CLINICS

REVIEW

Predictive factors for sperm retrieval and sperm injection outcomes in obstructive azoospermia: Do etiology, retrieval techniques and gamete source play a role?

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Obstructive azoospermia is a relatively common male infertility condition. The main etiologies of obstructive azoospermia include congenital, surgical-derived, traumatic and post-infectious cases. Although seminal tract reconstruction is a cost-effective treatment in most cases, this approach may not be feasible or desired in some cases. In such cases, assisted reproduction techniques offer a method for achieving pregnancy, notably via sperm retrieval and intracytoplasmic sperm injection. This process requires several considerations and decisions to be made, including the cause and duration of obstruction, which sperm retrieval technique to use, and whether to use fresh or frozen-thawed sperm. We present a review of obstructive azoospermia and assisted reproduction techniques, highlighting the most relevant aspects of the decision-making process for use in clinical practice.

KEYWORDS: Male Infertility; Obstructive Azoospermia; Sperm Retrieval; Intracytoplasmic Sperm Injection; Outcomes; Review.

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INTRODUCTION

Azoospermia, defined as the complete absence of sperm from the ejaculate, affects approximately 1% of all men and 5 to 10% of all subfertile males seeking care (1,2). Although the majority of cases are secondary to an impairment of testicular function, a bilateral obstruction of the male genital tract causes azoospermia in up to 20 to 40% of cases (3).

We review the currently available data regarding sperm retrieval in obstructive azoospermia (OA). Here, we present the current status of the literature concerning sperm retrieval and intracytoplasmic sperm injection (ICSI) outcomes in relation to the cause of obstruction, the method of sperm retrieval, and the gamete source.

OBSTRUCTIVE AZOOSPERMIA: AN OVERVIEW

Obstructive azoospermia is defined as the absence of spermatozoa in the ejaculate secondary to a physical disruption of the seminal tract, which may be congenital or caused by trauma (surgical or non-surgical) or infection. Generally, spermatogenesis is fully preserved.

The absence of sperm on routine examination must be confirmed by at least two semen samples collected 1-4 weeks apart and centrifuged at high speed (1500-1800 *g* for 15 min at room temperature) to distinguish azoospermia from cryptozoospermia; otherwise, an equivocal diagnosis may occur in up to 20% of cases (4,5). If retrograde ejaculation is suspected, a post-ejaculate urine specimen should be analyzed (6). Additional proposed criteria to diagnose obstructive azoospermia include a normal hormonal profile, normal-sized testis, and normal spermatogenesis, as evidenced by a testicular biopsy or an epididymal aspirate full of spermatozoa (7).

Ninety-six percent of men with OA are found to have FSH levels $\leq$7.6 mIU/mL or a testicular long axis $>$4.6 cm, whereas 89% of men with non-obstructive azoospermia (NOA) have FSH levels $>$7.6 mIU/mL or a testicular long axis $\leq$4.6 cm (8).

In patients with OA, although normal spermatogenesis is preserved, the quality of spermatozoa may be altered because the distal epididymis contains a high number of sperm fragments with macrophages (9). The number of macrophages progressively decreases toward the proximal epididymis and testis, while the quantity of motile sperm gradually increases. The concentration of motile spermatozoa in the epididymal fluid can be as high as 1 million sperm per μL (10).

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Vasectomy comprises the main cause of OA, and 2 to 6% of vasectomized men will seek reversal to restore fertility. In these cases, microsurgical reconstruction by either vasovasostomy or vasoepididimostomy is the standard of care (11-13). There is compelling evidence that vasectomy reversal is a more cost-effective approach than *in vitro* fertilization (IVF) for vasectomy-related OA (14,15). However, the final therapeutic decision should be individualized for each couple, as certain situations (e.g., significant female factor infertility or social factors) may favor sperm retrieval (SR) and IVF as more suitable treatment options.

Both men with OA who are not candidates for reconstructive surgery and those who fail to achieve success with such procedures have been historically considered hopelessly infertile. However, with the first reports of successful pregnancies achieved by the use of IVF in the early 1990s, a new perspective emerged (16,17). Currently, IVF is an established assisted reproductive technique (ART) with consistent results. The Society of Assisted Reproductive Technology (SART) publishes yearly data on the effectiveness of IVF in different patient populations. A total of 17% of all IVF cycles from 2000 to 2008 were performed due to male factor infertility. The live birth rate associated with the use of IVF is increasing. In fact, the delivery rate across all age groups increased from 34.5% in 2003 to 37.3% in 2008. The use of ICSI for male factor infertility also increased from 84 to 87% during this time period. Data from SART, however, do not offer fine enough resolution to distinguish IVF treatments undertaken for obstructive versus non-obstructive azoospermia (15,18). In Latin America, the latest report from the Red Latinoamericana de Reprodución Asistida (RedLara) in 2009 noted ICSI and IVF procedure proportions of 85 and 15%, respectively; a total of 27,174 IVF/ICSI cycles were initiated, resulting in 7,141 live births (19). Again, it is not possible to determine the proportion of such treatments applied to men with OA.

