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Testicular sperm aspiration (TESA) for infertile couples with severe or complete asthenozoospermia

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SUMMARY

The aim of the study was to evaluate reproductive outcomes in a cohort of infertile couples with severe and complete asthenozoospermia undergoing TESA (testicular sperm aspiration) with ICSI. We conducted a retrospective study of 28 couples with complete or severe asthenozoospermia who underwent TESA between January 2010 and December 2015. We compared TESA-ICSI outcomes of these couples to ejaculate ICSI outcomes of 40 couples with severe asthenozoospermia treated during the same time period at our institution. Couples with female factor infertility and/or female aged ≥39 were excluded. Sperm retrieval rates and ICSI outcomes [(MII oocytes, fertilization rate, good embryo rate (transferred and frozen), couples with embryo transfer (per cycle started), clinical pregnancy (per embryo transfer)] were recorded. Patients were grouped based on whether they had ejaculated (Ej-group) or testicular (TESA-group) spermatozoa used. Testicular sperm patients were further classified based on whether they had complete asthenozoospermia (0% total motility) (Tc-group) or severe asthenozoospermia (≤1% progressive motility) (Ts-group). Mean (±SD) male and female ages were 36 ± 6 and 32 ± 4, respectively. Sperm recovery by testicular sperm aspiration (TESA) was successful in 100% (28/28) of the men. The overall clinical pregnancy rate (CPR) per cycle started was 34% (23/68) with a mean of 1.1 ± 0.4 embryos transferred per transfer. Fertilization rates were significantly lower in TESA-group compared to Ej-group (52% vs. 67%, respectively; $p = 0.001$), while male age was significantly higher in TESA-group compared to Ej-group (34 ± 6 vs. 37 ± 6, respectively; $p = 0.03$). Moreover, female age was significantly higher in Tc-group compared to Ts-group (30 ± 4 vs. 33 ± 3, respectively; $p = 0.0285$). However, there were no significant difference in clinical pregnancy rate per embryo transfer in the Tc-group, Ts-group, and Ej-group (50% vs. 45% vs. 57%, respectively; $p = 0.8219$). The data suggest that testicular sperm-ICSI is no better than ejaculated sperm-ICSI in couples with severe or complete asthenozoospermia. Randomized, controlled trials comparing ejaculated vs. testicular spermatozoa are needed to assess the true benefit of TESA-ICSI in these couples.

INTRODUCTION

The introduction of ICSI by Palermo in 1992 has revolutionized infertility treatment, especially in cases of male factor infertility and repeated IVF failures (Palermo *et al.*, 1992). Palermo initially claimed ICSI success rate was not influenced by sperm concentration, morphology, and progressive motility (Palermo *et al.*, 1993). However, Nagy *et al.*, (1995) reported that injecting a totally immotile (and presumable dead) spermatozoon can negatively influence ICSI outcome; a finding which was replicated by other series (Liu *et al.*, 1995; Nagy *et al.*, 1995; Nijs *et al.*, 1996; Vandervorst *et al.*, 1997).

Kahraman *et al.*, (1996) reported the first successful clinical pregnancy in a couple with complete asthenozoospermia by utilization of testicular spermatozoa for ICSI. They reported that testicular spermatozoa gave more favorable ICSI pregnancy outcomes than ejaculated spermatozoa in these couples. Moreover, Shulman *et al.*, (1999) reported that ICSI pregnancy outcomes with testicular spermatozoa were comparable whether motile or complete immotile spermatozoa are used.

The etiology of asthenozoospermia is complex and not completely understood. Ultrastructural abnormalities resulting in flagellar dysfunctions (e.g. primary ciliary dyskinesia – PCD) are uncommon and can lead to complete asthenozoospermia. Kartagener's syndrome is a rare disorder characterized by immotile cilia, situs inversus, chronic sinusitis, and bronchiectasis. Other factors associated with varying degrees of asthenozoospermia are intrinsic factors [mitochondrial dysfunction (Folgero *et al.*, 1993; Shamsi *et al.*, 2008), prolonged anejaculation

(Wilton *et al.*, 1988)] or extrinsic factors [environmental toxins (Saradha & Mathur, 2006), pesticide exposure (Pflieger-Bruss *et al.*, 2004; Perry, 2008), cigarette smoking (Vine *et al.*, 1996), orchitis (Schuppe *et al.*, 2008), and epididymitis (Haidl *et al.*, 2008)].

