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The Motility of Epididymal or Testicular Spermatozoa Does Not Directly Affect IVF/ICSI Pregnancy Outcomes

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ABSTRACT: Our objective was to determine whether the presence of motility in surgically obtained sperm samples improves fertilization and pregnancy rates for patients undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI). This was a retrospective study in a hospital-based infertility center. Sixty-seven couples with a diagnosis of azoospermia or severe oligozoospermia who had undergone a sperm retrieval procedure in conjunction with 100 IVF/ICSI cycles from 1995 to 2004 were evaluated. The impact of sperm motility on fertilization and clinical pregnancy rates was determined. The motile and nonmotile sperm groups differed in the number of mature oocytes retrieved (10.7 ± 5.8 vs 13.4 ± 6.0), but fertilization (56.7% vs 59.1%) and embryo cryopreservation rates (35.9% vs 39.3%) were statistically similar. Clinical pregnancy rates did not differ between the motile (38.5%) and nonmotile (31.2%) groups, nor did they differ between obstructive and nonobstructive patients (35.3% vs 26.7%). There was also no statistical difference in pregnancy rates between testicular and epididymal aspiration (35.3% vs 26.7%), although epididymal sperm were significantly more likely to be motile than testicular sperm (100% vs 39.3%, $P < .0001$). Epididymal aspiration is more likely to produce motile sperm than testicular sperm retrieval. The use of motile sperm from epididymal or testicular samples, however, does not appear to enhance fertilization or clinical pregnancy rates.

Key words: Azoospermia, infertility, epididymal sperm, testicular sperm.

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Male factor is the cause of infertility in approximately one third of all couples seeking reproductive assistance. Azoospermia, observed during 5% of infertility evaluations, accounts for an important subgroup of male factor diagnoses (Irvine, 1998). For infertile men with azoospermia or severe oligozoospermia, the only therapeutic option had previously been donor sperm. Although the advent of intracytoplasmic sperm injection (ICSI) was a significant breakthrough (Palermo et al, 1992), the ability to recover and inject testicular and epididymal sperm has been a most noteworthy achievement in the treatment of azoospermic patients.

Several surgical approaches can be used to acquire sperm for assisted reproduction, including microsurgical epididymal aspiration (MESA), percutaneous epididymal aspiration (PESA), testicular sperm extraction (TESE), and testicular sperm aspiration (TESA) (Khorram et al, 2001). A number of studies have examined factors that might influence reproductive success with these techniques. Although some researchers suggest the use of testicular sperm leads to higher spontaneous abortion rates (Ghazzawi et al, 1998; Pasqualotto et al, 2001), these and other investigators have generally demonstrated similar in vitro fertilization (IVF) and pregnancy outcomes with testicular and epididymal samples (Rosenlund et al, 1997; Aytoz et al, 1998). A recent meta-analysis of surgical sperm retrieval in azoospermic patients further supports that sperm origin does not affect cycle outcome (Nicopoullos et al, 2004). Cycle results with cryopreserved sperm or tissue have also been examined and are comparable to those observed with fresh specimens (Friedler et al, 1998; Tournaye et al, 1999; Kupker et al, 2000; Fukunaga et al, 2001). Alternatively, nonobstructive azoospermia has been associated with poorer fertility outcomes than obstructive azoospermia, irrespective of epididymal or testicular specimen source (Palermo et al, 1999; Osmangaoglu et al, 2003; Vernaeve et al, 2003).

Although fertilization and pregnancy can occur with the use of immotile sperm (Shulman et al, 1999), motility is a particularly helpful parameter in the detection of live sperm. However, only a few studies to date have specifically examined IVF/ICSI cycle results as related to sperm aspirate motility, with conflicting results (Nagy et al, 1998; Shibahara et al, 1999; Park et al, 2003). The objective of this retrospective review was to determine if the presence or absence of motility in surgically obtained sperm specimens affects IVF/ICSI outcomes.

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Materials and Methods

Patients

Institutional review board approval was obtained before data collection. Couples were included when the male partner had a diagnosis of azoospermia or severe oligozoospermia (sperm concentrations <1 million/mL) and had undergone a sperm retrieval procedure; IVF and ICSI were subsequently performed by 4 embryologists through the Center for Reproductive Studies IVF laboratory at The Christ Hospital in Cincinnati, Ohio, from 1995 to 2004. Demographic parameters included female age, male age, male prior paternity, male diagnosis (obstructive or nonobstructive), female diagnosis, gravidity, and parity.

