Does the outcome of ICSI in cases of obstructive azoospermia depend on the origin of the retrieved spermatozoa or the cause of obstruction? A comparative analysis

Ahmed Kamal, M.D.,[a] *Ibrahim Fahmy, M.D.,*[b,d] *Ragaa Mansour, M.D., Ph.D.,*[b] *Gamal Serour, M.D., F.R.C.O.G.,*[b] *Mohamed Aboulghar, M.D.,*[b] *Liliana Ramos, Ph.D.,*[c] *and Jan Kremer, M.D., Ph.D.*[c]

[a] Assisted Reproduction and Gynecology Centre, London, United Kingdom; [b] Egyptian IVF-ET Center, Cairo, Egypt; [c] Department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; [d] Andrology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

Objective: To compare the outcomes of intracytoplasmic sperm injection (ICSI) for men with obstructive azoospermia and normal spermatogenesis, according to the use of epididymal or testicular spermatozoa and the cause of obstruction.
Design: Retrospective study.
Setting: Private infertility center.
Patient(s): A detailed chart review of a cohort of 1,121 men with obstructive azoospermia who underwent intracytoplamic sperm injection (ICSI) was performed.
Intervention(s): Patients were grouped according to the origin of spermatozoa: epididymal (n =331) or testicular (n =790). They were further classified into two subgroups according to the cause of obstruction: congenital bilateral absence of vas deferens (CBAVD; n = 434), and other causes of obstruction (n= 687).
Main Outcome Measure(s): Fertilization, clinical pregnancy, and miscarriage rates.
Result(s): Fertilization (64.2% vs. 68.0%), clinical pregnancy (42.3% vs. 43.2%), and miscarriage (17.6% vs. 18.4%) rates did not differ between epididymal spermatozoa and testicular spermatozoa, respectively. Fertilization, clinical pregnancy, and miscarriage rates were also similar in the patients with CBAVD or due to other causes of obstruction.
Conclusion(s): The source of sperm used for ICSI in cases of obstructive azoospermia and the etiology of the obstruction do not affect the outcome in terms of fertilization, pregnancy, or miscarriage rates. (Fertil Steril® 2010;94:2135–40. ©2010 by American Society for Reproductive Medicine.)

Key Words: Intracytoplasmic sperm injection, azoospermia, spermatozoa retrieval, pregnancy, abortion

Surgically unreconstructable obstruction of the male genital tract is a relatively common etiology of azoospermia among infertile men (1). Patients with obstructive azoospermia (OA) due to congenital bilateral absence of the vas deferens (CBAVD) and those who have suffered failure of reconstructive surgery have historically been considered hopelessly infertile. After the introduction of intracytoplasmic sperm injection (ICSI) as a means to achieve pregnancies with surgically retrieved sperm from the testis or the epididymis, paternal hope was restored to azoospermic men (2, 3).

Spermatozoa can be obtained by percutenous epididymal sperm aspiration (PESA) or microsurgical epididymal sperm aspiration (MESA) in cases of azoospermia due to CBAVD, failed vasoepididymostomy, or otherwise irreparable obstruction (4). Spermatozoa can also be collected straight from the testis if the epididymis is inaccessible or if no motile spermatozoa are present in the epididymis (5, 6). Good fertilization and pregnancy rates have been demonstrated in cases with obstructive and nonobstructive azoospermia (NOA) (7, 8), although the quality of spermatozoa in terms of DNA damage or maturation when collected from the epididymis may differ from that collected from the testis.

The reported incidence of miscarriages resulting from the use of testicular or epididymal spermatozoa varies greatly, from 5% to 40%, especially when the cause of the azoospermia (OA or NOA) is not well defined. High abortion and miscarriage rates were reported when testicular spermatozoa were used (9–11), suggesting that the male gamete could affect the developmental competence of the resulting embryos. However, in most studies, it was not possible to determine whether this reported high miscarriage rate (MR) associated with the use of testicular spermatozoa was related to a defect of spermatogenesis, sperm immaturity, or DNA damage. Alternatively, other studies reported high rates of failed embryo development after ICSI using epididymal spermatozoa compared with testicular spermatozoa (12). In contrast, Buffat et al. (11) suggested that the use of testicular spermatozoa, even those generated during normal spermatogenesis, alters embryonic development and that epididymal spermatozoa should be preferentially used, regardless of the etiology of OA.