As the number of ART procedures performed is expected to increase with the anticipated increase in the current 15% incidence of infertility over the next 20 years (20), some questions have emerged regarding the state-of-the-art methods used to perform sperm retrieval for use in IVF/ICSI.

THE IMPACT OF ETIOLOGY ON RETRIEVAL SUCCESS AND REPRODUCTIVE OUTCOMES AFTER ICSI

Common causes of OA include previous vasectomy, congenital bilateral absence of the vas deferens (CBAVD), infectious epididymitis/orchitis, Young's Syndrome, and testicular trauma (21). Some authors include ejaculatory dysfunctions in the OA category, including retrograde ejaculation and anejaculation (7,21). The appropriateness of the inclusion of these conditions in the OA category is, however, debatable, as there is no anatomical barrier precluding sperm from being ejaculated in these conditions.

The most common cause of iatrogenic ductal obstruction is vasectomy. Although vasectomy reversal is achievable in many cases, the scope of this review will be limited to vasectomized men who failed reconstruction or opted for sperm retrieval procedures associated with IVF treatment. After vasectomy, testicular histology changes, as noted by the presence of fibrosis and decreased spermatid number, which worsen with longer periods of obstruction; as such, pregnancy rates with ART treatment might also decline (22). In a study by Sukcharoen et al. comprising 17 patients and 21 ICSI cycles within a period of two years, subjects were divided into three groups according to the time elapsed since vasectomy: 0-10 years, 11-20 years, or more than 20 years. No difference could be found regarding fertilization rates (FRs), implantation rates (IRs), and pregnancy rates (PRs) between the groups. The authors of the aforementioned study concluded that the interval between vasectomy and surgical sperm retrieval associated with ICSI treatment had no impact on pregnancy outcomes, although it should be noted that the analyzed sample was small and likely lacked statistical power to enable definitive conclusions (23).

Congenital unilateral (CUAVD) and bilateral (CBAVD) absence of the vas deferens and congenital obstruction of the epididymides are all part of the spectrum of vasal aplasia. CBAVD affects 2% of infertile men and has been described as a primary genital form of cystic fibrosis (5). Approximately 80% of CBAVD patients exhibit definable mutations within at least one allele of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which is located on the short arm of chromosome 7 (24). This gene encodes a protein that functions as an ion channel but also affects the formation of the distal 2/3 of the epididymis, vas deferens, seminal vesicle and ejaculatory duct. An additional 10 to 15% of men with vasal aplasia exhibit renal anomalies (e.g., agenesis, failure of assent, and ectopia). The diagnosis of vasal aplasia must be suspected in infertile men with a low semen volume and with a low pH as a consequence of the absence of the seminal vesicles. Spermatogenesis in CBAVD appears normal, but sperm derived from the caput epididymis are thought to have a low fertilizing capacity in conventional IVF cycles because of their short passage through the epididymis (25). All men with CBAVD should be assumed to harbor *CFTR* mutations, as a negative genetic test does not rule out the mutation with 100% accuracy due to the gene length and testing limitations. However, genetic screening should be offered to the female partner because a 25 to 50% risk of inheritance of cystic fibrosis exists if she also carries a mutation (26). Because spermatogenesis is not altered by this condition, sperm can be retrieved from both the testicle and the epididymis without difficulty. In an earlier study by Chen et al. (27), 31 patients with obstructive azoospermia underwent epididymal aspiration procedures associated with ART (including IVF, zygote intra-Fallopian transfer, subzonal insemination). The fertilization rate for caput spermatozoa was lower than that for spermatozoa derived from other areas of the epididymis ($p<0.05$). In the aforementioned study, the CBAVD group exhibited a higher probability to achieve pregnancy compared with the acquired obstruction group (20 versus 5.9%, $p>0.05$). Currently, ICSI is used rather than conventional IVF after sperm retrieval because sperm injection has been shown to result in a significantly higher fertilization rate (28).

