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Morphology of Testicular Spermatozoa Obtained by Testicular Sperm Extraction in Obstructive and Nonobstructive Azoospermic Men and Its Relation to Fertilization Success in the In Vitro Fertilization–Intracytoplasmic Sperm Injection System

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ABSTRACT: The aim of the present study was to evaluate the morphology of testicular spermatozoa by 3 different determinants. Sperm cells were obtained and their morphology was evaluated from 27 testicular sperm extraction (TESE) operations, of which 20 men had nonobstructive azoospermia and 7 had obstructive azoospermia. In 17 cases, 2 biopsies were obtained from 2 different locations of the testis. Only mature spermatozoa presenting full-grown tail (tail dimension about 10-fold greater than the head dimension) were counted. Three characteristics of sperm morphology were evaluated: head dimensions, and acrosome and midpiece irregularities. The percentage of sperm cells with normal morphology (considering the 3 characteristics) in specimens from patients with obstructive and nonobstructive azoospermia were 47% ± 4.6% and 29 ± 1.8%, respectively ($P < .01$). The percentage of spermatozoa with normal head dimensions were 76% ± 3.2% and 63% ± 2.6% ($P > .05$), those with normal acrosome were 58% ± 4.6% and 41% ± 3.4% ($P < .05$), and those with normal midpiece were 74% ± 4.1% and 67% ± 1.6% ($P > .05$), in obstructive and nonobstructive azoospermia, respectively. No significant differences were observed in sperm morphology between different locations of the testis. Sperm morphological characteristics were not associated with fertilization rate in intracytoplasmic sperm injection (ICSI). Follicle-stimulation hormone and luteinizing hormone were inversely correlated with normal morphology of testicular spermatozoa ($r = -0.49$ and $r = -0.47$, respectively; $P < .05$). It can be concluded that a relatively high portion of testicular sperm are morphologically normal. The higher rate of normal spermatozoa in obstructive azoospermia compared with nonobstructive spermatozoa suggests that the factors leading to azoospermia may affect testicular sperm morphology. The morphological characteristics of testicular sperm do not affect fertilization rate in ICSI.

Key words: Testis, acrosome, azoospermia.
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The quality of spermatozoa is associated with genetic determinants, physiological characteristics, and morphological organization. The sperm cell attains its distinct morphological characteristics in the process of spermiogenesis, which comprises the final phase of spermatogenesis. Briefly, the process starts with a round spermatid that elongates, generates the acrosomal vesicle, and the nucleus condenses within the head. At the opposite pole, the centriole induces the formation of the flagellum. In between, a midpiece is organized with mitochondria, following the expulsion of the cytoplasm. Any defect in these processes may lead to abnormalities in the morphology of mature testicular spermatozoa.

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Little is known about the morphology of matured testicular spermatozoa. The common notion is that most of these cells are morphologically abnormal mainly because they have not yet completed epididymal maturation (Cosentino and Cockett, 1986; Yeung et al, 1997). The epididymis was considered to be obligatory site for completion of maturation, where sperm cells are supposed to gain motility and attain normal morphology; nevertheless, this is under debate (Cooper, 1995).

The recent successful use of isolated testicular spermatozoa by intracytoplasmatic sperm injection (ICSI) to achieve fertilization in vitro (Silber et al, 1996) challenges the latter claim. A large group of patients treated by ICSI with testicular spermatozoa had a fertilization rate of approximately 52% (Tarlatzis et al, 1998). Thus, testicular sperm have become an important source for ICSI, although limited information is currently available about its genetic and biological state in patients with obstructive and nonobstructive azoospermia (Silber et al, 1995; Meu-

leman and van-Moorselaar, 1998). Liu et al (1997) have demonstrated that following incubation for 3 days in medium, testicular spermatozoa gain motility and improved morphology. Still, this study was restricted to patients with obstructive azoospermia only.