Controlled Ovarian Hyperstimulation, Oocyte Retrieval, and Culture

Patients were administered gonadotropin-releasing hormone agonist either before (long protocol) or concurrent with (flare protocol) gonadotropin stimulation for pituitary down-regulation. Serial estradiol levels and transvaginal ultrasound evaluations were performed until an optimal number of mature follicles were observed. Approximately 35 to 36 hours after human chorionic gonadotropin administration, oocytes were retrieved under transvaginal ultrasound guidance.

Oocytes were then examined for maturation by means of an inverted microscope under high power and subsequently incubated at 37°C (5% CO_2 in air) in human tubal fluid (HTF) with 10% synthetic serum supplement and 5% CO_2 (InVitroCare Inc, Frederick, Md). In preparation for ICSI, corona radiata and cumulus cells were denuded through brief incubation with 80 IU/mL of hyaluronidase (Sigma Chemical Co, St Louis, Mo) approximately 3 to 4 hours after retrieval. Reviewed cycle data included baseline serum follicle-stimulating hormone (FSH) levels, total gonadotropin ampules used, number of retrieved oocytes, and number of mature oocytes.

Preparation and Cryopreservation of Testicular and Epididymal Sperm

Antecedent urologic consultation for male subjects consisted of physical examination, hormonal assessment, genetic testing, and selection of a sperm collection method. All specimens were obtained through one of three techniques: MESA, TESE, or TESA. For study purposes, samples were categorized as either epididymal (MESA) or testicular (TESE or TESA). The sperm motility before ICSI as documented by the embryologist was also noted, as was collection timing (fresh vs cryopreserved).

On the day of the sperm retrieval procedure, testicular and epididymal specimens were immediately collected in a petri dish with 3 mL of modified, CO_2-free HTF with HEPES buffer and 5% synthetic serum supplement (InVitroCare). After rinsing with media, seminiferous tubules from testicular samples were carefully dissected using two 26-gauge needles (Becton Dickinson and Company, Franklin Lakes, NJ) under high-power microscopy. If fresh ICSI was planned, expressed testicular sperm (or epididymal sperm) in media were then placed in conical tubes and centrifuged at 1200 × *g*. Following disposal of supernatant fluid, the sample was resuspended in a small volume (<0.5 mL) of media and placed in the incubator until the time of ICSI.

Unused seminiferous tubules (or aspirated sperm) were divided and placed in Vangard cryogenic vials (Sumitome Bakelite Co, Ltd, Tokyo, Japan) with cryopreservation medium (Irvine Scientific, Irvine, Calif); sperm presence was verified in testicular samples before freezing. The vials were cooled to 5°C for 30 minutes and then exposed to nitrogen vapor for 30 minutes before submersion in liquid nitrogen (−196°C) for long-term storage.

When cryopreserved sperm or tissue was used for ICSI, specimen vials were immersed and thawed in a 37°C water bath for 5 minutes on the day before ICSI. For testicular specimens, expression of sperm from thawed seminiferous tubules was performed as detailed previously. All specimens were then incubated overnight in culture media at 37°C (5% CO_2 in air) in preparation for ICSI the following day.

ICSI, Embryo Transfer, and Pregnancy

Efforts were made to identify motile or twitching spermatozoa under high-power microscopy. A hypo-osmotic swelling (HOS) test was performed when motile sperm could not be identified (Hafez, 1998). The specimen was described as nonmotile if all the retrieved mature oocytes were fertilized with sperm that were neither moving nor twitching despite normal HOS testing. Immobilization of selected spermatozoa was accomplished with a drop of 8% polyvinylpyrrolidone (Irvine Scientific), and individual sperm were then aspirated into the injection pipette. Using a Hoffman Modulation Contrast System (Modulation Optics Inc, Greenvale, NY) and a holding pipette, metaphase II oocytes were positioned with the polar body at either the 6 o'clock or 12 o'clock position and injected.

Following ICSI, oocytes were washed and incubated in a 1-mL drop of HTF at 37°C (5% CO_2 in air). Oocytes were assessed at 18 and 24 hours post-ICSI for fertilization. Final embryo cleavage and grade (Veeck, 1991) were observed 64 to 66 hours after ICSI. The fertilization rate, cryopreservation rate (percentage of cycles in which at least 1 embryo was frozen), mean embryo cell number and grade (for transferred embryos), and number of embryos transferred were noted.

Embryo transfer was performed on the third day after oocyte retrieval. Cycle results were categorized as implantation (documented increase in serum β-human chorionic gonadotropin) or clinical pregnancy (documented fetal heart motion on ultrasonography at 6–7 weeks of gestation).

Statistical Analysis

All laboratory procedures were standardized through collection of data from a single IVF and andrology laboratory site. Statistical analysis was performed with SAS version 8 (SAS Institute, Cary, NC). Pearson product correlation, χ^2, and Student's *t* tests were used, with $P < .05$ considered statistically significant.