Ejaculatory duct abnormalities can also result in obstruction. Congenital ejaculatory duct obstruction (EDO) represents an anomaly caused by maldevelopment of the portion of the Wolffian ductal system that courses through the central zone of the prostate and enters the prostatic urethra. Müllerian duct and prostatic utricle cysts can extrinsically compress the ejaculatory ducts and result in obstruction

(29). Acquired EDO causes include chronic prostatitis, prostatic calcification, prior prostatic biopsy, and iatrogenic damage to the ejaculatory ducts during urethral instrumentation (6,30). Transurethral resection of the ejaculatory ducts (TURED) has been the classic treatment for this disorder. Alternatively, sperm retrieval can be attempted with ICSI. Epididymal, testicular, and transperineal seminal vesical sperm retrievals have been applied in such cases with adequate yields of motile sperm for either immediate use with ICSI or cryopreservation (31). In cases of ejaculatory dysfunction, spermatogenesis is fully preserved, and gametes can be recovered either from the urine (in cases of retrograde ejaculation) or from the epididymides or testicles with any chosen surgical sperm retrieval technique (in cases of primary anorgasmia or failed urinary sperm recovery).

In a recent study, Esteves et al. (32) reported a cumulative sperm retrieval success rate (SRR) of 97.9% using percutaneous epididymal sperm aspiration (PESA), in association or not with testicular sperm aspiration (TESA), in men with OA, regardless of the cause of obstruction. PESA alone was able to retrieve sperm in more than 80% of the cases. Reproductive outcomes after ICSI were not affected by the source (epididymis versus testicle) or etiology (congenital, failed vasectomy reversal, post-infectious disease). Successful epididymal sperm retrievals were achieved in all congenital obstructive cases, whereas testicular retrievals were needed in approximately 1/3 of the cases classified in the other etiology groups (vasectomy, post-infectious obstructions).

In a meta-analysis involving 756 ICSI cycles using surgically retrieved sperm, Nicopoullos et al. compared the outcomes of ICSI between patients with congenital and acquired OA. The meta-analysis revealed no difference in either clinical pregnancy rate (relative risk [RR]: 1.03; 95% confidence interval [CI]: 0.75-1.31; $p = 0.87$) or live birth rate (RR: 1.03; 95% CI: 0.81-1.31; $p = 0.80$) between patients with congenital and acquired cases of OA. A significantly higher fertilization rate was noted in the acquired group (RR: 0.92; 95% CI: 0.84-1; $p = 0.05$), while a significantly higher miscarriage rate (MR) was noted in the congenital group (RR: 2.67). The authors concluded that in ICSI cycles for men with OA, the cause of OA appears to influence the outcome, with higher FRs and lower MRs observed in patients with acquired OA. However, tests of heterogeneity were significant, and it should be noted that the studies included had no power to detect clinically significant differences in the analyzed outcomes (21).

A more recent and larger series conducted by Kamal et al. involved 1,661 ICSI cycles in 1,121 men with proven histological OA. The outcomes were compared according to the sperm retrieval source and cause of obstruction. The fertilization rates (68.0% versus 64.2%, $p = 0.02$), implantation rates (19.9% versus 20.8%, $p = 0.41$), and frequencies of clinical pregnancies (43.2% versus 42.3%, $p = 0.84$) and miscarriages (18.4% vs. 17.6%, $p = 1.0$) resulting from the use of testicular and epididymal spermatozoa, respectively, were comparable in ICSI. Similar rates were maintained after stratification according to the cause of obstruction (CBAVD versus acquired obstruction), suggesting that neither the origin of surgically retrieved spermatozoa nor the cause of obstruction have any significant effect on the success of IVF/ICSI (7).

IMPACT OF THE METHOD OF COLLECTION ON RETRIEVAL SUCCESS AND REPRODUCTIVE OUTCOMES AFTER ICSI

The method of choice for sperm retrieval is based on the attending surgeon's preferences and the preferences of the embryologist involved in the patient's care. In a meta-analysis by Van Peperstraten et al., the technique for sperm retrieval (microsurgical epididymal sperm aspiration [MESA] versus epididymal micropuncture with perivascular nerve stimulation and ultrasound-guided TESA versus conventional TESA) and the sperm source (testis, epididymis, vas deferens, or seminal vesicle) did not seem to play a role in the pregnancy rates achieved with IVF/ICSI (33). All methods have been shown to provide sufficient viable sperm for ICSI and often also for cryopreservation (34).

The use of surgical techniques for sperm retrieval in OA men, as well as the use of retrieved spermatozoa in IVF treatments, dates back to the 1980s with the description of MESA by Temple-Smith et al. (35). Thereafter, many other techniques have been developed, including those meant to extract sperm from the epididymis and from the testis. Their use varies according to the azoospermia etiology and specific scenarios (36). Sperm retrieval should focus on three main goals:

1. To retrieve an adequate number of sperm for both immediate use and cryopreservation;
2. To obtain the highest quality sperm possible;
3. To minimize damage to the reproductive tract to not jeopardize future sperm retrieval attempts or testicular function (36,37).