The objectives of the present study were to evaluate the morphology of testicular spermatozoa in men with obstructive and nonobstructive azoospermia according to 3 different determinants; to assess possible variabilities in spermatozoon morphology within different locations of the testis; and finally, to establish the correlation between testicular sperm morphology, reproductive hormones, and fertilization rates in the in vitro fertilization (IVF)–ICSI system.

Materials and Methods

Patient Selection

Testicular spermatozoa were obtained from 50 azoospermic men by testicular sperm extraction (TESE). Each patient was diagnosed as azoospermic by strict definitions (eg, absence of spermatozoa in 2–3 ejaculates following high-speed centrifugation [8000 × *g*] and pellet analysis by phase-contrast microscopy and stained smears). Thirty men had spermatozoa in their testicular specimens and 27 (90%), who had at least 200 sperm cells per wet smear obtained during TESE, were enrolled in the study. Of the 27 patients, 7 were diagnosed as having obstructive azoospermia proven by scrotal exploration and normal spermatogenesis in testicular biopsy. The other 20 were defined by histology as having mixed atrophy (n = 15), hypospermatogenesis (n = 4), and incomplete spermatocyte arrest (n = 1). Mixed atrophy was defined by the appearance of spermatogenic foci side by side with different spermatogenic impairments as arrested spermatogenesis, or Sertoli cell only (Sigg and Hedinger, 1981; Nistal et al, 2000).

Testicular Sperm Extraction

TESE was performed as previously described by Hauser et al (1998). Briefly, under general anesthesia, the scrotum was excised, exposing the testicle. The tunica albuginea was incised in different locations, and seminiferous tissue was protruded by compressing the testis. In 17 cases, at least 2 pieces of tissue were excised from different locations of the same testis.

Preparation of Smears

Biopsies of seminiferous tissue from different locations of the testis were brought in contact with a glass slide and left to dry at room temperature. The dried specimens were fixed with an ether/ethanol (1:1) solution and stained according to the Papanicolaou procedure (Ragni et al, 1984).

Evaluation of Sperm Morphology

Two hundred sperm cells were counted on each slide, according to World Health Organization (1992) guidelines for evaluation of sperm morphology. Only mature spermatozoa presenting with a full-grown tail (tail dimension about 10-fold greater than the head dimension) were evaluated. The definitions of Kruger's strict criteria were used (Menkveld et al, 1990). Only 3 characteristics of sperm morphology were evaluated, according to the criteria of head dimensions, and acrosome and midpiece irregularities. The technician was blinded to knowledge of the cause of azoospermia (obstructive or nonobstructive), patients' clinical characteristics, and ICSI fertilization rates.

ICSI Procedure

The pieces of testicular tissue were put in a Petri dish with human tubal fluid (Irvine Scientific, Santa Ana, Calif). The tissue was mashed and excised by 2 needles, the supernatant was collected to a tube, and incubated further for a few hours.

ICSI was carried out according to the method published elsewhere by Van Steirteghem et al (1993). Briefly, spermatozoa were incubated in Medi-Cult Universal IVF medium (Medi-Cult, Copenhagen, Denmark) in 10-μL droplets under mineral oil for approximately 1 hour at 37°C and 5% CO_2 prior to examination for the presence of motile spermatozoa. A single motile spermatozoon was transferred to a droplet containing 10% polyvinylpyrrolidone. Spermatozoa were immobilized, aspirated into the injection pipette, and injected into an oocyte as previously described. The oocytes were then incubated for 16–20 hours and inspected for survival and fertilization. Fertilization was determined 24 hours postinsemination, when 2 pronuclei were observed.

Measurements of Hormones

Serum samples for hormone determination were obtained from 25 patients in the study group. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured with enzyme-linked immunosorbent assay with streptavidin technology (Enzymun-Test; Boehringer-Mannheim GmbH, Germany). The assay had a measuring range of approximately 0–150 mIU/mL and the lower detection limit was less than 0.5 mIU/mL.

