Sexual Function/Infertility

Reproductive Potential of Men with Obstructive Azoospermia Undergoing Percutaneous Sperm Retrieval and Intracytoplasmic Sperm Injection According to the Cause of Obstruction

Sandro C. Esteves,* Walter Lee, David J. Benjamin, Bill Seol, Sidney Verza, Jr. and Ashok Agarwal

From the Androfert, Center for Male Reproduction (SCE, BS, SV), Campinas, Brazil, and Center for Reproductive Medicine, Cleveland Clinic Foundation (WL, DJB, AA), Cleveland, Ohio

Abbreviations and Acronyms

CBAVD = congenital bilateral absence of vas deferens

FSH = follicle-stimulating hormone

ICSI = intracytoplasmic sperm injection

LH = luteinizing hormone

OA = obstructive azoospermia

PESA = percutaneous epididymal sperm aspiration

SR = sperm retrieval

SRR = SR rate

TESA = testicular sperm aspiration

Purpose: We evaluated the retrieval rates and reproductive outcomes of percutaneous sperm retrieval according to the cause of obstructive azoospermia.

Materials and Methods: We retrospectively studied the records of 146 men with obstructive azoospermia who underwent sperm retrieval for intracytoplasmic sperm injection. Patients were grouped by the cause of obstruction, including 32 with congenital bilateral absence of the vas deferens, 59 with vasectomy and 55 with obstruction due to post-infection disease. Sperm were retrieved percutaneously from the epididymis or testis. We compared retrieval rates and intracytoplasmic sperm injection outcomes, including neonatal results, in the groups of men with obstructive azoospermia.

Results: The success of sperm retrieval was similar among the etiology groups, including 100% for congenital bilateral absence of the vas deferens, 96.6% for vasectomy and 96.3% for previous infection. Significantly fewer men in the congenital bilateral absence of the vas deferens group needed testicular aspiration compared to those in the post-infection and vasectomy groups (3.1% vs 23.6% and 30.5%, respectively, p <0.001). Sperm cryopreservation was possible in 26.7% of the cases and did not significantly differ among the groups. Live birth rates after sperm injection were similar in the congenital (34.4%), vasectomy (32.2%) and previous infection (36.4%) groups. Birth parameters, prematurity and low birth weight rates were comparable among the groups.

Conclusions: Percutaneous sperm retrieval is an effective method to retrieve sperm in men with obstructive azoospermia irrespective of the cause of obstruction. The chance of achieving a live birth and the profile of neonates born after sperm injection do not seem to be related to the cause of obstruction.

Key Words: testis; infertility, male; sperm injections, intracytoplasmic; pregnancy; infant, newborn

AZOOSPERMIA is found in about 1% of all men and 10% to 15% of infertile men.[1] OA is attributable to mechanical blockage, which can occur anywhere along the reproductive tract, including the vas deferens, epididymis and ejaculatory duct.[1] OA is considered one of the most favorable prognostic conditions for male infertility since spermatogenesis is not disrupted, unlike in nonobstructive azoospermia.

Obstruction in the male reproductive system can be congenital or acquired. The most common cause of ac-

quired OA is vasectomy. Vasectomy reversal is generally considered a cost-effective treatment that results in the ability to conceive naturally.[2] Other OA acquired causes include infection and trauma. The most common congenital form of OA is CBAVD, which is linked to mutations in the cystic fibrosis transmembrane-conductance regulator gene.[3]

Despite being highly successful, microsurgical reconstruction may not be indicated in all men with OA, such as those with CBAVD and in certain men with post-infectious obstruction or failed vasectomy reversal. In such cases sperm retrieval can be performed for use with ICSI. Of the multiple sperm retrieval techniques we focused on 2 particular methods in this study, that is PESA and TESA. The 2 techniques are similar in that they require a needle to be percutaneously inserted into the respective sperm source.[4] In PESA the goal is to obtain epididymal fluid, which should contain sperm. In TESA the seminiferous tubules are removed. After sperm retrieval ICSI is done rather than standard in vitro fertilization because ICSI results in a significantly higher fertilization rate.[5]

The literature is rich in studies of the outcomes of sperm acquisition and ICSI.[5–18] Nevertheless, few groups have reported SR outcomes in men with OA according to obstruction etiology.[13,14,16–18] This is important to determine because it will help infertility physicians counsel such patients on the chances of SR and ICSI. Moreover, data on neonates born after sperm injection in OA cases are sparse and also lacking in detail as to the cause of obstruction.[16,19–22]

We compared percutaneous sperm retrieval and ICSI outcomes in men with OA according to the cause of obstruction. Neonatal outcomes of pregnancies in these categories of male infertility are provided.