The rationale for using testicular rather than ejaculated spermatozoa in complete asthenozoospermia is based on sperm vitality. Nagy *et al.*, (1995) hypothesized that immotile ejaculated spermatozoa are 'long' dead spermatozoa with poor vitality. On the other hand, immotile testicular spermatozoa are either immotile because of immaturity (with potential to gain motility as they mature) or immotile 'freshly' dead spermatozoa, both of which are presumed to possess a higher vitality rate than immotile ejaculated spermatozoa and hence higher fertilization potential.

In our study, we sought to investigate testicular sperm retrieval rates and ICSI outcomes using testicular spermatozoa in couples with severe and complete asthenozoospermia. We compared TESA-ICSI outcomes to ICSI outcomes using ejaculated spermatozoa at our institution.

MATERIALS AND METHODS

Patients

We conducted a retrospective analysis of 28 consecutive men, the majority having 'unexplained' complete or severe asthenozoospermia (diagnosed on routine semen analysis) who underwent TESA between January 2010 and December 2015 at the OVO fertility clinic in Montreal, Canada. Our control group was comprised of 40 men that underwent ICSI (during the same period) using ejaculated spermatozoa. All men in the ejaculated sperm (control) group had a diagnosis of severe asthenozoospermia (≤1% progressive motility). At our clinic, only motile spermatozoa are used for ICSI. As such, if the sample to be used for ICSI shows complete asthenozoospermia, an emergency TESA is performed whenever possible (based on surgeon availability). Otherwise, oocytes are frozen and a TESA is scheduled at the following cycle. For both groups (testicular and ejaculated spermatozoa), all couples with female factor infertility and/or female age ≥39-year-old were excluded.

Semen analysis was performed using a microptic SCA (Sperm Class Analyzer, Microptic, Barcelona, Spain) with sperm motility measurements taken at 37°C. Multiple fields are analyzed to ensure that a minimum of 200 spermatozoa are assessed. Once analyzed, the user reviews the video data and manually corrects any errors included by the SCA. Once complete, the entire process is repeated a second time to confirm the results. The mean of two motility assessments is reported.

Consent was not obtained from patients. The study was reviewed by the OVO research and development scientific committee and received approval as a quality control study. The principles of the Helsinki Declaration were followed.

A fellowship-trained male infertility specialist (Urologist) evaluated all men with a detailed history, physical examination, and appropriate laboratory testing (e.g. hormone evaluation). At our IVF Center, TESA (testicular sperm aspiration) is performed the day before oocyte retrieval because the embryologists prefer fresh (rather than frozen thawed) testicular spermatozoa for ICSI**.** Before proceeding to TESA-ICSI, each case was first reviewed by the clinical team (Urologist, Gynecologist, and Embryologist). Couples with unexplained severe to complete asthenozoospermia were deemed potential candidates for TESA-ICSI based on studies supporting the use of testicular spermatozoa in such cases (Kahraman *et al.*, 1996; Nijs *et al.*, 1996; Shulman *et al.*, 1999). Couples with complete and severe asthenozoospermia understood that on the day of ICSI, there was some possibility that no motile spermatozoa would be found and that the ICSI cycle would potentially be canceled. Couples were given the option to proceed to ICSI with ejaculated spermatozoa vs. ICSI with testicular spermatozoa with the understanding that the two options would be associated with comparable outcomes. Moreover, the couples were informed of the added known potential risks of TESA (bleeding, infection, pain, irreversible testicular dysfunction, and hypogonadism), as well as the unknown risks associated with use of testicular spermatozoa for ICSI (genetic and epigenetic risks).

The couples that underwent TESA-ICSI were divided into two groups. (Tc-Group): complete asthenozoospermia (0% total motility) and (Ts-Group): severe asthenozoospermia (≤1% progressive motility). Our control group consisted of couples with severe asthenozoospermia (≤1% progressive motility) who underwent ICSI using ejaculated spermatozoa (Ej-Group). Variables included in our analysis were patient age, partner age, FSH and total testosterone level, testicular volume, sperm retrieval rate, MII oocytes retrieved, fertilization rate (2PN), good embryos rate, and clinical pregnancy rate.