Several authors have examined the effectiveness of percutaneous procedures for sperm retrieval in OA. Sperm retrieval rates have been quoted at approximately 100% when percutaneous epididymal and testicular retrievals are combined (38-43) (Table 1). Glina et al. reported a series of 58 men with OA treated with ICSI who underwent percutaneous epididymal sperm retrievals (with rescue TESA whenever needed). The authors reported 100% recovery of motile sperm using these combined techniques. Successful repeated PESA was performed up to three times, with recovery of motile sperm in over 80% of the cases. Forty-three percent of PESA procedures yielded sufficient spermatozoa to allow cryopreservation (42). Esteves et al. reported a SRR of 97.9% among 142 men with OA. In these series, TESA as a rescue procedure after a failed PESA was performed in 17% of the cases. One-third of the retrievals yielded a sufficient number of spermatozoa for cryopreservation (32). Lin et al. analyzed 100 men with irreparable OA who underwent 109 ICSI cycles. The PESA SRR was 61%. MESA or testicular sperm extraction (TESE) were successfully performed if PESA failed. Fertilization and pregnancy rates were not significantly different for PESA-ICSI cycles (56 and 39%, respectively) and MESA-ICSI cycles (47 and 45%, respectively) (43). Despite the notably lower SRR for PESA the authors' data corroborate the ability to perform rescue procedures in cases of initial failure.

The reasons for epididymal retrieval failures include obstructions at the level of the rete testis, which, according to Pryor (44), can be found in up to 15% of OA men. Such individuals have normal spermatogenesis but no clinical findings suggestive of obstruction. Even grossly distended

Table 1 - Studies reporting sperm retrieval efficacy and/or pregnancy outcomes in men with obstructive azoospermia.

Author	Country	Design	No. of cycles	Mean Paternal Age (years)	Outcome	Main findings	Conclusion
Rosenlund et al. 1998	Sweden	Retrospective	NR	37	Recovery of viable sperm on repeated PESA	High sperm recovery rates were found on repeated PESA up to 4 times	PESA is simple, offers a high sperm recovery rate and can be safely repeated in OA men, yielding similar SRR results.
Levine et al. 1998	USA	Retrospective	37	NR	Efficacy and safety of percutaneous sperm retrieval and ICSI outcomes (CPR) using retrieved sperm	Efficacy and safety of percutaneous sperm retrieval techniques (PESA, TESA) were demonstrated	PESA and TESA are more effective alternatives compared with the more invasive MESA approach.
Dohle et al. 1998	The Netherlands	Retrospective	39	38.1	ICSI outcomes (ongoing pregnancy rates) using percutaneously or surgically-retrieved sperm	Both percutaneously and surgically retrieved spermatozoa provide adequate pregnancy outcomes.	High fertilization rate was obtained after ICSI regardless of the retrieval method
Janzen et al. 2000	USA	Retrospective	108	38.3	ICSI outcomes (CPR) using fresh or frozen epididymal sperm	Both fresh and cryo-thawed epididymal sperm harvested by MESA yielded similar CPR	Comparable pregnancy outcomes for fresh and frozen epididymal sperm, with logistic-related advantages for frozen sperm
Glina et al. 2003	Brazil	Retrospective	79	45	Success at obtaining sperm by PESA and CPR after ICSI	High SRR on repeated PESA up to 4 times	PESA is simple, offers a high sperm retrieval rate and can be safely repeated in men with OA.
Westlander et al. 2001	Sweden	Retrospective	22	34.8	ICSI outcomes up to ongoing pregnancy/ delivery rates after TESA	TESA can be repeated with no negative impact on the recovery of mature spermatozoa or pregnancy outcome	Repeated TESA is safe and effective
Levine et al. 2003	USA	Retrospective	112	37.1	ICSI outcomes (CPR) after percutaneous sperm retrieval	PESA and TESA are highly effective for sperm retrieval and offer similar pregnancy outcomes with ICSI.	Percutaneous sperm aspiration is effective, safe and reproducible
Dozortsev et al. 2006	Brazil	Retrospective	185	NR	ICSI outcomes (ongoing pregnancy rates) with percutaneous sperm retrieval	Higher FR in the PESA group and higher implantation rate in the TESA group were reported; trends toward higher PR and lower miscarriage rate in the TESA group	Embryo development was significantly better when testicular sperm was used for ICSI
Pasqualotto et al. 2006	Brazil	Retrospective	155	NR	ICSI outcomes according to etiology of OA	Higher FR and implantation rate in men with congenital OA; similar PR in all etiology categories	No impact of etiology of OA on CPR
Garg et al. 2008	USA	Retrospective	38	39.1	ICSI outcomes (LBR) using TESA	TESA is highly effective in recovering motile spermatozoa and offers adequate pregnancy outcomes.	TESA is an effective means of recovering mature motile sperm which are suitable for cryopreservation in most cases.
Kamal et al. 2010	Egypt, The Netherlands, United Kingdom	Retrospective	1,661	39.2	ICSI outcomes (CPR) according to sperm source and etiology of obstruction	Similar pregnancy outcomes for different sperm sources (testicular or epididymal) and causes of obstruction (congenital or acquired)	The source of sperm and the etiology of obstruction do not seem to influence pregnancy and miscarriage rates.
Kalsi et al. 2010	United Kingdom	Retrospective	258	NR	ICSI outcomes (live birth rate) using fresh or frozen-thawed retrieved sperm	Higher pregnancy and live birth rates were reported for frozen-thawed compared with fresh sperm	No negative impact of using frozen-thawed epididymal or testicular sperm for ICSI; a tendency for higher PR and live birth rate associated with frozen-thawed testicular sperm

