryos derived from the same patient.

RESULTS

A total of 169 cases met criteria for inclusion in the analysis. Of these, MESA was utilized in 45.0% (76/169) of cases and TESE was utilized in 55.0% of cases (93/169). Primary cycle characteristics comparing the couples who utilized MESA versus TESE sperm for ICSI are described in **Table 1**.

The LBR rate after the first embryo transfer was no different between the groups (48.6% *vs* 50.5%, $P = 0.77$). However, the overall fertilization rate after ICSI was higher when MESA sperm were utilized (78.3% *vs* 71.5%, $P < 0.01$). The percentage of 2PNs that converted to the usable blastocyst stage was also higher when MESA sperm were utilized (58.6% *vs* 49.3%, $P < 0.01$). As a result, the number of supernumerary blastocysts vitrified for potential future use was higher in the MESA group (**Table 2**).

To correct for correlations between oocytes derived from the same patient and to account for the effect of age on the likelihood of blastulation, a mixed effects logistic regression was also performed.

Table 1: Patient and cycle characteristics for frozen microsurgical epididymal sperm aspiration versus frozen testicular sperm extraction cases in men with obstructive azoospermia

Obstructive azoospermia case	Frozen MESA	Frozen TESE	P
Cycles (n)	76	93	
Demographics			
Female partner age (year)[a], mean±s.d.	34.3±4.7	34.9±4.9	0.45
Male partner age (year), mean±s.d.	39.6±9.0	40.5±9.9	0.55
Cycle characteristics			
Maximum day 3 FSH (IU ml^{-1}), mean±s.d.	8.5±3.3	8.3±3.2	0.22
Number of oocytes retrieved[b], median (1st to 3rd quartile range)	11 (7–21)	12 (6–18)	0.21
Postvasectomy, n (%)	40 (52.6)	38 (40.8)	0.17
CBAVD, n (%)	28 (36.8)	35 (37.6)	0.99
Other causes of obstructive azoospermia, n (%)	8 (10.5)	20 (21.5)	0.09
Cycles utilizing PGS, n (%)	34 (50.0)	35 (45.4)	0.77
Cycles undergoing fresh embryo transfer, n (%)	15 (19.7)	13 (13.9)	0.42
Cycles undergoing frozen embryo transfer, n (%)	61 (80.3)	80 (86.1)	0.42

[a]Age recorded on the day of oocyte retrieval of the first ICSI cycle. [b]Total number of oocytes obtained during vaginal oocyte retrieval. TESE: testicular sperm extraction; MESA: microsurgical epididymal sperm aspiration; CBAVD: congenital bilateral absence of the vas deferens; PGS: pre-implantation genetic screening; FSH: follicle-stimulating hormone; s.d.: standard deviation

Table 2: Laboratory and pregnancy outcomes of patients using frozen microsurgical epididymal sperm aspiration versus frozen testicular sperm extraction sperm for intracytoplasmic sperm injection in men with obstructive azoospermia

Obstructive azoospermia cases	Frozen MESA	Frozen TESE	P
Cycles (n)	76	93	
Embryologic characteristics			
Fertilization rate[a] (%)	78.3	71.5	<0.01
Usable blastocysts[b] (%)	58.6	49.3	<0.01
Euploidy rate (%)	69.6	67.4	0.74
Number of embryos transferred, mean	1.31	1.42	0.21
Reproductive outcomes			
Live birth rate per stimulation cycle start, n (%)	37 (48.6)	47 (50.5)	0.77
Sustained implantation rate, n (%)	57 (57.6)	73 (55.2)	0.84
Supernumerary blastocysts, median (1st to 3rd quartile range)	4 (2–7)	3 (2–6)	0.04

[a]Number of 2PN divided by number of oocytes that underwent ICSI; [b]Number of usable blastocysts divided by number of 2PN. TESE: testicular sperm extraction; MESA: microsurgical epididymal sperm aspiration; 2PN: two pronuclei

Table 3: Mixed effects logistic regression comparing frozen microsurgical epididymal sperm aspiration versus frozen testicular sperm extraction for intracytoplasmic sperm injection in men with obstructive azoospermia

Cycle outcome (MESA vs TESE)	Adjusted OR	95% CI	P
Live birth rate	0.97	0.68–1.81	0.73
Fertilization rate	1.37	1.05–1.81	0.02
Usable blastocyst per 2PN	1.41	1.10–1.85	0.01

TESE: testicular sperm extraction; MESA: microsurgical epididymal sperm aspiration; ICSI: intracytoplasmic sperm injection; CI: confidence interval; OR: odds ratio; 2PN: two pronuclei

The adjusted OR for live birth rate was no different between MESA- and TESE-derived sperm. However, the adjusted OR for fertilization and conversion to usable blastocyst was increased for MESA-derived sperm (**Table 3**).