Serum testosterone was measured using a radioimmunoassay kit for total testosterone (Elecsys; Roche, Basel, Switzerland). The reportable range is 2–1500 ng/dL. The sensitivity is 2 ng/dL and specificity is high, indicating very low cross-reactivity (eg, cross-reactivity with dihydrotestosterone of 1.8%).

Statistical Evaluation

Results are given as mean ± standard error of the mean (SEM), medians, and range. All the variables were analyzed for the normality of their distribution by the one-sample Kolmogorov-Smirnov test procedure and found to distribute normally. Nonetheless, due to the small number of cases, a significant difference was defined using the nonparametric Mann-Whitney *U*-test (or Wilcoxon test for related samples). Correlations were calculated using the Pearson's correlation test. All statistics were performed using SPSS for Windows version 8.0.

Results

Morphology of Testicular Spermatozoa

The histological classifications according to TESE results were as follows: mixed atrophy (n = 15), hyposperma-

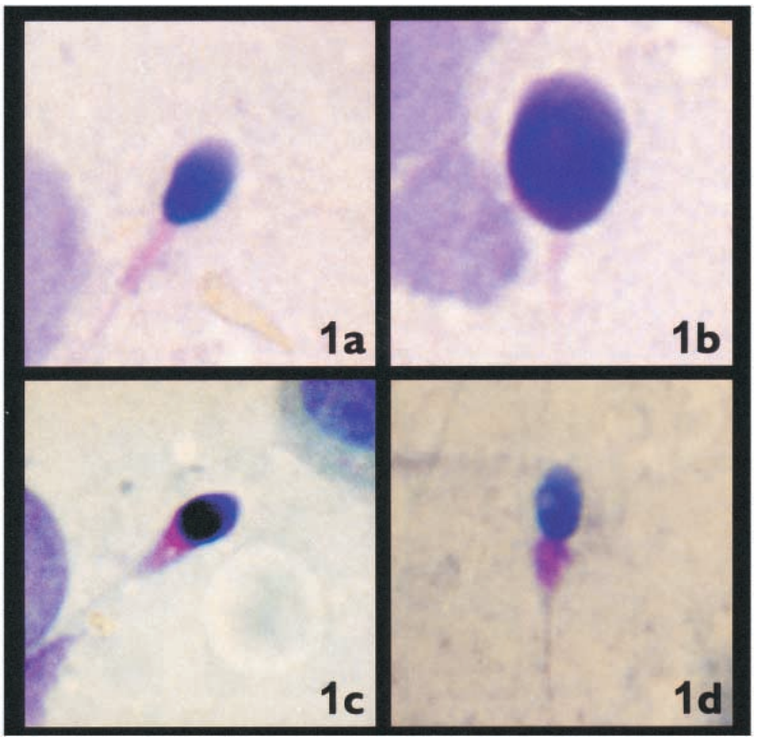

Figure 1. Morphology of testicular spermatozoa. **(a)** Normal sperm cell, **(b)** macrocephalic sperm cell, **(c)** sperm cell with midpiece defect (cytoplasmic droplet), **(d)** acrosomal defects and cytoplasmic droplet in testicular spermatozoa.

togenesis (n = 4), and incomplete spermatocyte arrest (n = 1). Figure 1 represents testicular spermatozoa with different abnormalities. The mean (± SEM), median, and range of percentage of sperm cells with normal morphology in obstructive and nonobstructive azoospermia and the rate of normal head dimensions, normal acrosome, and normal midpiece are presented in Table 1. Nearly half of the sperm cells in the obstructive group were normal (47% ± 4.6% compared with 29% ± 1.8% in the nonobstructive group [$P < .01$]). Owing to the small number of cases in each histological subtype, we could not compare them.

Sperm Morphology Obtained from Different Locations of the Testis

The mean (± SEM), median, and range of percentage of sperm cells with normal morphology, normal head dimensions, normal acrosome, and normal midpiece when obtained from 2 different locations of the testis were compared. No significant differences were observed in sperm morphology obtained from different locations of the testis.