MATERIALS AND METHODS

Patient Selection
We retrospectively analyzed the records of 146 consecutive patients with OA who underwent percutaneous SR and ICSI from January 2002 to December 2008. Patients were grouped by obstruction etiology, including group 1—32 with CBAVD, group 2—59 with vasectomy and group 3—55 after infection. The results of a complete evaluation, including history, physical examination, and hormone profile with serum FSH, LH and total testosterone were available for all patients. Genetic screening for CFTR gene mutations was performed in all men with CBAVD.[3] Azoospermia was confirmed on at least 2 centrifuged ejaculates according to WHO guidelines.[23] Signed informed consent was obtained from patients to use data for analysis. The study was approved by our institutional review board. Only the first ICSI cycle of each patient using fresh sperm for injections was included in analysis.

Sperm
Acquisition. A single senior urologist (SCE) performed all sperm retrieval. Our approach was to attempt PESA first. If we failed to retrieve motile sperm by PESA after at least 2 attempts in each epididymis, TESA was used.[4] Retrieval was done on an outpatient basis with the patient under local anesthesia in association with an intravenous bolus infusion of propofol. We used loupe magnification during aspiration to avoid small vessels seen through the skin. Aspirated epididymal fluid or testicular tissue was flushed into a tube containing sperm medium and sent to the embryology laboratory for examination. Successful retrieval was defined as the presence of motile sperm.

Sperm injection. Ovarian stimulation, oocyte retrieval, sperm processing, sperm injection and embryo transfer were performed as previously described.[15,24] Briefly, the ovaries were stimulated using a long down-regulation gonadotropin-releasing hormone agonist protocol, followed by recombinant FSH administration. Fertilized oocytes were cultured until embryo transfer to the uterine cavity, which was guided by abdominal ultrasound on day 3 of embryo culture. Clinical pregnancy was confirmed by a gestational sac with an embryo showing cardiac activity on ultrasound at weeks 5 to 7. Miscarriage was defined as nonviable clinical pregnancy noted on ultrasound followup up to gestational week 20. The live birth rate was defined as the ratio between the number of deliveries resulting in at least 1 live birth and the number of embryo transfers. Multiple pregnancies included pregnancies resulting in more than 1 live birth. Gestational age at birth, birth weight and malformations were documented. Perinatal mortality included stillbirth (death after 20 weeks of gestation) and neonatal death (death within the first 28 days after birth).

Statistical Analysis
The relationship between qualitative variables was evaluated by the chi-square or Fisher exact test. The Kruskal-Wallis test was used to compare quantitative variables. Differences were analyzed using the Dunn multiple comparisons test. We performed linear and logistic regression analyses for quantitative and dichotomous outcomes, respectively, that included the cause of OA. We selected male and female factors as covariates. For SR the covariates included male age, infertility duration and hormone levels. For embryonic and clinical outcomes the covariates also included female age and an associated female fertility problem. Significance was considered at p <0.05. All statistical data were processed with SPSS®, version 13.0.

RESULTS
From January 2002 to December 2008 at our institution 318 couples, of whom the male partner had azoospermia, underwent ICSI. Of these men 146 (45.9%) were classified with OA and were assessed.

The proportion of patients with congenital, vasectomy and post-infection obstruction was 21.9%, 40.4% and 37.7%, respectively. In the vasectomy group the interval between vasectomy and ICSI was

1 to 29 years (median 10). Of these men 16 (27.1%) had a history of failed reversals. In the post-infection group only 1 man had a history of failed reconstruction.

The cumulative SRR was 97.3%. It did not differ among the CBAVD (100%), vasectomy (96.6%) and post-infection (96.3%) groups (table 1). The success of PESA was higher in the CBAVD group than in the vasectomy and post-infection groups (96.8% vs 69.5% and 76.4%, respectively, p = 0.001). In a logistic regression model adjusting for male factors (age, infertility duration and serum hormones) the groups continued to show a statistically significant difference in the SRR (p = 0.02). Six patients (4.2%) did not have sperm retrieved, while in 26 (17.8%) only immotile sperm were retrieved after PESA. TESA was performed as a rescue procedure to attempt to obtain motile sperm in these 32 patients (21.9%) with an overall SRR of 87.5%. Only 4 patients (2.7%) did not have motile sperm retrieved after PESA and TESA attempts. In these cases immotile sperm were obtained and ICSI was done using the hyposmotic swelling test to assess sperm vitality.[4] Significantly fewer men in the CBAVD group needed TESA than men in the post-infection and vasectomy groups (3.1% vs 23.6% and 30.5%, respectively, p <0.001).