DISCUSSION

These data demonstrate no improvement in live birth rate among blastocysts derived from testicular versus epididymal source after the first transfer in men with obstructive azoospermia. However, oocytes injected with epididymal sperm were more likely to successfully fertilize the oocyte and produce a clinically usable blastocyst. As a result, epididymal sperm produced superior efficiency per oocyte in a laboratory exclusively utilizing extended culture.

These findings are important for programs utilizing extended culture as a means to promote synchrony, enhance embryo selection, or utilize genetic diagnostics. No study to date has compared embryologic parameters in blastocyst-only transfer settings. While the outcomes of the first transfer appear no different between sperm sources, the true benefit of epididymal sperm may only be seen if cumulative pregnancy rates are assessed on all embryos from a single cohort. Furthermore, patients interested in having multiple children from one retrieval cycle may also see the overall benefit of epididymal sperm in extended culture as more blastocysts may be available for a second transfer. In clinical practice, assessing the overall reproductive potential of an entire cohort is difficult to do as many patients have different plans regarding the number and timing of subsequent transfers. These data serve as a glimpse into the current state of a cohort of patients after the first embryo transfer.

The data support some previous studies comparing epididymal to testicular sperm, although this study provides a new degree of insight into outcomes following blastocyst transfer rather than day-3 transfer. A recent study by van Wely *et al.*[3] demonstrated a superior pregnancy rate among embryos created with epididymal sperm in men with OA. In the van Wely report, all transfers occurred on day 3 of development and the average number of transferred embryos was 3.4 in the MESA group and 3.5 in the TESE group. The adjusted odds ratio of live birth in a multivariable logistic regression demonstrated greater success with MESA-derived sperm (adjusted OR: 1.82, 95% CI: 1.05–3.67, $P = 0.01$). The data presented in our study demonstrate the possibility of utilizing extended culture to reduce transfer order as only 1.3 and 1.4 embryos were transferred on average in this cohort. While the current study did not demonstrate an improvement in live birth per cycle start, the greater odds of blastulation and larger number of supernumerary blastocysts support the notion of improved developmental potential with epididymal sperm. Accumulation of pregnancies with multiple transfers may ultimately support van Wely's data, although confirmation of these findings requires further study.[3] In addition, while the MESA and TESE groups did not appear to differ in our

study based on the characteristics of male and female age on the date of oocyte retrieval, number of oocytes retrieved, day-3 follicle-stimulating hormone (FSH) level, and other factors included in **Table 1**, a more comprehensive comparison of patient characteristics may be worthwhile in future studies that investigate this issue.

The data presented in our study also have important biological implications for understanding the contribution of the male gamete to successful embryogenesis. Differential fertilization and blastulation success suggests that the male contribution to embryogenesis impacts both early and late events in preimplantation development. However, once an embryo successfully becomes a high-quality blastocyst and is transferred, there is no difference in embryo performance.

It is also important to highlight recent reports, suggesting that testicular sperm may harbor less DNA fragmentation than epididymal sperm.[6] This intriguing line of research has suggested that, in men with evidence of high levels of DNA fragmentation, retrieving testicular sperm may improve outcomes as high DNA fragmentation is associated with poor embryo development and pregnancy loss.[13–15] The important difference between these data and our study is the population in question. Men with known high levels of DNA fragmentation in ejaculated sperm inherently differ from those with OA. However, if men with OA demonstrate poor embryologic or pregnancy outcomes after epididymal sperm retrieval, it may be useful to study DNA fragmentation from a portion of retrieved sperm and consider testicular retrieval in subsequent cycles if such damage exists. Indeed, data now exist that support the notion that elevated DNA fragmentation may also be present in epididymal sperm of OA men, even without prior evidence of high DNA fragmentation levels.[7]

The retrospective nature of this study introduces some limitations to the findings. It is likely that, in many cases, TESE was performed only after an epididymal approach failed to yield any sperm. As a result, the urologic surgeon was not necessarily presented with an option between epididymal versus testicular retrieval. Thus, while per oocyte efficiency in the laboratory may have been decreased in cases in which TESE was used, many patients had no other option to generate spermatozoa for ICSI.

In addition, the lack of details related to the male evaluation such as hormone levels and testicular volume introduces the possibility that patients with no sperm present in the epididymis may have also had an element of impaired spermatogenesis that necessitated testicular retrieval. While all attempts were made to clarify a diagnosis of NOA or OA prior to inclusion in the study, it is possible that the number of patients requiring TESE in this study was higher than expected due to unrecognized, unintentional inclusion of NOA patients. It is well documented from studies comparing outcomes between men with NOA and OA that impaired spermatogenesis is a risk factor for poor embryologic development.[16] In addition, it has been previously shown that azoospermic men with congenital bilateral absence of the vas deferens (CBAVD) who have undergone a TESE procedure for sperm extraction exhibit normal spermatogenesis in only 38.8% of cases, whereas an impaired spermatogenesis can be observed in 61.1% of cases. Fertilization rates have also been shown to be significantly lower in hypospermatogenic men with CBAVD than in normospermatogenic men following TESE.[17] In this study, the TESE group may have been overrepresented with cases comprised some element of spermatogenic dysfunction because TESE was only performed after a failed epididymal approach. This overrepresentation may have influenced the results.