Furthermore, it has been shown that DNA fragmentation, as measured by the Comet assay, was higher in epididymal than in testicular spermatozoa from men with OA (12). This finding has led to the

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Reprint requests: Ahmed Kamal, M.D., Embryology Laboratory, 13 Upper Wimpole Street, London W1G 6LP, United Kingdom (FAX: +44 (0) 207 486 1232; E-mail: ahmedivf@hotmail.com).

recommendation to use testicular spermatozoa rather than epididymal spermatozoa in cases of OA (12). However, when using morphologically normal and motile epididymal sperm, Ramos et al. (13) found no increase in sperm with DNA damage compared with ejaculated sperm. Taking all these results together, we conclude that not only the source but the type of sperm used (normal/abnormal; motile/immotile) may influence the outcome of ICSI in OA. Although chromatin maturity of epididymal sperm is more like that of ejaculated sperm, these sperm are at risk of DNA damage due to detrimental influence of radical oxygen species in the obstructed epididymis (14).

As a result of the contradicting outcomes and recommendations for OA, it is still not clear which source of sperm should be preferably used in cases of OA. Therefore, the results presented in this study are of essential clinical relevance for determining the best strategy for treatment.

MATERIALS AND METHODS

Study Population

A detailed chart review of ICSI cycles between 1994 and 2006 at the Egyptian IVF-ET Center revealed that a total of 1,661 ICSI cycles were performed for patients with OA using surgically retrieved sperm. Institutional Review Board approval was obtained for this study. The mean age of the men in the cohort was 39.28 (±7.47) years (range 20–69 years) at the time of sperm retrieval.

Establishing a correct diagnosis of the azoospermia is mandatory for adequate clinical counseling. Preoperative andrologic work-up consisted of physical examination, testicular volume assessment, and hormone analysis (2× FSH, LH, T). Serum estrogens, PRL, and adrenal steroids were measured only if clinically indicated, e.g., low serum T, decreased libido, gynecomastia, or a history of precocious puberty.

Semen analysis was performed twice on each man according to World Health Organization (15) criteria. Azoospermia was diagnosed when no spermatozoa were found after samples were centrifuged (2,000*g*, 10 min) and retrograde ejaculation had been excluded. Karyotype analysis was recommended when indicated. The incidence of cystic fibrosis (CF) is very low in Egypt (16). Despite the high incidence of CBAVD in Egypt, the specific mutations resulting in CBAVD remain unknown. It appears that the mutation causing/resulting in CBAVD in Egypt are not associated with the severe forms of CF. Therefore, we only counsel patients (especially those with positive consanguinity) about the possibility of having a child with CBAVD.

The mean age of the female partners was 30.90 (±5.67) years (range 17–45 years). General examination, pelvic examination, and transvaginal ultrasonography were routinely performed according to the center's standard operation protocols.

To avoid possible sources of bias, only the first fresh cycle per couple was included in this study. In addition, patients were excluded if their clinical history included any of the following: repeated ICSI cycles, ICSI using cryopreserved-thawed sperm, and/or no histopathologic confirmation of the cause of azoospermia.

Ovulation Induction

The protocol for ovulation induction was as published in detail elsewhere (17). In brief, ovulation induction started with down-regulation using a midcycle GnRH long protocol. Once down-regulation was confirmed, ovarian stimulation was induced with human menopausal gonadotropins and/or recombinant FSH. Once the 3 leading follicles reached ~18 mm in mean diameter, 10,000 IU hCG were given IM to induce final oocyte maturation. Oocyte retrieval was performed 36 hours later, through transvaginal ultrasonography as previously described (18).

Sperm Retrieval Techniques

Initially, microsurgical epididymal aspiration (MESA) was used to retrieve spermatozoa from all obstructive cases (n = 24). In 1995, testicular sperm extraction (TESE) was adopted for all obstructive cases, because it was easier and cheaper to perform (n = 135). In 1996, percutenous epididymal aspiration (PESA) was introduced to obtain spermatozoa for cases with congenitally absent vas and failed or unreconstructable acquired cases (n = 178). PESA was attempted if clinical examination showed a full distended epididymal head. Testicular sperm retrieval was performed if the epididymis was collapsed or when no motile epididymal sperm could be retrieved by PESA (n = 71). Later (2002), we introduced the technique of large-needle aspiration using intravenous catheter instead of open biopsy to retrieve testicular spermatozoa (n = 218).