Retrieval techniques

TESA was performed under local anesthesia, and the same surgeon (AZ) performed all procedures. Before undergoing TESA, all patients were re-examined for assessment of testicular volume. The choice of testicular side to be aspirated was based on testicular volume and testicular sensitivity (the larger and less sensitive testicle was aspirated first). All patients received a spermatic cord block with 1% lidocaine hydrochloride (HCl) (without epinephrine). Typically, 10 mL of lidocaine HCl is injected into the spermatic cord with another 3–5 mL in the scrotal skin and dartos muscle superficial to the testis; then, the needle is advanced through tunica albuginea and 0.5–1.0 mL of lidocaine HCl is injected directly into the testis (Kamal *et al.*, 2002). The testis becomes firmer after injection. A 16-gauge clear angiocatheter needle (1-1/4^{00} CATHLON I.V Catheter; Smiths Medical International Ltd, Rossendale, UK) is directed through the scrotal skin into the testis. The needle is withdrawn and the angiocatheter is kept in place. A 10 mL syringe containing 1–2 mL of sperm buffer is attached to the angiocatheter. Negative pressure is created and the angiocatheter is gently withdrawn and then pushed back into the testis until testicular tissue appears in the syringe. At this point, the angiocatheter is withdrawn completely while maintaining negative pressure and the remaining tissue is pulled using a smooth forceps. The testicular tissue is then expelled into a sterile dish. The specimen is immediately dissected and then examined under the microscope to confirm the presence of spermatozoa. The same technique is repeated (up to three aspirations per side) until adequate numbers of spermatozoa are recovered. If no spermatozoa are found, the same procedure is performed on the contralateral side (if necessary). Immediately after withdrawing the angiocatheter needle, gentle pressure is applied to the puncture site for 2–3 min, so as to minimize the risk of bleeding.

Sperm retrieval outcomes

Sperm retrieval outcomes were recorded by the embryologist (in consultation with the treating urologist-AZ) within 10 min after the TESA and reported as positive (presence of mature spermatozoa) or negative (absence of mature spermatozoa).

ICSI outcomes

For each ICSI cycle, MII oocytes, fertilization rate (2PN), good embryo rate, couples with embryo transfer (ET), and clinical pregnancy (per embryo transfer) were recorded. Clinical pregnancy was established by detection of fetal heartbeat on ultrasound after 8 weeks of gestation.

Statistical analysis

Descriptive analyses were used to represent the various parameters (e.g. male age, testicular volume, serum FSH, sperm retrieval rates). Continuous variables are presented as mean ± SD and were compared using the Mann–Whitney rank sum test or Kruskal–Wallis test when applicable. Dichotomous variables are presented as percentages and were compared using Fisher's exact test or Chi-squared test when applicable. The relationship between variables was estimated by Pearson's correlation coefficient. All statistical analyses were performed using the SAS statistical software (version 9.2; SAS Institute Inc., Cary, NC, USA).

RESULTS

We identified a total of 28 couples that underwent ICSI using testicular spermatozoa and 40 couples that underwent ICSI using ejaculated spermatozoa. The clinical characteristics of the cohorts are shown in Tables 1 and 2. The mean paternal and maternal ages were 36 ± 6 and 32 ± 4 years, respectively. The mean paternal age was significantly lower in the TESA-group compared to Ej-group (34 ± 6 vs. 37 ± 6, respectively; $p = 0.0308$). The mean maternal age was comparable between TESA-group and Ej-group, however, the mean maternal age of Tc-group was significantly lower than Ts-group (30 ± 4 vs. 33 ± 3, respectively; $p = 0.0285$). Additionally, the right and left testicular volumes in TESA-group were significantly higher than Ej-group (18 ± 2 and 18 ± 3 vs. 16 ± 5 vs. 15 ± 4, respectively; $p = 0.002$, $p = 0.006$).

The Tc-group had a significantly higher mean sperm concentration than both the Ts-group and Ej-group (49 ± 49 vs. 10 ± 11 and 23 ± 63, respectively; $p \leq 0.0001$). Otherwise, the three groups had comparable semen volume and percent normal forms (see Table 3).

Of the 11 men presenting with complete asthenozoospermia, two had secondary infertility while the rest had primary infertility. Two men had poor sperm viability, one had diabetes mellitus, one had partial ejaculatory duct obstruction, and one man had Kartagener's syndrome.

Of the 17 men presenting with severe asthenozoospermia, six had secondary infertility while the rest had primary infertility. Six men had a prior ICSI failure using ejaculated spermatozoa, two of these men had high sperm DNA fragmentation index (DFI ≥70%).

The 40 couples who underwent ICSI using ejaculated spermatozoa all had severe asthenozoospermia (≤1 progressive motility). Seven men had secondary infertility while the rest had primary infertility.