SRR: sperm retrieval rate; ICSI: intracytoplasmic sperm injection; IVF: in vitro fertilization; PESA: percutaneous epididymal sperm aspiration; TESA: testicular sperm aspiration; MESA: microsurgical epididymal sperm aspiration; FR: fertilization rate; PR: pregnancy rate; CPR: clinical pregnancy rate; LBR: live birth rate; NR: not reported.

epididymides represent a poor clinical sign of obstruction and can be misleading. Successful epididymal sperm retrieval in these cases can be achieved in approximately 70% of cases (40). The PESA SRR is influenced by the anatomical conformation of the epididymis, and PESA may be more difficult in small and loosely attached epididymides, which can make isolation more difficult. Needle width can also affect the results and must be selected properly. Levine et al. reported their experience using a 23-gauge butterfly needle to aspirate spermatozoa from the epididymis with satisfactory sperm quantity for ICSI use (45). In a study by Mallidis and Baker (46) comparing devices for fine needle tissue aspiration biopsy of the testis, the authors observed that the 20-gauge biopsy needles with stylet penetrated easier within the testis and caused the least amount of tissue distortion.

Repeat percutaneous procedures have been associated with successful sperm retrieval. Repeated PESA up to three times on the same unilateral epididymis with equivalent fertilization rates was described as being successful by Rosenlund et al. in a retrospective series involving 27 men with OA or ejaculatory dysfunction (47). Sperm retrieval rates of 91, 89, and 86% were reported at the first, second and third PESA, respectively. Repeated TESA has also been reported to yield SRRs of 100 and 96% for first and second attempts, respectively. No significant impacts on fertilization rates were observed by the authors of the study.

PESA and TESA have some disadvantages compared with open surgical sperm retrievals (36). Both methods have an increased risk of hematoma compared with open techniques (48). Nevertheless, except for minor pain and local swelling, there are no reports of clinically significant perioperative or postoperative complications leading to medical treatment or hospital care when percutaneous techniques are used. The time interval between the procedures (classified as less than three months, three to six months, and more than six months) did not influence the outcome (sperm recovery, fertilization, and pregnancy rates). It should be noted, however, that the TESA technique is not standardized in the published studies, and in most centers, TESA is considered a rescue procedure for OA cases (48,49).

Other factors associated with the preference for one technique over the other include the quantity of retrieved spermatozoa and the ability to cryopreserve the excess retrieved sperm. PESA has been associated with better recovery of motile spermatozoa compared with testicular retrieval (TESA) (100% versus 39.3%, $p<0.0001$) in patients with azoospermia or severe oligozoospermia (33). However, the ability to cryopreserve excess retrieved sperm is lowest in percutaneous compared with open sperm retrievals (33). Interestingly, Garg et al. (50) reported a 97.5% success rate in motile sperm recovery that was adequate for intentional cryopreservation in 40 men with either OA or ejaculatory dysfunction using TESA. Cryopreservation is important because it prevents the need for future retrieval procedures in the event that ICSI fails.

IMPACT OF GAMETE SOURCE (EPIDIDYMIS OR TESTICLE) ON REPRODUCTIVE OUTCOMES WITH ICSI

As stated earlier, spermatozoa can be recovered from either the epididymis or the testicle with a high probability of success in men diagnosed with obstructive azoospermia, regardless of the cause. In early ICSI series, the fertilization and pregnancy rates using surgically retrieved spermatozoa obtained from the epididymis or testis were comparable to the results of ejaculated sperm (51,52). As both sources were proven adequate for ICSI, the possible treatment outcome advantages of one over the other have been explored.