Testicular Sperm Morphology, Serum Hormones, and Clinical Characteristics

Table 2 presents the mean, median, and range of patient ages, testicular volumes, and serum hormones. Only serum gonadotropins (FSH and LH) were shown to differ significantly between the obstructive and nonobstructive azoospermia groups, and were higher in the latter group.

No significant correlations were found between the rate of normal sperm morphology and patient age or testis volume (−0.22 and 0.1, respectively; not significant by the Pearson test). Serum gonadotropins, FSH and LH, were significantly correlated with the rate of normal sperm morphology ($r = -0.49$, and $r = -0.47$, respectively [$P < .05$]). No significant correlation was found between the rate of normal sperm morphology and serum testosterone ($r = -0.27$, $P = .9$).

Correlation Between Testicular Sperm Morphology and Fertilization Rate

A total of 55 IVF-ICSI cycles were performed; in most of them (76%), frozen-thawed sperm cells were used. The mean number of retrieved oocytes was 10 ± 0.7 per cycle. The mean rate of 2 pronuclei oocytes was 40% ± 2.9%, and 4 (15%) ongoing pregnancies were achieved. There were no significant differences in mean age of women, mean number of retrieved oocytes, or number of ICSI cycles between the obstructive and nonobstructive azoospermia groups. Mean fertilization rates did not differ between the obstructive and nonobstructive azoospermia groups (42% ± 5.1% and 43% ± 3.8%, respectively [$P = .73$]). In addition, there was no significant correlation

Table 1. *Percentage of normal morphology of testicular sperm characteristics of the azoospermic patients in the 2 subgroups: obstructive and nonobstructive*

	Normal Head Dimension, %	Normal Acrosome, %	Normal Midpiece, %	Total Normal Forms, %
Obstructive azoospermia, n = 7				
(mean ± SEM)	76 ± 3.2	58 ± 4.6*	74 ± 4.1	47 ± 4.6†
Median (range)	77 (60–89)	55 (43–76)	76 (56–88)	48 (30–62)
Nonobstructive azoospermia, n = 20				
(mean ± SEM)	63 ± 2.6	41 ± 4.3*	67 ± 1.6	29 ± 1.8†
Median (range)	71 (14–86)	37 (14–68)	72 (20–86)	31 (10–44)

* Significantly different by $P < .05$ between the 2 groups.
† Significantly different by $P < .01$ between the 2 groups.

between the rate of normal forms and ICSI fertilization rates ($r = -0.30$, $P = .89$, by the Pearson test).

Discussion

ICSI, first introduced in 1992 (Palermo et al, 1992), has put into doubt many of the common concepts regarding physiology of spermatozoa. One of the concepts is that morphology has an effect on the ability of sperm to fertilize an egg. Up to now most studies failed to establish any correlation between the rate of normal sperm morphology and fertilization rate (Kupker et al, 1995; Nagy et al, 1995; Hammadeh et al, 1996; Svalander et al, 1996; Lundin et al, 1997; Novero et al, 1997; Tasdemir et al, 1997; Sukcharoen et al, 1998). In all these studies, ejaculated spermatozoa were used and none has assessed the correlation regarding testicular spermatozoa. Our findings regarding testicular spermatozoa are in agreement with the reports on ejaculated spermatozoa (ie, sperm morphology has no effect on the success of ICSI).