TESA outcomes

All men (28/28) underwent a unilateral procedure (TESA) with a mean number of 1.7 ± 0.9 aspirates (attempts) per procedure. A sperm retrieval rate of 100% (28/28) was achieved. None of the men required a bilateral TESA. Although no complications were

Table 1 Clinical characteristics of the couples with testicular (TESA-group) and ejaculated spermatozoa (Ej-group)

Parameters	Total	TESA spermatozoa (complete + severe asthenozoospermia)	Ejaculate spermatozoa (severe asthenozoospermia)	*p*-value
Number	68	28	40	-
Mean (±SD) male age	36 ± 6	34 ± 6	37 ± 6	0.0308[a]
Mean (±SD) female age	32 ± 4	32 ± 4	32 ± 4	NS[a]
Mean (±SD) FSH (IU/L)	7 ± 7	4 ± 2	10 ± 8	NS[a]
Mean (±SD) total testosterone (nmol/L)	16 ± 8	17 ± 9	16 ± 7	NS[a]
Mean (±SD) right testicular volume (mL)	17 ± 4	18 ± 2	16 ± 5	0.0028[a]
Mean (±SD) left testicular volume (mL)	16 ± 4	18 ± 3	15 ± 4	0.0006[a]

NS, not significant ($p \geq 0.05$). [a]Mann–Whitney rank sum test (Comparison between TESA and ejaculated spermatozoa).

Table 2 Clinical characteristics of the couples with testicular (Tc-group, Ts-group) and ejaculated (Ej-group) spermatozoa

Parameters	TESA spermatozoa		Ejaculate spermatozoa	*p*-value
	Complete asthenozoospermia	Severe asthenozoospermia	(Control)	
Number	11	17	40	-
Mean (±SD) male age	34 ± 5	34 ± 7	37 ± 6	NS[c]
Mean (±SD) female age	30 ± 4[a]	33 ± 3[b]	32 ± 4	0.028[c]
Mean (±SD) FSH (IU/L)	4 ± 2	5 ± 2	10 ± 8	NS[c]
Mean (±SD) total testosterone (nmol/L)	18 ± 12	16 ± 6	16 ± 7	NS[c]
Mean (±SD) right testicular volume (mL)	19 ± 1[b]	18 ± 3[b]	16 ± 5[a]	0.005[c]
Mean (±SD) left testicular volume (mL)	19 ± 2[b]	17 ± 3[b]	15 ± 4[a]	0.001[c]

NS, not significant ($p \geq 0.05$). [a,b]Different letters indicate significant difference between subgroups. [c]Kruskal–Wallis one-way ANOVA on ranks.

reported, we could not adequately assess the TESA complication rate because the majority of the men did not return for follow-up after TESA.

TESA vs. ejaculated spermatozoa ICSI outcomes

Table 4 presents the ICSI outcomes of the Tc-group (complete asthenozoospermia), Ts-group (severe asthenozoospermia), and the Ej-group (ejaculated spermatozoa). The overall per cycle started clinical pregnancy rate (CPR) was 34% (23/68), while CPR per embryo transfer was 52% (23/44) with a mean of 1.1 ± 0.4 total embryos transferred per transfer. Fertilization rates were significantly lower in both the Tc-group and Ts-group compared to the Ej-group (55 and 48% vs. 67%, respectively; $p = 0.003$). However, there was no significant difference in CPR per embryo transfer in the Tc-group, Ts-group, and Ej-group (50% vs. 45% vs. 57%, respectively; $p = 0.8219$).

DISCUSSION

The data indicate that sperm retrieval with TESA was successful in 100% (28/28) of the asthenozoospermic men in our study, with a mean number of 1.7 ± 0.9 aspirates (attempts) per procedure. Moreover, none of these men required a bilateral TESA. We found that the 28 couples in our study had favorable outcomes with TESA-ICSI. Indeed, the overall fertilization rate and clinical pregnancy rate (per embryo transfer) in the 28 couples were 52 and 48%, respectively, with a mean of 1.3 ± 0.6 embryos transferred (per transfer) in the TESA-ICSI groups.

The fertilization rate and clinical pregnancy rate with TESA-ICSI in the cohort with complete asthenozoospermia (Tc-group, $n = 11$), were 55 and 50%, respectively, with a mean of 1.4 ± 0.7 embryos transferred per transfer. The fertilization rate in our complete asthenozoospermia cohort (55%) is comparable to that reported by Kahraman *et al.*, (1996) and Nijs *et al.*, (1996) with testicular sperm-ICSI (53.5 and 65%, respectively). The mean maternal age of our cohort with complete asthenozoospermia (30 ± 4) was also comparable to that reported by Kahraman *et al.*, (1996) (28.8 ± 5.4). Moreover, the mean number of embryos transferred in the cohort with complete asthenozoospermia undergoing TESA-ICSI is lower than that reported by Nijs *et al.*, (1996) with testicular sperm-ICSI (1.4 ± 0.7 vs. 3.3 embryos/transfer, respectively).

