

Severe Hypospermatogenesis in Cases of Nonobstructive Azoospermia: Should We Use Fresh or Frozen Testicular Spermatozoa?

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ABSTRACT: The aim of this comparative clinical study was to examine whether the fertilizing potential of frozen-thawed testicular sperm in the most severe cases of hypospermatogenesis is reduced compared with fresh testicular sperm. The results could determine the necessity of using fresh testicular sperm cells, which mandates involving the spouse by performing simultaneous in vitro fertilization intracytoplasmic sperm injection (IVF-ICSI) treatment in this subgroup of nonobstructive azoospermia (NOA) patients. We studied 13 couples in which the husband was diagnosed as having NOA and few motile testicular sperm cells or only immotile testicular sperm cells were isolated by testicular sperm extraction (TESE). Each couple underwent both an ICSI cycle, in which fresh testicular sperm that were retrieved shortly beforehand were injected, and a consecutive cycle, which used frozen-thawed sperm that were retrieved in the original TESE procedure but were cryopreserved and stored until use. We found that motility was lost during the freezing and thawing

process in some cases, which resulted in significantly more cycles with only immotile sperm cells for injection in the frozen-thawed sperm group (38.5%) than in the fresh sperm group (7.7%; $P < .05$). Availability of only immotile sperm cells significantly reduced fertilization rates in both fresh and frozen-thawed groups, but the respective overall fertilization rate (44.9% vs 41.1%) and quality of embryos and pregnancy rate (18.2% vs 15.4%) were not significantly different between groups. Implantation rates were more favorable in the fresh sperm group (10.5% vs 5.9%), but not significantly so. We conclude that, although cryopreservation does impair motility, which results in significantly more cycles with only immotile sperm cells for ICSI in the most severe forms of hypospermatogenesis, fertilization and pregnancy rates are not significantly compromised.

Key words: Testicular sperm extraction, fertilization, implantation, IVF-ICSI.

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Only a few testicular sperm cells might be extracted in certain cases of nonobstructive azoospermia (NOA) with what we considered as being “severe” hypospermatogenesis (see the following criteria). Freezing these limited numbers of sperm cells for future use demands special experience and techniques (Romero et al, 1996; Cohen et al, 1997). Although previous studies have shown that the fertilizing potential of testicular sperm cells in NOA patients is not reduced by the freezing-thawing procedure (Ben Yosef et al, 1999; Gil-Salom et al, 2000), postthaw quality and fertilization potential are not certain when dealing with an extremely low quality and quantity of testicular sperm cells. It could be reasoned that the use of fresh testicular samples should be preferred if the postthaw fertilizing potential of those severe cases is proven to be reduced. Because there are no definite preoperative diagnostic tests to predict which are the cas-

es with severe hypospermatogenesis, this rationale may influence the treatment strategy of all NOA patients, with the intent of not compromising the severe ones among them.

It is well established that mature spermatozoa can be found in approximately 50%–60% of men with NOA, even in those characterized by high follicle-stimulating hormone (FSH) levels (Hauser et al, 1995; Seo and Ko, 2001; Friedler et al, 2002b). Because spermatogenesis may be focal due to nonhomogenous testicular histology in about 50% of those cases, sperm detection is enhanced by the performance of multiple testicular biopsies in each testis (Hauser et al, 1998) or by repeated testicular sperm extraction (TESE) procedures (Friedler et al, 2002a). In a previous study that included NOA patients, motile spermatozoa were available for intracytoplasmic sperm injection (ICSI) in 47 of 57 cycles (82.5%; Ben-Yosef et al, 1999). However, only a few sperm cells and, occasionally, totally immotile ones are found after a thorough search and multiple biopsies in severe NOA cases.