Results

From July 1995 through April 2004, we identified complete cycle data for 67 couples undergoing 100 IVF/ICSI cycles in which the male partner had undergone surgical

Table 1. *Patient demographic information for motile and nonmotile sperm groups*

	Motile (n = 39 cycles)	Nonmotile (n = 61 cycles)	*P*
Female age (y ± SD)	33.4 ± 3.7	32.9 ± 4.2	NS*
Male age (y ± SD)	39.1 ± 6.8	38.9 ± 7.4	NS
Male diagnosis—obstructive	35 (89.7%)	50 (81.9%)	NS
Male diagnosis—nonobstructive	4 (10.3%)	11 (18.1%)	NS
Female partner—infertility diagnosis present	5 (12.8%)	6 (14.7%)	NS
Female partner—prior conception	19 (48.7%)	28 (45.9%)	NS
Female partner—prior live birth	12 (30.7%)	16 (26.2)	NS
Prior paternity	26 (66.6%)	36 (59.0%)	NS

* NS indicates not significant.

sperm retrieval. There were no significant differences in patient demographic parameters between cycles using motile vs nonmotile sperm (Table 1). Overall, the majority of patient cycles involved male partners with an obstructive diagnosis and proven paternity (85% and 62% of cycles, respectively), most commonly before vasectomy. Men with a diagnosis of obstructive azoospermia were older than those with nonobstructive azoospermia (39.9 ± 7.1 vs 33.7 ± 5.2, $P < .01$), but there was no correlation between male age and pregnancy outcome.

Analysis of cycle stimulation factors revealed similar baseline serum FSH levels and gonadotropin dose requirements for female partners in motile and nonmotile sperm cycles. There were, however, significantly higher mean numbers of retrieved and mature oocytes in nonmotile sperm cycles than in motile sperm cycles (Table 2). While this difference further resulted in a significantly greater number of 2 pronuclei stage embryos in nonmotile sperm cycles (7.8 ± 4.5 vs 5.9 ± 3.5, $P < .05$), both the cryopreservation rates (35.9% vs 39.3%—motile vs nonmotile, $P > .05$) and mean number of embryos cryopreserved (1.6 ± 2.6 vs 2.1 ± 3.6—motile vs nonmotile, $P > .05$) were similar. Furthermore, there was no difference in the mean cleavage cell number, mean embryo grade, or mean number of embryos transferred between motile and nonmotile sperm cycles (Table 2). Finally, implantation rates (51.3% vs 44.3%—motile vs nonmotile, $P > .05$) and clinical pregnancy rates (38.5% vs 31.2%—motile vs nonmotile, $P > .05$) were statistically comparable between these 2 cycle groups (Table 2).

Table 2. *Cycle stimulation and outcome data for motile and nonmotile sperm groups**

	Motile (n = 39 cycles)	Nonmotile (n = 61 cycles)
Basline serum FSH*	6.3 ± 1.7	5.5 ± 1.7
Total number of ampules used*	37.3 ± 10.3	33.7 ± 9.8
Total number of retrieved oocytes*	12.7 ± 7.1	16.2 ± 7.3†
Total number of mature oocytes*	10.7 ± 5.8	13.4 ± 6.0‡
Fertilization rate	56.7%	59.1%
Cryopreservation rate§	35.9%	39.3%
Number of cells at embryo transfer (72 h)*	6.26 ± 1.46	6.08 ± 1.48
Grade of transferred embryos*	2.01 ± 0.84	2.15 ± 0.73
Number of transferred embryos*	2.97 ± 0.87	3.20 ± 1.06
Implantation rate	51.3%	44.3%
Clinical pregnancy rate	38.5%	31.2%

* Data presented as mean ± SD. FSH indicates follicle-stimulating hormone.
† $P = .02$.
‡ $P = .03$.
§ Percentage of cycles in which at least 1 embryo was frozen.

Among motile and nonmotile sperm cycles, similar percentages of fresh (44% vs 36%, respectively, $P > .05$) and cryopreserved (56% vs 64%, respectively, $P > .05$) specimens were used. Clinical pregnancy rates were not statistically different with the use of fresh vs cryopreserved specimens (28.2% vs 37.8%, $P > .05$). Epididymal aspirates were much more likely to contain motile sperm than testicular specimens (100% vs 39.3%, $P < .0001$). Nonetheless, clinical pregnancy rates did not differ between cycles using epididymal vs testicular sperm (26.7% vs 35.3%, respectively, $P > .05$); the aforementioned clinical pregnancy rates also applied to obstructive vs nonobstructive diagnosis.