It is important to note that Nijs *et al.*, (1996) further subdivided their couples with complete asthenozoospermia into two sub-cohorts: those with (i) 'initially immotile spermatozoa' (immotile at initial evaluation but subsequently rare motile spermatozoa were found at time of ICSI) and (ii) 'totally immotile spermatozoa' (immotile at initial evaluation and immotile spermatozoa at ICSI). Nijs *et al.*, (1996) reported that the outcomes (i.e. clinical pregnancy rate) were more favorable in the cohort with 'initially immotile spermatozoa' than the cohort with 'totally immotile spermatozoa'. Nijs *et al.*, determined that 38% of the couples that presented with complete asthenozoospermia in their ejaculate sample were subsequently found to have 'totally immotile spermatozoa'. This means that in clinical practice, if one is unable to obtain testicular spermatozoa on demand, there is a 38% chance that if these couples undergo ICSI (with ejaculated spermatozoa), they would be expected to have poor pregnancy rates. In our study, we did not further subdivide patients into these sub-cohorts (initially immotile and totally immotile) because of the practical difficulty in performing

Table 3 Semen parameters of the couples undergoing ICSI with testicular spermatozoa (Tc-group, Ts-group) and ejaculated spermatozoa (Ej-group)

Parameter	Total	TESA spermatozoa		Ejaculate spermatozoa	*p*-value
		Complete asthenozoospermia	Severe asthenozoospermia	(Control)	
Number	68	11	17	40	-
Mean (±SD) volume (mL)	2.6 ± 1.3	2.8 ± 1.4	2.7 ± 1.0	2.5 ± 1.4	NS[c]
Mean (±SD) concentration (M/mL)	24 ± 53	49 ± 49 [a]	10 ± 11[b]	23 ± 62 [b]	<0.0001[c]
Mean (±SD) normal morphology (%)	6 ± 11	8 ± 12	4 ± 6	6 ± 12	NS[c]

NS, not significant ($p \geq 0.05$); ET, embryo transfer; CPR, clinical pregnancy rate. [a,b]Different letters indicate significant difference between subgroups. [c]Kruskal–Wallis one-way ANOVA on ranks.

Table 4 ICSI outcomes in the couples with testicular (Tc-group, Ts-group) and ejaculated (Ej-group) spermatozoa

Parameter	Total	TESA spermatozoa		Ejaculate spermatozoa	*p*-value
		Complete asthenozoospermia	Severe asthenozoospermia	(Control)	
Number of couples	68	11	17	40	-
Total MII oocytes	461	110	94	257	-
Mean (±SD) MII oocytes	6.8 ± 5.7	10 ± 7.0	5.5 ± 4.0	6.4 ± 5.8	NS[e]
Fertilization rate (%)	277 (60%)	61 (55%)[c]	45 (48%)[c]	171 (67%) [d]	0.003[f]
Mean (±SD) fertilized oocytes	4.1 ± 4.0	5.5 ± 4.7	2.6 ± 3.1	4.3 ± 4.0	NS[e]
Good embryo rate (%)	130 (47%)	32 (52%)	23 (51%)	75 (44%)	NS[f]
Mean (±SD) good embryos	3.0 ± 1.5	3.2 ± 1.6	2.1 ± 0.9 [b]	3.3 ± 1.5 [a]	0.025[e]
ET per cycle started (%)	44/68 (65%)	10/11 (90%)	11/17 (65%)	23/40 (58%)	NS[f]
Mean (±SD) embryos per ET	1.1 ± 0.4	1.4 ± 0.7	1.2 ± 0.4	1.0 + 0	NS[e]
CPR per ET (%)	23/44 (52%)	5/10 (50%)	5/11 (45%)	13/23 (57%)	NS[f]

NS, not significant ($p \geq 0.05$); ET, embryo transfer; CPR, clinical pregnancy rate. [a,b,c,d]Different letters indicate significant difference between subgroups. [e]Kruskal–Wallis one-way ANOVA on ranks. [f]Fisher's exact test.

testicular sperm retrieval at the time of egg retrieval (because an urologist is not readily available to perform testicular sperm retrieval on demand).