All of the aforementioned techniques were carried out under local infiltration anesthesia cord block with a mixture of 1:1 bupovacaine and lidocaine. In anxious patients, sedation was achieved by injecting medazolam 5 mg IM 15 minutes before surgery. General anesthesia was performed rarely when requested by the patient or when traction on the testes during examination caused intolerable pain.

These procedures are described in detail elsewhere (7). Only PESA and TESA will be described briefly below.

Percutaneous Epididymal Sperm Aspiration (PESA)

We modified the previously described technique (7) by using a G30 tuberculin syringe containing 0.5 mL Hepes-buffered Earle medium (Sigma, St. Louis, MO) instead of a butterfly needle to aspirate the epididymis. The epididymis was held between the surgeon's thumb and index finger. The needle was introduced in the epididymis head (caput), the closest to the testicle. Only 2–3 punctures were performed, always changing syringes and needles for each puncture. The samples were examined under an optical microscope in the operating room to verify the presence of spermatozoa. Counting of spermatozoa was not accomplished, owing to the small quantity available. The material was stored at 37°C until the ICSI procedure.

TESA/Testicular Biopsy Procedures

Our TESA procedure is described in detail in a previous publication (19). In summary, an assistant held the testis firmly, taking care to keep the epididymis at a posterior position. An IV catheter (size G14 or G16) was introduced into the anterior surface at the testis to a depth of 1–2 cm. The metal needle was removed and the catheter was connected to a 20 mL syringe to create a negative pressure. The catheter was then withdrawn slowly, while maintaining the negative pressure. When fluid or testicular tissue was seen through the transparent wall, a clamp was applied to the catheter while still maintaining the negative pressure.

The bulging core of tissue was cut from the testis skin by sharp scissors and the catheter content was evacuated into a Petri dish. The quantity of any retrieved tissues was estimated from the length of the core of aspirated tissue. The retrieved testicular tissues were minced together with the aspirated fluid in a Petri dish containing 1–2 mL medium (Sigma), depending on the amount of tissue.

The testicular homogenate was examined under the high power of an inverted microscope. If, after a rapid search, no normal motile spermatozoa could be detected, another aspiration was performed. If three aspirations from the same testis were negative for motile spermatozoa, an open biopsy was performed.

ICSI procedure

The ICSI was carried out on the stage of an inverted microscope (Nikon) at ×400 magnification using the Hoffman modulation contrast system. It was performed on all morphologically intact metaphase II oocytes (second metaphase) and always with immobilized motile spermatozoa (20). Only motile sperm was used in all injections. After performing the ICSI, the remaining testicular or epididymal sperm were routinely frozen for future use unless declined by patients who refused freezing. Oocytes were examined 16–18 hours after microinjection. Normal fertilization was defined as the presence of two distinct pronuclei and a second polar body. Embryo cleavage was assessed 24 hours later and embryos scored according to the number of blastomeres and the percentage of enucleate fragments (I: no fragments; II: 1&–20% fragments; III: 21%–50% fragments; IV: >50% fragments). Up to three, and in

rare instances four, embryos were transferred into the uterine cavity 48–72 hours after the ICSI procedure.

Follow-Up

Male patients were asked to return after 1 week and also after 6 months prospectively for follow-up. Pain and hematoma were considered to be a short-term postoperative complication.

Clinical pregnancy are defined by the presence of gestational sac (with heartbeat) observed by ultrasound scanning ~7 weeks after embryo transfer.

Miscarriage was defined as any spontaneous interruption of clinical pregnancy not related to extrauterine implantation or medical termination of pregnancy.

Statistical Analysis

Statistical test of significance were two-sided and tested at the 5% level; values of $P<.05$ were considered to indicate significant differences. Continuous variables were tested for normal distribution using the F test. When data were found to be parametric, the results of the two groups were compared using the *t* test. Qualitative variables were compared with chi-square test with Yates correction or Fisher exact test, when necessary. Clinical and demographic data are also presented as mean (SD) or as frequency distribution for simplicity. Statistical analysis was performed using the computer statistical package StatsDirect (StatsDirect, Cheshire, U.K.).

Results

A total of 1,661 ICSI cycles were performed for patients with OA using surgically retrieved sperm in the period from 1994 and 2006. In this retrospective cohort study, only patients with their first fresh ICSI cycles were included. Repeated ICSI cycles, cycles using cryopreserved-thawed spermatozoa or embryos, or cycles with incomplete data (n = 540) were not included in the study.