Discussion

The purpose of our study was to determine whether the presence or absence of motility in surgically obtained sperm specimens has an impact on IVF/ICSI outcomes. Our results suggest that both fertilization and pregnancy rates are similar, regardless of whether motile or nonmotile sperm are used. Among our patients, epididymal sperm were significantly more motile than testicular sperm. This is particularly relevant because epididymal samples are more costly and invasive to obtain (Friedler et al, 1998). Conversely, specimens without motile sperm require increased time and micromanipulation to identify sperm suitable for ICSI. Since our findings suggest that

cycle outcomes are comparable with the use of epididymal or testicular sperm, consistent with the meta-analysis mentioned earlier (Nicopoullos et al, 2004), the increased morbidity and expense of epididymal aspiration may not be warranted.

Another important observation is that patients with azoospermia or severe oligozoospermia can benefit from cryopreservation of surgically retrieved sperm. This approach permits assessment of spermatogenesis before ovarian stimulation (Kupker et al, 2000), facilitates ovarian stimulation cycle scheduling, and serves to avoid cancellation of egg retrieval in cases for which spermatozoa cannot be retrieved. Furthermore, when repeat IVF/ICSI cycles are desired, this can be accomplished without an additional sperm retrieval procedure (Fukunaga et al, 2001). That we observed no decline in pregnancy rates with the use of cryopreserved sperm further reinforces the acceptability of this method.

Only a few others have investigated the motility of surgically obtained sperm and its effect on IVF/ICSI success rates. In a small patient sample, one study examined motility among previously cryopreserved epididymal aspirates (Shibahara et al, 1999). While fertilization rates were higher for motile vs nonmotile specimens, pregnancy rates between the 2 groups were not different. A second study evaluated aspirated spermatozoa, correlating motility with embryo quality, fertilization rates, and clinical pregnancy rates (Nagy et al, 1998). Fertilization rates were again significantly higher for subjects with motile sperm, but embryo quality and clinical pregnancy rates, as in our study, were similar between motile and nonmotile groups. Although we did not demonstrate any differences in fertilization data, the smaller number of nonmotile sperm cycles included in the studies above (6 and 14, respectively) may explain this discrepancy.

A recent retrospective analysis of 261 IVF/ICSI cycles provided evidence to suggest that the use of motile testicular sperm in men with obstructive azoospermia improves both fertilization and pregnancy outcomes (Park et al, 2003). An examination of our data shows that, since there was no statistical difference in the mean number, mean cell number, or mean grade of transferred embryos between the motile and nonmotile groups, it is unlikely that the group differences in total mature oocytes explain our differing conclusions. As our study did include patients with both obstructive and nonobstructive azoospermia, group heterogeneity between our 2 studies may have been a contributing factor. In addition, our sample size was smaller, possibly reflecting a beta error; assuming 80% power with an α of .05, 557 cycles per group would be required for the difference in clinical pregnancy rates between our motile and nonmotile groups (7.3%) to be statistically significant. For this reason, our ability to clinically generalize these findings is somewhat limited, but they nonetheless merit further exploration.

With regard to nonmotile cycle and patient numbers, our study provides the second largest sample size of any investigation on the subjects of motility, surgically obtained sperm, and ICSI outcomes reported in the literature. Interestingly, testicular specimens from our subjects exhibited comparatively lower motility than those in the Park study (39.3% vs 71.6%) but did not influence overall cycle outcomes. It is plausible that in laboratories, such as ours, with relatively moderate cycle volume, the embryologists are afforded greater time for the identification of live spermatozoa; one methodology difference in sperm preparation from the Park study was the thaw or culture of specimens 3 to 5 hours before ICSI, in contrast to overnight incubation and subsequent reassessment in our laboratory. Moreover, our findings lend credence to current research into other techniques to determine sperm suitability for assisted reproductive technologies. For example, a recent article (Ramos et al, 2004) examined the use of a computerized karyometric image analysis (CKIA) system, demonstrating that current routine ICSI selection criteria (including motility) are not sufficient for selecting normal condensed nuclei from sperm samples in obstructive azoospermic patients. Collectively, there is increasing evidence that factors other than sperm motility may have a more substantial impact on sperm selection and subsequent cycle outcome in these patients.

In summary, our study results suggest that the motility of surgically obtained sperm prior to IVF/ICSI does not directly affect cycle outcomes, an observation that may be particularly relevant to programs with a small-to-moderate cycle volume. These findings would certainly benefit from further investigation in larger fertility centers. Ultimately, continued research and advances in sperm assessment beyond motility should enhance the ability to choose the best approaches for sperm attainment and selection in azoospermic men.

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