The surprisingly high rate of spermatozoa with normal forms in men with obstructive azoospermia as well as those with nonobstructive azoospermia have never been mentioned before. These results should be taken with caution due to the small size of both groups studied, as well as the fact that men with nonobstructive azoospermia had at least 200 mature sperm in their specimens. Liu et al (1997) observed a similar, relatively high rate of normal forms of testicular spermatozoa in patients with obstructive azoospermia after 3 days of medium incubation. When these sperm cells were evaluated immediately after their extraction from the testis, as in our study, the percentage of normal morphology was dramatically lower, up to 3% (Liu et al, 1997). The apparent disagreement in the rate of normal sperm morphology may be the result of the different definitions of normal morphology; we have determined what may have appeared as tail abnormalities to actually be immature forms during the process of spermiogenesis, thus we did not consider them as abnormal. This in turn may cause an overestimation of the rate of normal sperm morphology. Hence, we did not use ''Kruger strict criteria,'' but rather only 3 criteria of normal morphology. We have chosen specific criteria that usually represent testicular spermatozoa and used them for both obstructive and nonobstructive groups. Therefore, the magnitude of the percentage of spermatozoa with normal forms in our study must be taken cautiously and is surely higher compared with the standard strict criteria. Despite this, the fact that testicular spermatozoa in obstructive and nonobstructive azoospermic men possess a high percentage of normal morphology is apparent. This is supported in a recent study by Nogueira et al (1999) who described normal head morphology (including the nuclei and acrosomal cap) of testicular spermatozoa by

Table 2. *Characteristics of the 2 subgroups: obstructive and nonobstructive azoospermic men**

	Age, y	Mean Testis Volume, mL	FSH, mIU/mL	LH, mIU/mL	Testosterone, ng/mL
Obstructive azoospermia, n = 7					
(mean ± SEM)	32 ± 0.8	25 ± 1.0	4 ± 0.7†	4 ± 0.6†	5.2 ± 0.7
Median (range)	32 (29–35)	25 (18–27)	3 (2–7)	3 (2–6)	5 (2–9)
Nonobstructive azoospermia, n = 20					
(mean ± SEM)	36 ± 1.5	20 ± 1.5	16 ± 2.3†	7 ± 0.6†	5.7 ± 0.7
Median (range)	34 (27–52)	20 (12–25)	13 (2–36)	7 (4–15)	5 (3–10)

* Serum hormones were assessed only in 25 men—7 with obstructive azoospermia and 18 with nonobstructive azoospermia. FSH indicates follicle-stimulating hormone; LH, luteinizing hormone.
† Significantly different between the 2 groups by $P < .01$.

light and electron microscopy. Unfortunately, these important data were not quantified.

It is interesting that the mean rate of normal testicular spermatozoa in men with obstructive azoospermia was above the normal rate in ejaculated specimens. One can carefully speculate that spermatogenesis commonly yields normal spermatozoa, which then might be damaged during transit through the rete testis, epididymis, and vas deferens. However, this does not concur with previous observations (Yeung et al, 1997). Another common debate regarding TESE is homogeneity versus heterogeneity in regard to spermatogenesis in the testis of men with azoospermia (Silber et al, 1997). This question relates to yet another debate regarding the number and size of biopsies one has to perform in order to obtain spermatozoa that will be used successfully in ICSI (Hauser et al, 1998). Here we have shown that whenever spermatozoa are obtained from different locations of the testis they possess about the same morphological characteristics.

The significant correlation found between serum gonadotropins and sperm morphology is not surprising and is even expected, though not clinically relevant. In fact, this study reconfirmed that viable, mature spermatozoa can be retrieved in sufficient numbers for ICSI even in cases of azoospermia with extremely elevated levels of serum FSH (in this group up to 36 mIU/mL). Regardless, the correlation can be explained as the combined outcome of the higher rate of normal-shaped spermatozoa in patients with obstructive azoospermia and the well-known observation that gonadotropin levels are generally higher in men with nonobstructive azoospermia compared with those with obstructive azoospermia (Bergmann et al, 1994), possibly due to abnormal histology (eg, spermatogenic arrest) and low levels of inhibin.

Although there were no significant differences in ICSI fertilization rates between the obstructive and nonobstructive azoospermia groups, we prefer not to draw any conclusions on this finding due to the small study group. The lack of association between testicular sperm morphology and ICSI fertilization rates reveals that morphology of testicular spermatozoa may not be as important a predictor of ICSI fertilization rate as it is with regard to standard IVF.

It can be concluded that a relatively high portion of testicular spermatozoa are morphologically normal in men with obstructive or nonobstructive azoospermia, and that isolated sperm cells have the same potential to fertilize oocytes in ICSI, regardless of their morphology.

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