The fertilization rate and clinical pregnancy rate with TESA-ICSI in our cohort with severe asthenozoospermia (Ts-group, $n = 17$) were 48 and 45%, respectively, with a mean of 1.2 ± 0.4 embryos transferred (per transfer). The rationale for performing TESA in this cohort is based on the observation that many of the couples with severe asthenozoospermia (≤1% motility) can present with complete asthenozoospermia on the day of ICSI (unpublished observations). Indeed, Vandervorst *et al.*, (1997) have reported that complete asthenozoospermia is a sporadic condition, such that on occasion, these couples will have severe asthenozoospermia. Moreover, variability in laboratory reporting can occasionally result in the incorrect assignment of these couples into the complete or severe asthenozoospermia cohort (Vandervorst *et al.*, 1997).

Direct comparison of our study groups shows a significantly higher fertilization rate in the Ej-group compared to the TESA-group (67% vs. 52%, respectively; $p = 0.001$). However, the proportion of mature embryos available to be transferred or frozen, as well as the proportion of couples with embryos transferred was higher in the TESA-group compared to the Ej-group (52% vs. 44% and 75% vs. 58%, respectively), although this difference did not reach statistical significance. We suspect that the relatively poor embryo development in the Ej-group may be because of an abnormality in the sperm (paternal) genome. Simon *et al.*, (2014) suggested that the paternal genome is inactive during early embryonic development stages (pre-fertilization, fertilization) and becomes actively involved at a later stage (D3 cleavage stage onwards) of embryonic development, a phenomenon that was labeled as 'late paternal effect'. The late paternal effect on embryo maturation has been positively correlated with sperm DNA fragmentation (Virro *et al.*, 2004; Simon *et al.*, 2014). Sperm DNA fragmentation has also been positively correlated with advance paternal age in infertile couples (Zorn *et al.*, 2012; Das *et al.*, 2013), asthenozoospermia (Lin *et al.*, 2008; Belloc *et al.*, 2014) as well as low testicular volume (Zorn *et al.*, 2012; Condorelli *et al.*, 2013). Unfortunately, we did not measure systematically sperm DNA fragmentation in our cohort of patients. However, paternal age was significantly higher in the Ej-group compared to the TESA-group (37 ± 6 vs. 34 ± 6, respectively; $p = 0.0308$). Moreover, right and left testicular volumes were significantly lower in the Ej-group compared to the TESA-group (16 ± 5 vs. 18 ± 2; $p = 0.002$ and 15 ± 4 vs. 18 ± 3; $p = 0.006$, respectively). Indeed, these finding raise concern about sperm quality (sperm chromatin integrity) in the Ej-group and further support the 'late paternal effect' hypothesis.

Our data show that the clinical pregnancy rate (CPR) in the Tc-group, Ts-group, and Ej-group are comparable (50% vs. 45% vs. 57%, respectively). Although our sample size is small, the data suggest that TESA-ICSI outcomes are not influenced by the severity of the asthenozoospermia and are comparable to ICSI outcome using ejaculated spermatozoa. Moreover, the functional capacity of testicular spermatozoa (in the context of ICSI) is independent of the underlying nature of the sperm motility defect.

Although TESA is a simple, local anesthetic procedure, it is associated with complications. As such, the potential benefits of using testicular spermatozoa rather than ejaculated spermatozoa must be weighed against the risks of testicular sperm retrieval. There are known risks of bleeding, infection, pain, and hypogonadism. Also, there are unknown risks associated with use of testicular spermatozoa for ICSI (genetic and epigenetic risks). A few studies have raised concerns that testicular spermatozoa have higher aneuploidy rate when compared to ejaculated spermatozoa (Gianaroli *et al.*, 2005; Moskovtsev *et al.*, 2012; Vozdova *et al.*, 2012). Moreover, Platteau *et al.* (2004) showed that use of testicular spermatozoa in ICSI resulted in higher aneuploidy frequencies in embryos.

In conclusion, several studies support the use of testicular over ejaculated spermatozoa for ICSI in couple with complete asthenozoospermia (Kahraman *et al.*, 1996; Nijs *et al.*, 1996; Shulman *et al.*, 1999). Although testicular sperm retrieval may ensure that viable spermatozoa are available on the day of ICSI, our data indicate that reproductive outcomes with testicular sperm-ICSI are no better than with ejaculated sperm-ICSI in men with severe or complete asthenozoospermia. Randomized, controlled trials comparing ejaculated vs. testicular spermatozoa are needed to assess the true benefit of TESA-ICSI in these couples.

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