Data from a total of 1,121 patients undergoing ICSI cycles were available for this study. A total of 790 ICSI cycles were performed using testicular sperm, and 331 ICSI cycles were performed using epididymal sperm (Table 1). The etiology for obstruction was CBAVD (n =434) or other causes of acquired obstruction (n = 687).

Azoospermia was confirmed on at least two occasions. We did not do a karyotype for all patients. We started the karyotype at a later stage. It is not routinely asked for all patients with OA. It is mandatory only in NOA and in patients with small testes.

Testicular volume was assessed clinically and all patients had moderate (8–12 mL), normal (12–18 mL), or large testes (>18 mL). We used the Prader orchidometer only to measure the testicular size for patients with NOA and small testes (<8 mL). Regarding histopathology, many patients referred to our center have already had the biopsy done in other centers. We revised the slides if available. Otherwise a new biopsy was taken before or during the ICSI/TESE cycle. However, in patients undergoing PESA and who had no testicular biopsy, the presence of many sperm in the epididymal aspirate was taken as evidence to confirm normal spermatogenesis.

Therefore our criteria for diagnosing obstructive azoospermia are the presence of the following: azoospermia, normal hormonal profile, normal-sized testis, and normal spermatogenesis evident by a normal testicular biopsy or an epididymal aspirate full of spermatozoa.

Testicular biopsy revealed normal spermatogenesis in all cases for which it was performed. We did not do any study about how the patients tolerate each technique. We did not encounter any major complications from different techniques. We had very few minor complications with TESE/TESA, including small postoperative hematoma, either in the spermatic cord or subcutaneous, and minor wound infection.

Patient demographics between the two groups (epididymal and testicular) were similar (Table 1). Overall, 9,751 oocytes were retrieved. The mean fertilization rate was 65.2%. The overall implantation rate and clinical pregnancy rate were 20.2% and 42.9% per cycle started (Table 1). No statistical differences were found in the ICSI outcome regarding the source of sperm used in OA.

Again, the analysis of the ICSI outcome regarding the etiology of obstruction (congenital, CBAVD, or acquired) showed no differences in implantation, pregnancy, and miscarriage rates. Subgroup analyses according to the etiology of obstruction revealed similar results (Tables 2 and 3).

DISCUSSION

The strength of this study, compared with others and meta-analysis publications regarding the use of epididymal or testicular sperm in cases of OA, is the large population analyzed. The data presented here show that the preferential use of testicular sperm or epididymal sperm in cases of OA is unfounded. Neither the source nor etiology seems to affect the outcome of ICSI.

The use of surgical sperm retrieval and ICSI has greatly improved the treatment of azoospermic men, but the consequences of using immature sperm retrieved directly from the testis with normal or defective function are not fully known. We studied ICSI outcomes

TABLE 1

Intracytoplasmic sperm injection outcomes according to the origin of spermatozoa.

	Testicular spermatozoa	Epididymal spermatozoa	*P* value
No. of oocyte retrievals	790	331	
Age of women, y (mean ± SD)	31.05 ± 5.73	30.53 ± 5.51	.16
Age of men, y (mean ± SD)	39.73 ± 7.56	38.25 ± 7.04	.003
Period of infertility, y (mean ± SD)	9.08 ± 5.92	9.06 ± 5.73	.98
No. of oocytes retrieved (mean ± SD)	10.75 ± 6.17	11.30 ± 6.21	.23
No. of metaphase II oocytes injected (mean ± SD)	8.36 ± 4.88	8.73 ± 5.13	.26
No. of 2-pronuclei oocytes (mean ± SD)	5.69 ± 3.48	5.70 ± 3.42	.98
Fertilization rate (%)	68.03%	64.22%	.02
No. of transferred embryos (mean ± SD/transfer)	3.11 ± 1.15	3.10 ± 1.15	.93
Implantation rate (%)[a]	19.93%	20.77%	.41
No. of clinical pregnancies (%)[b]	341 (43.16%)	140 (42.30%)	.84
No. of miscarriages (%)[c]	49 (18.35%)	19 (17.59%)	1.00

[a] Ratio between the number of gestational sacs and the number of transferred embryos.
[b] Ratio between the number of clinical pregnancies and the number of oocyte retrievals.
[c] Ratio between the number of spontaneous pregnancy losses before 20 weeks of gestation and number of the clinical pregnancies.