Testicular sperm aspiration pregnancy rates with ICSI are reportedly as high as 31%, with a calculated delivery rate of 27% considering a miscarriage rate of 11.6% (38). Similarly, pregnancy rates with ICSI using epididymal sperm are reported to be as high as 43%, with a calculated delivery rate of 38% if a similar miscarriage rate is assumed (53-55). In a meta-analysis by Nicopoullos et al. (56), fertilization rates were reported to vary from 45 to 72% for epididymal and 34 to 81% for testicular sperm. No differences in cleavage, PRs or IRs were reported in any of the individual articles. Relative risk (RR) ratios of 1.08, 1.01, and 0.71 were described for the fertilization rate, clinical pregnancy rate and live birth rate, respectively, for epididymal compared with testicular sperm ($p>0.05$). The authors of the aforementioned studies therefore concluded that when a diagnosis of OA is made, epididymal aspiration should be the retrieval method of choice in view of the possible complications of testicular extraction, including inflammation, hematoma, devascularization (57) and decreased serum testosterone levels (58).

Similar results were reported by Kamal et al. (7) in a large cohort involving 1,121 patients with OA undergoing ICSI. These authors argued that the preferential use of testicular sperm or epididymal sperm in cases of OA is unfounded, as neither the source nor the etiology seems to affect sperm injection outcomes.

Conversely, Dozortsev et al. reported higher FRs using spermatozoa retrieved from the epididymis compared with the testis in OA cases (77.2 versus 67.5%, $p=0.0005$). However, patients in the testicular sperm group exhibited significantly higher IR (20.8 versus 32.8%, $p=0.008$), with a trend toward higher ongoing PR and lower miscarriage rate. These authors speculate that motile sperm randomly taken from the epididymis have lower reproductive potential than random sperm taken from the testicle, and argue that the prolonged presence of sperm within the epididymis may lead to structural chromosomal aberrations that can compromise the reproductive potential of the such cells (59). To corroborate these findings, recent studies have demonstrated that epididymal sperm exhibit more DNA damage than sperm retrieved directly from the testis. The frequency of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive cells according to the sperm cell origin has been reported as follows: $9.3\pm2.3\%$ in the testis, $17.4\pm4.0\%$ in the epididymis, and $29.2\pm6.7\%$ in the vas deferens (60,61). Despite these reports, sperm retrieved directly from the testis are generally limited in number and are often immotile or non-progressively motile, although they are still viable and almost always functional for use in ART (48).

IMPACT OF GAMETE STATUS (FRESH OR FROZEN-THAWED) ON REPRODUCTIVE OUTCOMES AFTER ICSI

The cryopreservation of epididymal and testicular sperm offers the advantage of a single retrieval procedure for

sperm collection, thereby enabling the use of stored spermatozoa in multiple IVF cycles. Cryopreservation avoids the logistical difficulties associated with attempts to coordinate sperm retrieval and ART on the same day and permits infectious triage prior to insemination (48). However, cryopreservation has been shown to negatively affect motility, acrosome integrity, acrosin activity, and morphology of both testicular and ejaculated spermatozoa. For example, Prabakaran et al. noted a 25% decrease in post-thaw testicular sperm motility (62). Decreased sperm morphology, mitochondrial function, and viability have also been described by O'Connell et al. after testicular sperm cryopreservation and thawing (63). The cryopreservation and thawing process has also been associated with the lipid peroxidation of sperm plasma membranes, which may facilitate free radical oxygen species access to sperm nuclei and ultimately disrupt DNA integrity (64).

Despite these potential downsides, the clinical pregnancy rate of 141 ICSI cycles using frozen-thawed (n = 33) or fresh (n = 108) spermatozoa retrieved from men with OA were comparable (60.6% vs. 66.7%, respectively) in the series of Jansen et al. (65). Garg et al. (50) also reported similar fertilization rates and biochemical pregnancy rates resulting from the use of frozen-thawed or fresh testicular spermatozoa. Conversely, a significantly higher clinical pregnancy rate was observed for fresh epididymal sperm versus frozen-thawed counterparts in a meta-analysis study, despite the fact that the fertilization (RR: 1.02; 95% CI: 0.96-1.08; $p = 0.61$) and implantation (RR: 1.20; 95% CI: 1.0-1.42; $p = 0.05$) potential of both gametes were equivalent (33).