The availability of so few sperm cells that are also of low quality poses a dilemma. On the one hand, when they are fresh, these sperm cells can be used for the ICSI pro-

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cedure immediately after isolation. This requires synchronous ovulation induction and oocyte retrieval on the part of the spouse, even though there is a 40%–50% chance that no sperm cells will be found (Hauser et al, 1998; Seo and Ko, 2001; Friedler et al, 2002b). On the other hand, the TESE procedure could be performed on an elective basis, and the sperm cells could be cryopreserved for future use. In that event, the couple's ability to have children may be decreased if the freezing-thawing process reduces the fertility potential of testicular sperm.

The present study was conducted to compare the outcome of ICSI treatments using fresh or frozen-thawed testicular sperm cells in cases of severe hypospermatogenesis. We aimed to verify whether the use of frozen-thawed testicular sperm for ICSI compromises or otherwise impairs the results in the most severe cases of hypospermatogenesis, where extremely low numbers of mature motile sperm cells have been detected in few (even in a single) testicular locations. We ensured that cryopreservation of sperm cells was the only major variable by including couples in whom testicular sperm cells extraction was performed concomitantly with oocyte retrieval and their fresh sperm was used for ICSI, and another ICSI cycle using their stored frozen-thawed sperm was performed later. Our findings may contribute to the determination of treatment strategy for all NOA patients.

Materials and Methods

Male Patients and the TESE Procedure

All the couples with NOA who were enrolled in our Andrology Unit between January 1997 and April 2004 and underwent TESE procedures were eligible. In all cases, azoospermia was confirmed by repeated semen analysis, and cryptozoospermia was excluded prior to the operative procedure using high-speed centrifugation. An open-operative procedure with multiple biopsies was performed in all cases, as described elsewhere (Hauser et al, 1998). Each extracted testicular sample weighed approximately 50 grams. A single testicular sample was taken from the central biopsy of each testis for histopathologic analysis. A severe form of hypospermatogenesis was diagnosed according to all 3 of the following criteria in 38 cycles (21.1% of all NOA patients): 1) the presence of an extremely low number of mature sperm cells or only immotile spermatozoa, 2) the detection of sperm cells in some (even in a single) of the testicular locations, and 3) isolation of sperm cells in the extracted testicular material mandated hours of careful searching by experienced embryologists.

To analyze only paired data, the study group consisted of the 13 couples in which 2 consecutive *in vitro* fertilization (IVF)-ICSI cycles were performed: the first one using fresh sperm cells and the second one using frozen-thawed testicular sperm cells. The sperm cells used for both IVF-ICSI cycles were retrieved in the same TESE procedure. Thus, cryopreservation of sperm cells is the only major variable between the 2 approaches.

Sperm Preparation, Cryopreservation, and Thawing Technique

TESE procedures were performed under general anesthesia. Three biopsies were taken from each testis, as described elsewhere (Hauser et al, 1998). Wet preparations were performed during the operative procedure and immediately examined. The retrieved testicular material was transferred to the laboratory and underwent mincing of the tissue, centrifugation of the sperm suspension, and thorough search under high-power magnification with an inverted microscope, as previously described (Ben Yosef et al, 1999). Sperm variables such as total number, motility, and progressive motility were recorded whenever possible. Excesses of testicular material were frozen for subsequent thawed sperm ICSI cycles in all cases. The minced testicular tissue was diluted by the addition of medium composed of human tubal fluid (Irvine Scientific, Santa Ana, Calif) with 1% human serum albumin (Kamada, Kibbutz Beit-Kama, Israel) and an equal volume of freezing medium test yolk buffer (Irvine Scientific) added in a droplet fashion. After dilution, the mixture was equilibrated for 15 minutes at room temperature, then sealed in 1.0-mL tubes (Nunc, Roskilde, Denmark) and cooled in a semiprogrammable freezer (Nicol, LM-10; Air Liquid, Paris, France). The tubes were cooled from room temperature to -6°C at a rate of $1.7^{\circ}\text{C}/\text{min}$, then to -100°C at a rate of $5^{\circ}\text{C}/\text{min}$. The tubes were then directly soaked in liquid nitrogen (-196°C) for storage (Yogev et al, 2004). Thawing of testicular tissue was performed at room temperature on the day of ICSI. To remove the cryopreservation medium, the sample was diluted with medium and centrifuged, and the pellet was resuspended in fresh medium and examined for the presence of motile spermatozoa in the same manner as was the fresh testicular specimen. Part of the spermatozoa acquired motility within 1–2 hours of culture.