Kamal. ICSI outcome in obstructive azoospermia. Fertil Steril 2010.

TABLE 2

Intracytoplasmic sperm injection outcomes in patients with congenital absence of the vas deferens.

	Testicular spermatozoa	Epididymal spermatozoa	*P* value
No. of oocyte retrievals	221	213	
Age of women, y (mean ± SD)	29.96 ± 6.11	29.70 ± 5.60	.64
Age of men, y (mean ± SD)	36.69 ± 7.12	36.59 ± 6.36	.91
Period of infertility, y (mean ± SD)	8.10 ± 5.55	8.44 ± 5.56	.53
No. of oocytes retrieved (mean ± SD)	10.90 ± 6.36	11.40 ± 6.46	.47
No. of metaphase II oocytes injected (mean ± SD)	8.57 ± 4.77	8.90 ± 5.15	.49
No. of 2-pronuclei oocytes (mean ± SD)	5.78 ± 3.41	5.77 ± 3.43	.98
Fertilization rate (%)	68.21%	63.35%	.10
No. of transferred embryos (mean ± SD/transfer)	3.10 ± 1.00	3.06 ± 1.09	.74
Implantation rate (%)[a]	21.56%	23.43%	.99
No. of clinical pregnancies (%)[b]	102 (46.15%)	92 (43.19%)	.60
No. of miscarriages (%)[c]	17 (22.24%)	14 (19.18%)	1.00

[a] Ratio between the number of gestational sacs and the number of transferred embryos.
[b] Ratio between the number of clinical pregnancies and the number of oocyte retrievals.
[c] Ratio between the number of spontaneous pregnancy losses before 20 weeks of gestation and number of the clinical pregnancies.

Kamal. ICSI outcome in obstructive azoospermia. Fertil Steril 2010.

according to the site of sperm retrieval (testis or epididymis) and the etiology of the obstructive azoospermia by analyzing more than 1,100 consecutive ICSI cycles involving men with normal spermatogenesis. To the best of our knowledge, this is the largest series since a recent published report of a series of 171 ICSI cycles in cases of obstructive azoospermia (11).

Various factors may affect the results of ICSI cycles using surgically extracted sperm. Among these are the method of sperm retrieval, the maturity and motility status of retrieved gametes, the timing of sperm retrieval in relation to oocyte collection, and the possibility of freezing the retrieved male gametes for repeated use (3).

It is reported that the fertilization rate is influenced not only by sperm quality but also by the embryo and blastocyst development (21–23). Furthermore, it has also been reported that the implantation, pregnancy, and even spontaneous abortion rates are affected by the sperm quality (23).

Published results concerning fertilization, pregnancy, and miscarriage rates after ICSI with testicular spermatozoa are discordant. Some studies have reported similar fertilization and pregnancy rates for ICSI with ejaculated and epididymal spermatozoa (9, 24–26), whereas other studies reported impaired fertilization rates with epididymal spermatozoa but similar pregnancy rates (8, 27). Moreover, others documented similar fertilization rates but impaired pregnancy rates (28, 29). In addition, the MRs after ICSI with testicular spermatozoa have been reported to be higher than (9, 10) or similar to (8, 21, 24, 26) ICSI with ejaculated or epididymal sperm.

The only report that demonstrated no significant difference in any outcome measure between the use of epididymal and the use of testicular sperm in men with OA was the meta-analysis published by Nicopoullos et al. in 2004 (30). They first analyzed their data and then used a meta-analysis of published data (including their data) to compare the outcome of ICSI in OA, classified as congenital or

TABLE 3

Intracytoplasmic sperm injection outcomes in patients with acquired obstruction.