The overall complication rate following retrieval was 5.5% and it did not differ among the groups. Pain was the most common complaint (5 patients). Only 1 patient each had hydrocele, infection and swelling. Complications occurred in 6.2% of the patients who underwent TESA compared to 3.4% of those treated with PESA (p = 0.21). Excess retrieved sperm were cryopreserved in 26.7% of cases and results did not differ among the groups (table 1).

The groups were homogeneous regarding female factors, such as age, endocrine profile and the proportion of females with an associated fertility problem. There was no significant difference in the number of retrieved oocytes or in embryonic outcomes among the groups. A total of 50 live births resulted from 142 ICSI cycles (34.2%). Live birth rates did not differ among the groups, including 33.3% for CBAVD, 32% for vasectomy and 43.8% after infection (p = 0.69). Miscarriage rates were not different among the groups (table 2).

A total of 65 neonates were delivered (table 3). Mean ± SD neonatal weight (2,777.6 ± 673.9 gm) and mean gestational age at birth (37.0 ± 2.3 weeks) were higher in the vasectomy group than in the other groups but the difference was not significant. One case of perinatal death due to renal agenesis was reported in the CBAVD group, while 1 malformation (1.5%) (hypospadias) was reported in this series. None of the covariate adjusted comparisons yielded a statistically significant difference among the causes of OA.

DISCUSSION

Percutaneous SR was a reliable method to obtain sperm for ICSI from men with OA. A cumulative SRR of 97.3% was achieved and results did not differ among the CBAVD, vasectomy and post-infection groups. Epididymal sperm retrieval succeeded in 78.0% of cases. Testicular retrieval during subsequent attempts rescued the majority of failed epididymal retrievals. Significantly fewer men in the CBAVD group needed a TESA procedure compared to those in the post-infection and vasectomy groups. Results indicate that PESA is sufficient for SR in CBAVD cases but TESA is likely to be required in

Table 1. *SRR of percutaneous retrieval in men with OA by obstruction cause*

	CBAVD	Vasectomy	After Infection		p Value
No. pts	32	59	55		
Mean ± SD male age	34.6 ± 4.6	46.7 ± 6.7	42.9 ± 10.1	<0.001	(Kruskal-Wallis test)*
Mean ± SD infertility duration (yrs)	3.2 ± 2.3	6.4 ± 5.6	4.7 ± 2.1	0.003	(Kruskal-Wallis test)†
Mean ± SD male endocrine profile:				<0.001	(Kruskal-Wallis test)*
FSH (mIU/ml)	2.3 ± 0.5	6.6 ± 2.5	4.5 ± 2.0		
LH (mIU/ml)	2.9 ± 0.9	4.7 ± 1.1	3.9 ± 1.5		
Total testosterone (ng/dl)	631.4 ± 170.5	459.9 ± 163.4	533.2 ± 169.2		
No. successful sperm retrievals (%)‡					
PESA only§	31 (96.8)	41 (69.5)	42 (76.4)	0.001	(Fisher exact test)†
Cumulative PESA + TESA	32 (100.0)	57 (96.6)	53 (96.3)	0.68	(Fisher exact test)
No. complications (%)	1 (3.1)	4 (6.8)	3 (5.5)	0.90	(Fisher exact test)
No. excess retrieved sperm cryopreservation (%)	7 (21.9)	17 (28.8)	15 (27.3)	0.77	(Pearson chi-square test)

* CBAVD vs vasectomy and after infection, and vasectomy vs after infection.
† CBAVD vs vasectomy and after infection.
‡ Defined at obtaining motile sperm.
§ In logistic regression model adjusting for male age, infertility duration, and serum FSH, LH and testosterone overall comparison of SRR by PESA among groups continued to be statistically significantly different (p = 0.021) as well as pairwise comparisons with no covariate adjustment done to analyze cumulative SRR due to high success rate of this outcome.