ICSI Procedure and Embryonic Development

Spermatozoa were continuously assessed for any indication of movement (ie, "twitching" of head or tail) in the sperm medium droplet without polyvinyl pyrrolidone (PVP). In most cases, a sluggish local motility was observed, which served as a sign of viability. A single motile spermatozoon was aspirated from the separate sperm droplet into the injection pipette and then transferred to the 10% PVP droplet to separate it from attaching cells and debris. When only completely immotile sperm cells were obtained, even after an exhaustive search, spermatozoa with normal morphology were selected for the microinjection procedure. Both motile and immotile spermatozoa were immobilized before their aspiration into the injection pipette. The isolated testicular spermatozoon was then injected into the metaphase II (MII) oocyte, which was retrieved from the spouse. Oocytes and embryos were cultured in the glucose-free P1 medium supplemented with 20% synthetic serum substitute (Irvine Scientific).

After the ICSI procedure, the oocytes were returned to the culture dish for further incubation and inspected 16–20 hours later under a stereomicroscope (SZH 10 Research stereo, Olympus; Tokyo, Japan) for survival and fertilization. Embryo cleavage rate and morphology were evaluated on either day 2 or 3 of development, prior to transfer. Embryo morphology was scored from 1–4 according to the shape of the blastomeres and the amount of detached anuclear fragments. Embryo development

Table 1. Comparison of ICSI results using fresh vs frozen-thawed testicular sperm in 13 cases of severe hypospermatogenesis*

	Fresh	Cryopreserved	P
Oocytes retrieved (total no.)	200	146	...
MII oocytes (total no.)	146	128	...
MII oocytes/cycle (total no.)	11.5 ± 4.1	9.8 ± 5.7	NS†
MII (%) oocytes retrieved	81.9 ± 20.3	88.4 ± 25.5	NS‡
Oocytes injected with motile spermatozoon/ cycle (total no.)	7.7 ± 5.6 (100)	2.7 ± 3.5 (35)	<i>P</i> < .001‡
Oocytes injected with immotile spermatozoon/ cycle (total no.)	2.9 ± 4.1 (37)§,	6.8 ± 6.0 (89)§	<i>P</i> < .05†
Cycles (%) with 0 motile sperm (no.)	7.7% (1)	38.5% (5)	<i>P</i> < .05¶

* Values are means ± SD. ICSI indicates intracytoplasmic sperm injection; MII, metaphase II; and NS, not significant.

† Wilcoxon nonparametric test for quantitative variables.

‡ Chi-squared test.

§ In 2 cycles, there were insufficient spermatozoa to inject all retrieved matured oocytes, and 16 oocytes were inseminated with donor spermatozoa.

|| In 1 cycle, there was insufficient spermatozoa to inject all retrieved matured oocytes, and 20 oocytes were frozen.

¶ McNemar test for dichotomous variables.

was categorized according to cleavage rate into 3 groups: fast cleaving (>4 cells on day 2 or >8 cells on day 3), normal cleaving (4 cells on day 2 or 8 cells on day 3), or delayed cleaving (<4 cells on day 2 or <8 cells on day 3). Embryos with the highest scores were selected for transfer using a Cook catheter (Cook, Brisbane, Australia). Clinical pregnancies were defined by a sonographic demonstration of a gestational sac. The implantation rate was defined as the number of gestational sacs divided by the total number of transferred embryos.