	Testicular spermatozoa	Epididymal spermatozoa	*P* value
No. of oocyte retrievals	569	118	
Age of women, y (mean ± SD)	31.48 ± 5.53	32.03 ± 5.01	.16
Age of men, y (mean ± SD)	40.93 ± 7.39	41.29 ± 7.24	.64
Period of infertility, y (mean ± SD)	9.46 ± 6.02	10.19 ± 5.90	.23
No. of oocytes retrieved (mean ± SD)	10.69 ± 6.10	11.13 ± 5.76	.52
No. of metaphase II oocytes injected (mean ± SD)	8.28 ± 4.93	8.42 ± 5.09	.78
No. of 2-pronuclei (PN) oocytes (mean ± SD)	5.66 ± 3.51	5.57 ± 3.42	.80
Fertilization rate (%)	67.96%	63.99%	.12
No. of transferred embryos (mean ± SD/transfer)	3.12 ± 1.20	3.18 ± 1.24	.61
Implantation rate (%)[a]	20.58%	20.77%	.95
No. of clinical pregnancies (%)[b]	239 (42.00%)	48 (40.68%)	.87
No. of miscarriages (%)[c]	32 (17.49%)	5 (14.29%)	.93

[a] Ratio between the number of gestational sacs and the number of transferred embryos.
[b] Ratio between the number of clinical pregnancies and the number of oocyte retrievals.
[c] Ratio between the number of spontaneous pregnancy losses before 20 weeks of gestation and number of the clinical pregnancies.

Kamal. ICSI outcome in obstructive azoospermia. Fertil Steril 2010.

acquired causes. Their study comprised 82 couples who underwent 127 ICSI cycles using surgically retrieved sperm. The meta-analysis comparing congenital (CBAVD) and acquired causes showed a significantly increased fertilization rate (95% confidence interval, 0.84–1) compared with acquired causes. Meta-analysis of the three papers reporting delivery outcome showed no difference in live birth rate but a significantly higher MR in the congenital group (relative risk 2.67). They concluded that in ICSI cycles for men with OA, the cause appears to influence the outcome, but the outcome is not affected by whether the retrieved sperm is fresh, frozen, epididymal, or testicular. The meta-analysis suggested a higher fertilization rate and lower MR in acquired causes of OA.

Because of the conflicting findings reported in the literature, it is difficult to interpret and clinically implement these results, because apart from three of the aforementioned studies (8, 11, 21) there was no systematic distinction between OA and NOA. This prevents consideration of the respective influence of spermatogenesis defects and of spermatozoa immaturity in cases of spontaneous miscarriage.

To avoid possible sources of bias, the present study included only cases with proven histopathologic obstructive azoospermia, with only one cycle per couple. Despite the fact that the large number of this study is considered to be a very strong point, besides the strict exclusion criteria we decided to adopt, there are some weak points represented in the retrospective nature of the study; moreover, another selection may have taken place by the stepwise nature of the procedures undertaken.

In this study, the overall 17.59% MR observed in pregnancies obtained with epididymal spermatozoa in cases of OA is within the previously reported range varying from 4.6% reported by Friedler et al. (26) to 25% reported by Balban et al. (31).

The study done by Griffith et al. (32), comparing ICSI results from epididymal or testicular sperm from obstructive azoospermic sperm, did not find any difference in the MR according to the origin of spermatozoa; however, that study was criticized because of the small series number (28 treatment cycles and 12 pregnancies with epididymal sperm and 14 treatment cycles and 6 pregnancies with testicular sperm), and, consequently, it is difficult to draw any firm conclusion from that particular study.

The overall 18.35% MR observed in pregnancies obtained with testicular spermatozoa is also in the previously reported range, which varies from 12.5% reported by Palermo et al. (8) to 40% reported by Nicopoullos et al. (30) whatever the nature of azoospermia.

Our results show that testicular spermatozoa from normal spermatogenesis allow similar fertilization and clinical pregnancy rates to epididymal spermatozoa. More interestingly, they are not responsible for a significantly higher MR, as previously suggested (10, 11). In the latter study, the authors could not explain the reason for their higher MR and could not attribute the failure of embryo development after ICSI to spermatozoa DNA damage, because it was previously proven that DNA fragmentation as measured by TUNEL assay and Comet assay was higher in epididymal than in testicular spermatozoa of men with OA (12, 13). Those findings are therefore contradictory.

We also found that the etiology of obstruction had no influence on the ICSI outcome when using surgically retrieved spermatozoa. The fertilization, pregnancy, and miscarriage rates were similar after ICSI with epididymal or testicular spermatozoa in cases of acquired or congenital obstruction.

In summary, the present results show that neither the origin of surgically retrieved spermatozoa nor the cause of obstruction has any significant effect on the success of assisted reproduction, especially when spermatogenesis is presumably normal. The paternal influence on embryo development and long-term follow-up studies are needed to prove the safety of surgically retrieved sperm in assisted reproductive techniques.

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