Table 2. *ICSI outcome in men with OA by obstruction cause*

	CBAVD	Vasectomy	After Infection	p Value*	
Mean ± SD female:					
Age	31.4 ± 5.0	32.6 ± 6.2	32.9 ± 5.9	0.48	(Kruskal-Wallis test)
Baseline FSH (IU/l)	6.7 ± 2.7	5.8 ± 3.2	5.4 ± 2.9	0.06	(Kruskal-Wallis test)
Mean ± SD No. oocytes:					
Retrieved	12.1 ± 9.3	11.9 ± 7.6	12.9 ± 7.3	0.49	(Kruskal-Wallis test)
Metaphase II	9.4 ± 7.0	9.6 ± 5.9	10.4 ± 5.9	0.50	(Kruskal-Wallis test)
Mean ± SD % 2 pronuclei fertilization	61.9 ± 25.0	64.1 ± 22.1	61.1 ± 23.9	0.94	(Kruskal-Wallis test)
Mean ± SD embryo:					
% High quality	51.6 ± 30.1	55.9 ± 31.1	50.1 ± 29.2	0.93	(Kruskal-Wallis test)†
No. transferred	2.9 ± 1.4	2.6 ± 1.0	3.0 ± 1.4	0.36	(Kruskal-Wallis test)†
No. clinical pregnancy (%)	16 (55.2)	26 (44.8)	25 (46.3)	0.71	(Pearson chi-square test)†
No. miscarriage (%)	4 (25.0)	7 (26.9)	5 (20.0)	0.77	(Fisher exact test)
No. live birth (%)	11 (34.4)‡	19 (32.2)	20 (36.4)	0.85	(Fisher exact test)†

* In linear regression model adjusting for male age, infertility duration, and serum FSH, LH and testosterone, in addition to female age and female factor infertility, overall comparison among groups continued to show no statistical significant difference as well as pairwise comparisons.
† After covariate adjustment.
‡ One stillbirth.

about a third of the cases in the other etiology groups. These findings suggest that the epididymis may be severely damaged in cases of vasectomy and post-infection disease.

Adopting strict criteria to diagnose OA is crucial to achieve a high SRR using percutaneous techniques. Ideally, normal spermatogenesis is confirmed by histological evaluation. However, in most patients OA may be distinguished clinically from nonobstructive azoospermia by a thorough analysis of diagnostic parameters. Our method included history, physical examination findings, endocrine profile and the presence of spermatozoa at epididymal retrieval. Of men with OA 96% have FSH less than 7.6 mIU/ml and/or a testicular long axis of 4.6 cm or greater.[25] Moreover, the presence of spermatozoa at epididymal retrieval yields 93% sensitivity and 94% specificity to predict normal spermatogenesis.[26]

Our study depended primarily on percutaneous methods of SR, which yielded a high SSR for all etiology categories and showed only minor complications. Success rates were calculated based on the finding of motile sperm. Thus, failure to retrieve motile sperm was not necessarily technical failure because immotile sperm were obtained. In fact, only 6 men (4.2%) had no epididymal sperm retrieved, of whom TESA rescued 2 and ICSI was performed using immotile sperm in the remainder.

We used percutaneous retrieval techniques due to cost-effectiveness and minimal post-procedural discomfort. While we were successful overall in our retrieval attempts, PESA and TESA have some disadvantages compared to open surgical methods.[4] The 2 methods carry an increased risk of hematoma compared to open techniques,[27] although no patient was affected in our series. Complications occurred more often in patients undergoing TESA than PESA (12.5% vs 5.7%) but our numbers did not attain statistical significance. We could not identify a correlation between any complication and the cause of

Table 3. *Outcome of neonates born after ICSI in OA cases by obstruction cause*

	CBAVD	Vasectomy	After Infection	p Value*	
No. deliveries (%):	12	19	20		
Singletons	9 (75.0)	15 (78.9)	15 (75.0)	0.68	(Fisher exact test)
Twins	3 (25.0)	4 (21.1)	3 (15.0)	0.96	(Fisher exact test)
Triplets	0	0	2 (10.0)	0.76	(Fisher exact test)
No. neonates born	15	23	27		—
Mean ± SD gestational age (wks)	35.8 ± 2.7	37.0 ± 2.2	35.54 ± 4.1	0.42	(Kruskal-Wallis test)†
Mean ± SD birth weight (gm)	2,655 ± 601	2,777.6 ± 673.9	2,504 ± 829	0.42	(Kruskal-Wallis test)†
No. male/female	2.2/1	1/1.7	1.4/1		—
No. perinatal deaths (%)‡	1 (6.7)	0	0	0.27	(Fisher exact test)
No. malformation (%)	0	1 (4.3)	0	0.70	(Fisher exact test)