Statistical Methods

Comparison of outcomes between fresh and thawed sperm IVF was performed using paired tests: Wilcoxon's nonparametric test for quantitative variables and McNemar's test for dichotomous variables. The outcomes of "2PN embryos/injected oocytes with motile sperm" and "2PN pronuclei embryos/injected oocytes with immotile sperm" were missing for some patients and were, therefore, compared using the Mann-Whitney nonparametric test. Percentages of matured oocytes were compared by the χ^2 test. Pregnancy outcome and implantation rates were compared by the Fisher test.

Results

Study Group

Of all the patients with NOA who underwent TESE procedures, 38 were diagnosed as having severe hypospermatogenesis. Our study group consisted of 13 of these couples in whom a consecutive frozen-thawed ICSI cycle was performed using the excess of testicular sperm cells that were cryopreserved after the first cycle with fresh testicular sperm cells. The frozen-thawed cycles were performed between 2–16 months following the fresh cycles. The use of frozen sperm in the second treatment cycle was aimed at achieving another pregnancy in 2 couples who achieved a pregnancy in the first treatment cycle.

The mean age ± SD of the males was 30.2 ± 4.3 years (range, 26–39), and their average FSH level was 15.9 ±

9.5 mIU/mL (normal range, up to 11.0 mIU/mL). Testosterone levels were within the normal range in all but 2 men whose levels were slightly below normal. The mean testicular volume was 14.3 ± 3.6 and 14.4 ± 5.2 for the right and left testicle, respectively. The mean age of the females was 30.0 ± 4.2 years (range, 23–38), and their average FSH level was 6.3 ± 5.2 mIU/mL (on day 3 of the menstrual cycle). Karyotyping was carried out in 11 men, and the results were normal in all of them.

Histopathology of the testes (taken from a single location in each testis) revealed Sertoli cell-only in most cases. Focal spermatogenesis was bilaterally identified in 5 cases and unilaterally identified in 2 cases.

ICSI Outcomes in Fresh vs Frozen-Thawed Cycles of the Same Couple

A total of 200 oocytes were retrieved in the fresh cycles and 146 in the frozen-thawed cycles among the 13 couples that composed the study group. Most of the retrieved oocytes were at MII (81.9% ± 20.3 fresh vs 88.4% ± 25.5 thawed; Table 1). There were not enough spermatozoa to microinject all retrieved matured oocytes in 3 cycles. An additional 16 oocytes were inseminated with donor spermatozoa after microinjecting all available testicular spermatozoa in 2 cycles. In another case, another 20 oocytes were frozen after microinjecting 10 oocytes with the husband's testicular sperm cells because the couple refused the use of donor samples.

Solely immotile sperm cells were found for microinjection into the oocytes that were retrieved in 1 out of 13 fresh cycles compared with 5 out of 13 frozen-thawed cycles. Thus, significantly more oocytes were injected with motile spermatozoa in the fresh vs thawed cycles (7.7 ± 5.6 vs 2.7 ± 3.5, respectively; *P* < .05; Table 1).

Fertilization rates were significantly lower when immotile fresh or frozen-thawed sperm were used for ICSI compared with motile spermatozoa (*P* < .03 and *P* < .01,

Table 2. Fertilization and embryo cleavage rates following ICSI using fresh and cryopreserved spermatozoa in the same couple with severe nonobstructive azoospermia*

	Fresh	Cryopreserved	P
2PN embryos formed (%)			
2PN embryos (%)/injected oocytes with motile sperm (no. of cycles)	49.0 ± 25.1 (12)†	53.8 ± 28.7 (8)‡	>.05§
2PN embryos (%)/injected oocytes with immotile sperm (no. of cycles)	20.1 ± 22.0 (8)†	24.4 ± 14.7 (10)‡	>.05§
2PN embryos (total no.)	61	45	
2PN/MII % (no. of cycles)	44.9 ± 15.6 (13)	41.1 ± 21.1 (13)	>.05
IPN rate (%)			
Motile	12.1 ± 18.6	0	
Immotile	0	4.4 ± 10.7	
3PN rate (%)			
Motile	3.0 ± 6.2	0	
Immotile	0	0	
Quality of transferred embryos			
Cleavage rate			
Normal#-cleaved embryos (no.)	27 (51.9%)	34 (75.5%)	>.05¶
Fast**-cleaved embryos (no.)	2 (3.8%)	1 (2.2%)	>.05¶
Delayed††-cleaved embryos (no.)	23 (44.2%)	10 (22.2%)	>.05¶
Grading			
I-II	32 (61.5%)	30 (66.7%)	>.05¶
III-IV	20 (38.5%)	15 (33.3%)	>.05¶