As more programs utilize extended culture, there is a greater need for understanding of factors that influence the likelihood of an embryo reaching the blastocyst stage. Given the lack of consensus regarding the ideal approach to surgical sperm retrieval in OA patients, data on postcompaction embryo development in these patients is valuable in helping clarify the ideal treatment for each patient. While a direct comparison of fresh and frozen embryo transfers using different sperm retrieval approaches would also be informative, the relatively small number of frozen embryo transfers in this patient population was prohibitive. These data suggest a benefit to the epididymal approach.[18] However, prospective data are needed to confirm these findings.

AUTHOR CONTRIBUTIONS

All authors contributed in a meaningful way to the creation of this manuscript. SJM was the primary author involved with generation of the concept of the study, manuscript writing, and data analysis. BMH, CRJ, and SAN assisted with data analysis and performed a majority of the manuscript writing and editing. BMH also assisted with formatting for manuscript submission. JNL performed raw data extraction and assisted with data analysis. RTS and JMH oversaw the project and provided feedback regarding the overall concept. They also checked the statistical analysis and data and performed manuscript editing.

COMPETING INTERESTS

All authors declared no competing interests.

REFERENCES

1   The Practice Committee of the American Society for Reproductive Medicine. Sperm retrieval for obstructive azoospermia. *Fertil Steril* 2008; 90: S213–8.
2   Palermo GD, Schlegel PN, Hariprashad JJ, Ergun B, Mienik A, *et al*. Fertilization and pregnancy outcome with intracytoplasmic sperm injection for azoospermic men. *Hum Reprod* 1999; 14: 741–8.
3   van Wely M, Barby N, Meissner A, Repping S, Silber SJ. Live birth rates after MESA or TESE in men with obstructive azoospermia: is there a difference? *Hum Reprod* 2015; 30: 761–6.
4   Nicopoullos JD, Gilling-Smith C, Almeida PA, Norman-Taylor J, Grace I, *et al*. Use of surgical sperm retrieval in azoospermic men: a meta-analysis. *Fertil Steril* 2004; 82: 691–701.
5   O'Connell M, McClure N, Lewis SE. Mitochondrial DNA deletions and nuclear DNA fragmentation in testicular and epididymal sperm. *Hum Reprod* 2002; 17: 1565–70.
6   Esteves SC, Roque M, Bradley CK, Garrido N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. *Fertil Steril* 2017; 108: 456–67.
7   Hammoud I, Bailly M, Bergere M, Wainer R, Izard V, *et al*. Testicular spermatozoa are of better quality than epididymal spermatozoa in patients with obstructive azoospermia. *Urology* 2017; 103: 106–11.
8   Balaban B, Urman B, Isiklar A, Alatas C, Mercan R, *et al*. Blastocyst transfer following intracytoplasmic injection of ejaculated, epididymal, or testicular spermatozoa. *Hum Reprod* 2001; 16: 125–9.
9   Maheshwari A, Hamilton M, Bhattacharya S. Should we be promoting embryo transfer at blastocyst stage. *Reprod Biomed Online* 2016; 32: 142–6.
10   Scott RT Jr., Upham KM, Forman EJ, Hong KH, Scott KL, *et al*. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases *in vitro* fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril* 2013; 100: 697–703.
11   Franasiak JM, Ruiz-Alonso M, Scott RT, Simon C. Both slowly developing embryos and a variable pace of luteal endometrial progression may conspire to prevent normal birth in spite of a capable embryo. *Fertil Steril* 2016; 105: 861–6.
12   Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril* 2000; 73: 1155–8.
13   Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. *Hum Reprod* 2004; 19: 611–5.
14   Seli E, Gardner DK, Schoolcraft WB, Moffatt O, Sakkas D. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after *in vitro*

226

fertilization. *Fertil Steril* 2004; 82: 378–83.

15 Zini A, Boman JM, Bezile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. *Hum Reprod* 2008; 23: 2663–8.

16 Vernaeve V, Tournaye H, Osmanagaougly K, Verheyen G, Van Steirteghem A, *et al*. Intracytoplasmic sperm injection with testicular spermatozoa is less successful in men with nonobstructive azoospermia than in men with obstructive azoospermia. *Fertil Steril* 2003; 79: 529–33.

17 Llabador MA, Pagin A, Lefebvre-Maunoury C, Marcelli F, Leroy-Martin B, *et al*. Congenital bilateral absence of the vas deferens: the impact of spermatogenesis quality on intracytoplasmic sperm injection outcomes in 108 men. *Andrology* 2015; 3: 473–80.

18 Morin SJ, Juneau CR, Neal SA, Landis JN, Scott RT Jr., *et al*. Comparison of the relative efficiency of ICSI and extended culture with epididymal sperm versus testicular sperm in patients with obstructive azoospermia. *Fertil Steril* 2017; 108: Se6.