* In linear regression model adjusting for male age, infertility duration, and serum FSH, LH and testosterone, in addition to female age and female factor infertility, overall comparison among groups continued to show no statistical significant difference as well as pairwise comparisons with overall malformation and perinatal death rates too low to allow for covariate comparisons using logistic model.
† After covariate adjustments.
‡ Includes stillbirth and neonatal deaths with frequency calculated as number of perinatal deaths/number of neonates born.

obstruction. We used PESA preferentially over TESA to avoid the risk of hematoma and atrophy, which have occasionally been reported with TESA.[4,27] Percutaneous retrieval has been claimed not to be as successful as more invasive techniques.[27] However, in our study only 4 patients (2.7%) might have benefited from an open procedure but even in those men it is possible that only immotile sperm would have been available for ICSI.

In this series sperm cryopreservation was possible in a third of the cases irrespective of the cause of obstruction. Cryopreservation may prevent the need for future retrieval if ICSI fails. Although increased cryopreservation rates have been reported for open SR, the cost of open SR is significantly higher.[27] On the other hand, percutaneous approaches can be performed using local anesthesia on an outpatient basis. If needed, repeat percutaneous procedures may result in successful SR.[9] However, it is debatable whether percutaneous retrieval is more cost efficient than microsurgical epididymal sperm aspiration.

To our knowledge no study has yet compared cumulative pregnancy rates after repeat cycles of percutaneous retrieval and ICSI with a single microsurgical epididymal sperm aspiration attempt at intentional sperm cryopreservation coupled with multiple subsequent ICSI cycles. Nonetheless, ICSI outcomes are comparable using frozen thawed or fresh sperm retrieved from men with OA.[28]

There are conflicting data on the influence of the cause of obstruction on ICSI success.[6–8,13,14,16,17] In a meta-analysis comparing ICSI outcomes between congenital and acquired causes of OA fertilization rates were decreased, whereas miscarriage rates were increased in the congenital group.[13] This suggests that sperm retrieved from men with congenital obstruction has decreased reproductive potential. In our series no differences were observed in any outcome analyzed among the different etiology categories of OA. Our results are corroborated by a large series of 1,121 men in whom similar rates of fertilization, clinical pregnancy and miscarriage were achieved irrespective of obstruction being acquired or congenital.[18] We add to the existing literature by reporting the short-term outcomes of neonates born after percutaneous SR and ICSI in men with OA. Although the limited evidence of pregnancy and postnatal ICSI outcomes has been reassuring for the overall population of men with OA,[16,19–22] such data were lacking when the cause of obstruction was considered.

Neonatal profiles were similar for all obstruction etiologies. Moreover, our 1.5% malformation and perinatal death rates were similar to the 1.3% to 5.2% reported in larger cohorts.[16,19–22] Despite being higher than in the general population, the incidence of hypospadias (1 of 65 cases) in our series was shown to be associated with male infertility.[21,22] However, given the relative rarity of specific birth defects, identifying an association between specific exposure and subsequent risk is difficult. Moreover, not all major malformations are found at birth and the neonates described in this series were not followed. Therefore, the number of malformations in our study may have been underestimated.

Due to the retrospective nature of this study, our patient populations may have had some inherent selection bias. Patients in the vasectomy group had an expected older age and longer infertility status since they had achieved previous pregnancies before vasectomy. FSH and LH were highest and testosterone was lowest in the vasectomy group, although they were within the normal range. These differences were likely due to the increase in FSH and LH, and the decrease in testosterone with male aging.[29] However, after adjusting for these relevant factors we confirmed that they had no impact on the statistical results of the univariable comparisons. Our findings agree with studies showing that paternal age and the interval since vasectomy are not significant determinants of ICSI success.[10,12] On the other hand, female factors such as age, basal FSH and the number of oocytes retrieved did not differ among our patient populations. All of these factors reflect ovarian function and are the most robust predictors of pregnancy after assisted reproductive technology.[30]

CONCLUSIONS

Our data reaffirm the existing knowledge that percutaneous retrieval is highly successful in men with OA regardless of the cause of obstruction. They also add to the limited evidence about the outcomes of neonates born of such fathers. Results suggest that neonates born after ICSI using nonejaculated sperm from men with OA due to CBAVD, vasectomy and post-infection disease have similar short-term outcomes because the integrity of the male gamete is not differentially affected by OA.

ACKNOWLEDGMENTS

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