* Values are means ± SD; PN indicates pronuclei.

† $P < .03$, Mann-Whitney nonparametric test (between motile and immotile fresh sperm).

‡ $P < .01$, Mann-Whitney nonparametric test (between motile and immotile cryopreserved sperm).

§ Mann-Whitney nonparametric test.

|| Wilcoxon nonparametric test for quantitative variables.

¶ McNemar test for dichotomous variables.

2-4 cells on day 2 or 6-8 cells on day 3.

** >4 cells on day 2 or >8 cells on day 3.

†† <2 cells on day 2 or <6 cells on day 3.

respectively; Mann-Whitney test; Table 2). There was no significant difference, however, between the fertilizing ability of fresh vs frozen-thawed motile spermatozoa. Similarly, fresh and frozen-thawed immotile spermatozoa yielded comparable results (Table 2). Cleavage rates and embryo morphology were not significantly different between the fresh and frozen-thawed groups (Table 2).

At least 1 embryo was available for transfer in all cycles in each group. All the embryos were frozen rather than transferred in 2 fresh cycles, however, because the female partner suffered from ovarian hyperstimulation syndrome. Altogether, 4 clinical pregnancies were achieved, 2 with fresh sperm cells and 2 with frozen-thawed sperm cells. One of the pregnancies in the fresh sperm group was initially a triplet, but 1 embryo ceased to develop, resulting in a twin delivery. All other pregnancies were singletons. One pregnancy in the frozen-thawed group was achieved using only immotile sperm cells. Pregnancy rates were similar but implantation rates of the fresh sperm group were higher, although the difference did not reach a level of statistical significance (Table 3).

Search for Predictive Parameters for Loss of Motility After Thawing

All the detected sperm cells in 1 fresh cycle were immotile, but cryopreservation did impair the availability of motile spermatozoa for ICSI in another 4 cycles, and spermatozoa that were motile in the fresh cycle lost their motility during the freezing-thawing procedure. The percentage of motile sperm and the presence of progressive motility in the fresh sample of these 4 cases were not different from those in the 8 cases that preserved motility after thawing and, therefore, freezing followed by thawing cannot be used as a predictive factor for motility loss. Similarly, the fertilization rates in the fresh ICSI cycles could not be used as a predictive parameter because they were similar among the 8 cases that preserved motility ($39.4 \pm 22.5\%$) and the 4 cases that lost it after thawing ($45.3 \pm 31.7\%$).

Discussion

The lack of reliable predictive preoperative tests for the existence of spermatogenesis in men with NOA mandates

Table 3. Pregnancy rates when either fresh or cryopreserved spermatozoa was used in the same severe nonobstructive azoospermia patient

	Fresh	Cryopreserved	P
Embryo transfer rate (%)	11/13 (84.6%)*	13/13 (100%)	...
Embryos transferred (n)	38	34	...
Implantation rate (%)	4/38 (10.5%)	2/34 (5.9%)	NS
No. ongoing pregnancies per embryo transfer (%)	2/11 (18.2%) 1 singleton, 1 set of triplets	2/13 (15.4%) 2 singletons	NS
Take-home baby rate (%)	2/11 (18.2%) 1 singleton, 1 set of twins	2/13 (15.4%) 2 singletons	NS

* In 2 cycles, embryos were frozen rather than transferred because the female partner suffered from ovarian hyperstimulation syndrome. NS indicates $P > .05$ (Fisher exact test).

the performance of operative sperm retrieval procedures in all cases. Ovulation induction and ova pickup on the part of the spouse is, however, not necessary in about 40%–50% of the couples in whom no sperm are found (Hauser et al, 1998; Seo and Ko, 2001; Friedler et al, 2002b), and justification for performing those procedures should be based solely on a demonstrable advantage of using fresh sperm vs thawed testicular sperm in NOA patients for achieving pregnancy.