Intriguingly, a recent retrospective study by Kalsi et al. compared 493 ICSI cycles with the use of fresh versus frozen sperm by stratifying results according to the testicular and epididymal sources. Unexpectedly higher PR (60.0% versus 32.1%, $p<0.05$) and live birth rate (60.0% versus 28.6%, $p<0.05$) were observed for frozen-thawed testicular sperm compared with fresh testicular sperm. The miscarriage rates were equivalent (25.0% versus 29.2% for fresh and frozen-thawed sperm, respectively) (66).

■ ASSESSMENT OF CHILDREN BORN WITH ICSI USING SPERM RETRIEVED FROM MEN WITH OA

Concern has been raised regarding the risk of using non-ejaculated spermatozoa in assisted reproduction. Epididymal sperm may be immature or senescent because of the long stay in the obstructed epididymis, which may lead to genetic risks if these sperm are used for fertilization (61). Testicular sperm, in turn, have the potential risk of incomplete genomic imprinting, incomplete chromatin condensation, and incomplete protamination (67,68).

In a recent Dutch prospective, multicenter study by Woldringh et al. (69), 378 children born from ICSI cycles using retrieved epididymal sperm were evaluated. More than 1,000 children born as a result of ICSI or IVF using ejaculated sperm were available for comparison. Assessments were performed at birth and at 1 and 4 years of age using mailed questionnaires. Moreover, follow-up visits of 2-year-old children were carried out to evaluate motor performance and mental-language development. The epididymal sperm group did not exhibit a higher incidence of stillbirths, malformations or poor development compared with the reference group of children born after ICSI or IVF using ejaculated sperm.

■ EXPERT COMMENTARY

In a group of 2,383 infertile men attending our tertiary center for male reproduction, 835 (35%) were identified as having azoospermia; approximately 36% of those cases resulted from obstruction in the ductal system.

The adoption of strict criteria to diagnose OA is crucial for obtaining a high retrieval success rate in the range of 90-100% using percutaneous techniques. In our experience, percutaneous sperm retrieval is a highly effective method for collecting sperm in men with OA. Successful sperm retrieval was achieved in over 85% of the cases using PESA, but more than one aspiration was often required (32). In cases of failed PESA, TESA was adequate to obtain sperm in nearly all cases. Motile spermatozoa were obtained in approximately 73% of the cases after the first or second PESA aspiration, and TESA was performed as a rescue procedure after failed PESA in approximately 14% of the individuals (32). Our results show that ICSI outcomes using spermatozoa collected by PESA or TESA are similar, thus suggesting that the reproductive potential of those gametes is independent of their source in OA. However, epididymal spermatozoa are easier to handle in the IVF laboratory compared with testicular sperm, and it is more likely that there will be excess sperm for freezing if epididymal retrievals are performed. In our group of men with OA, fertilization and live birth rates did not differ between individuals who had vasectomy/failed reversal, CBAVD or infection as the cause of obstruction (32).

Despite the fact that higher rates of sperm cryopreservation have been reported for open sperm retrieval compared to percutaneous retrieval, the costs of the former procedure are significantly higher. Cryopreservation is important because it prevents the need for future retrieval procedures in the case that ICSI fails. However, if needed, repeat percutaneous procedures result in successful sperm retrieval. This is encouraging because the need for open procedures is lessened. It is debatable whether percutaneous retrievals are more cost-efficient than MESA, and no study to date has compared the cumulative pregnancy rate per couple using repeat percutaneous retrievals and fresh sperm injections with a single MESA attempt and intentional sperm cryopreservation for use in multiple subsequent ICSI attempts.

Percutaneous sperm retrieval techniques can be performed for both diagnostic and therapeutic purposes. For the latter purpose, sperm retrieval is often performed on the same day of oocyte retrieval or on the preceding day. Using PESA, our approach is to perform the first aspiration at the epididymis corpus and proceed to the caput if necessary because aspirates from the cauda are usually of poor quality and contain senescent spermatozoa, debris, and macrophages. Most cases of PESA failures are not necessarily technical failures because immotile spermatozoa is usually retrieved. As a diagnostic procedure, the presence of spermatozoa at the time of percutaneous epididymal retrieval has shown to have 93% sensitivity and 94% specificity to diagnose obstructive azoospermia (70). Usually, percutaneous sperm retrievals are performed on an outpatient basis. Patients are discharged one hour later and can return to normal activities the following day. Oral analgesics are prescribed, but pain complaints are minimal. The most common complication is fibrosis at the aspiration site. Other potential complications include hematoma,

bleeding and infection, but these complications are rare. On rare occasions, we perform MESA for sperm retrieval in OA men with coagulation disorders.

While PESA is sufficient for the majority of patients, TESA is needed for more difficult cases. We noted that significantly fewer men with CBAVD required a TESA procedure compared with the post-infectious and vasectomy groups. Our results indicate that PESA is sufficient for sperm retrieval in CBAVD cases but that TESA will need to be performed in approximately 1/3 of cases in the other etiology groups (32). In our series, the higher percentage of vasectomy and post-infectious patients, compared to those with congenital obstruction, requiring a rescue TESA procedure after the initial failed epididymal attempt is likely due to the epididymis being obstructed after vasectomy or failed reversals or being severely damaged after infection, thus making it difficult to percutaneously retrieve motile sperm. The concept of cryopreservation may be used in association with sperm retrieval procedures. Epididymal and testicular spermatozoa can be cryopreserved using protocols routinely used for ejaculated sperm. Some centers prefer to retrieve and intentionally cryopreserve sperm for future use. If sperm are frozen, thawing can be performed at any time, thus obviating the need to organize two operations (oocyte and sperm retrieval) on the same day. Additionally, cryopreservation may be an interesting method for storing leftover specimens that would otherwise be discarded after ICSI, especially if the treatment cycle does not result in a pregnancy. Future ICSI attempts may be performed without repeated surgical retrievals. We routinely freeze excess motile epididymal spermatozoa that are not needed for the current ICSI cycle. Most often, motile sperm will be available after thawing in such cases, and ICSI outcomes using motile fresh or frozen epididymal sperm do not appear to differ.

The best currently available evidence regarding the influence of the cause of obstruction on ICSI results is provided by a meta-analysis that was entirely based on a retrospective heterogenic series (21). Therefore, further studies are needed, particularly studies with a prospective design. Moreover, few studies have considered the impact of maternal age on fecundity. Vasectomy reversal may not be the first line therapy for couples in which the female partner is of advanced maternal age because sperm retrieval and ICSI expedites the time to pregnancy and delivery. Moreover, when analyzing studies that compare pregnancy outcomes, it must be noted that a significant proportion of them consider only the results achieved for the first ICSI cycle. These results can be misleading, as success can be achieved in subsequent cycles as a result of optimized ovarian stimulation of the female partner or the replacement of cryopreserved embryos.

Finally, there is little high-quality evidence on short- and long-term pregnancy outcomes with ICSI using spermatozoa from men with OA. From the limited data available, no major differences have been reported in the short-term neonatal outcomes of children born from such fathers. ICSI with epididymal sperm does not lead to a higher incidence of stillbirths or congenital malformations compared with IVF and ICSI with ejaculated sperm and does not lead to poor childhood development (69). In this sense, the Dutch follow-up study is reassuring, but obtaining more data on fetal, neonatal and long-term outcomes should be identified as a major research priority.

Due to the importance of obstructive azoospermia in the context of male infertility, there is a need for the execution of well-designed multi-center studies with adequate sample sizes and follow-up durations, the development of standard data sets to enable the differentiation of men into the various OA subgroups, the consideration of the cause of obstruction and the source of the male gamete, and the use of control groups of children conceived naturally or with ICSI using ejaculated sperm. For now, the continued monitoring of children born after ICSI using non-ejaculated sperm from men with OA should be set as the minimum standard.

KEY ISSUES

- In men with OA, sperm production is normal, and gametes can be easily retrieved from the epididymis or testis in the vast majority of cases.
- The sperm retrieval technique and the cause of obstruction have little impact on sperm retrieval success rates.
- Percutaneous sperm retrievals are simple and effective methods for collecting epididymal or testicular spermatozoa in OA. TESA should be performed as a rescue procedure because TESA may impose a higher risk of complications.
- MESA may yield a higher number of motile sperm but is not demonstrably cost-effective and is more technically demanding compared with percutaneous retrieval methods.
- The time interval since vasectomy and the paternal age do not seem to affect the sperm retrieval success rate or pregnancy outcomes from sperm injections.
- The current evidence demonstrates equivalent pregnancy outcomes in OA men when comparing epididymal and testicular sperm, fresh and frozen-thawed sperm, and different causes of obstruction.
- Sperm chromatin integrity seems to be decreased toward the distal portion of an obstructed seminal tract.
- Motile sperm retrieved from men with OA should be cryopreserved whenever possible for future use, thereby sparing men from unnecessary procedures.
- No major difference has been reported in the short-term neonatal outcomes of children born from fathers with OA. ICSI performed with epididymal sperm does not lead to a higher rate of stillbirths or congenital malformations compared with IVF and ICSI with ejaculated sperm and does not lead to poor childhood development. However, the current data are limited, and the continued monitoring of children born to OA fathers is of utmost importance.

AUTHOR CONTRIBUTIONS

Miyaoka R and Esteves SC were involved in the acquisition and analysis of the data, as well as the drafting and revision of the manuscript.

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