Previous studies comparing ICSI outcomes in fresh vs frozen-thawed cycles in patients with NOA of all degrees of severity demonstrated that pregnancy rates were similar (Ben Yosef et al, 1999). Lack of motility, however, resulted in significantly lower fertilization rates (Friedler et al, 1997; Liu et al, 1997). The justification of exposing the spouse to ovulation induction treatment and ova pickup simultaneously with the husband's sperm retrieval procedure would appear to be open to question.

We encountered cases in which a few, and sometimes exclusively nonmotile, testicular sperm cells were isolated. Freezing of these isolated sperm cells imposed technical difficulties because it was not easy to ensure the recovery of those cells after thawing. Furthermore, doubts were raised concerning a possible loss of motility during the process and the subsequent reduction of fertility potential of those very precious spermatozoa. In 1 study in which a few thawed testicular sperm cells were used in 2 cases, no pregnancies were achieved in spite of a 60% fertilization rate (Romero et al, 1996). In another report, 2 pregnancies were achieved with thawed nonmotile testicular sperm cells that were chosen based on their tail shape (Ma et al, 2000).

Various creative solutions for the freezing of a few isolated sperm cells have been offered. One approach was to inject isolated sperm cells into an empty zona pellucida of a hamster oocyte and place it between 2 air bubbles inside a straw, a technique that helped in postthaw localization (Cohen et al, 1997; Hsieh et al, 2000). Others suggested the freezing of the minute sample under a layer of paraffin oil with glycerol (Craft and Tsirigotis, 1995). Romero et al (1996) described a frozen "testicular pill" composed of a mixture of some sperm cells and testicular

tissue. Aside from these solutions to technical obstacles, our literature search failed to reveal any published data on the influence of the freezing and thawing procedure on the fertilizing potential of sperm cells isolated by TESE in the unique subgroup of NOA patients with severe hypospermatogenesis.

In the present study, we evaluated our experience with the most difficult subgroup of NOA patients, that is, those in whom very few, and sometimes exclusively immotile, sperm cells were isolated ("severe hypospermatogenesis"). Two groups were compared: the first employing fresh testicular sperm cells that were extracted concomitantly with oocyte retrieval, and the second in which an ICSI cycle was performed later on using the subjects' stored frozen-thawed sperm. The results indicated that pregnancies can be achieved at similar rates as with fresh testicular sperm, even when motility is lost during the cryopreservation process. Motility had been completely lost in 4 of our cases, resulting in a significantly lower number of motile sperm cells available for ICSI in the frozen-thawed cycles (Table 1). The lack of motility correlated with a significant reduction in fertilization rates and with similar magnitude for both the fresh and frozen-thawed cycles. Embryo quality expressed by cleavage rate and grading did not, however, differ between the groups (Table 2), nor did fertilization and pregnancy rates. The implantation rate was more favorable in the fresh sperm group, albeit not significantly so (Table 3). Although embryos generated by microinjection of these severe hypospermatogenesis patients' testis were of good morphology, their implantation rates did not correlate with the tested morphologic parameters. This might be due to the fact that testicular sperm obtained from NOA patients score lower in terms of quality and quantity, resulting in a lower number of embryos for selection (Platteau et al, 2004). In addition, the aneuploidy frequency of sperm originating from NOA men is much higher than from ejaculated sperm, which may result in a higher rate of aneuploidy embryos that is not always in correlation with the morphologic appearance of the derived embryo. Given that more than 40% of morphologically normal embryos have chromosomal abnormalities (Márquez et al, 2000), mor-

phologic analysis of the embryo is not sufficient to predict its implantation potential (Gianaroli et al, 1997; Magli et al, 1998).

These results suggest that postthaw loss of motility of testicular sperm cells should be considered differently than primary lack of motility of fresh sperm cells. Motile sperm cells that lost motility during the freezing-thawing process might still be viable and may preserve their fertilizing capacity in an IVF-ICSI setting. This capacity may be better than the fertilizing capacity of sperm cells that are primarily immotile when retrieved from the testes. Due to the paucity of sperm cells in the severe hypospermatogenesis group of patients, we did not perform viability tests to validate the survival rate of these testicular sperm cells.

Insofar as the motility of sperm cells indicates their viability, different supravital stains and procedures have been suggested to differentiate dead spermatozoa from viable, but immotile, spermatozoa. The use of the hypo-osmotic swelling (HOS) test has been proposed to select immotile, but viable, spermatozoa from the ejaculated processed semen sample (reviewed by Sallam et al, 2001). Sallam et al (2001) showed that, in couples with immotile ejaculated or testicular spermatozoa treated with ICSI, the selection of the viable spermatozoa by a modified HOS solution can achieve comparable pregnancy rates in both groups of patients. Although the HOS test can be successfully applied with absolutely immotile spermatozoa, Vandervorst et al (1997) questioned its toxicity.

Another approach to identify viable spermatozoa is by incubating them with pentoxifylline. Pentoxifylline was approved by the American Food and Drug Administration for administration to humans. It is a nonspecific inhibitor of phosphodiesterase that increases intracellular cAMP levels and has been shown to affect sperm motility and motion characteristics, such as velocity or hyperactivity. The beneficial effect of pentoxifylline on sperm motility has often been described for both fresh and cryopreserved spermatozoa (reviewed by Henkel and Schill, 2003). It was also suggested as a pretreatment to stimulate epididymal and testicular sperm motility (Terriou et al, 2000). There are, however, conflicting results regarding the effectiveness of pentoxifylline treatment. We are currently running a study on the same group of patients with severe hypospermatogenesis using pentoxifylline to enhance detection of viable sperm for ICSI.

Sallam et al (2001) compared the results of 12 ICSI cycles using immotile testicular sperm cells of NOA patients (1 fresh and 11 frozen-thawed) with ICSI being performed with immotile ejaculated sperm cells. Fertilization rates, embryo quality, and pregnancies were not significantly different. There are other reports on the fertility performance of immotile testicular cells in azoospermic patients (Nagy et al, 1998; Rives et al, 1998; Shul-

man et al, 1999; Baukloh, 2002; de Oliveira et al, 2004), but they included either patients with obstructive azoospermia or only cases in which fresh testicular sperm cells were used.

The patients included in the current study were highly selected and comprise the most difficult cases of NOA. Only the ones with repeated consecutive ICSI cycles using frozen-thawed testicular sperm cells were included to allow paired analysis. Such stringent recruiting criteria resulted in a relatively small study group. We would, of course, prefer to adopt a treatment strategy that does not compromise the fertility outcome of even the most severe cases. Based on our results, and taking into consideration that our study group is relatively small to draw any firm conclusions, we suggest performing testicular sperm extraction without simultaneous ovulation induction and ova aspiration to the spouse in a setting of experienced andrology and embryology laboratories and good sperm-freezing techniques. This approach would also enable more flexibility for the operating team because the procedure can be scheduled on an elective basis. It is also more comfortable for the treated couple and may spare the spouse unnecessary treatments in cases when testicular sperm cells could not be retrieved.

Conclusion

Pregnancy outcome is not compromised after freezing and thawing of testicular sperm cells, even in the most severe forms of hypospermatogenesis, although motility is sometimes lost during cryopreservation.

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