The Impact of Early Testicular Sperm Extraction or Cryopreservation on the Outcome of Intracytoplasmic Sperm Injection—A Randomized Controlled Study

M. Dirnfeld,¹ M. Paz,¹ D. Yshai,¹ I. Calderon,¹ S. Lahav-Baratz,¹ M. Koifman,¹
H. Shiloh,¹ H. Abramovici,¹ D. Bider^{2,3,4}

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Purpose: To compare the outcome of sperm extraction 24 h before ovum pickup and on the day of oocyte retrieval.

Methods: A controlled study was performed to compare the outcome of 90 sperm extractions and in vitro sperm injection cycles performed in 54 patients.

Results: Available fresh sperm for the sperm injection procedure and cryopreservation obtained on the day of ovum pickup were similar to sperm collected 1 day before (33.3% vs. 39.4%, respectively). Fertilization rate obtained with fresh sperm was also similar (48.9% vs. 54%), respectively. Clinical pregnancy rate was 38% vs. 22% per embryo transfer, respectively ($P = 0.235$). When comparing an additional 24 cycles with cryopreservation of sperm retrieved on the day of ovum pickup, as well as a day previously, no significance was noted in the parameters.

Conclusions: Sperm retrieved 24 h before oocyte retrieval and used as fresh or frozen–thawed for sperm injection are as effective as those used on the day of ovum pickup.

KEY WORDS: Cryopreservation; ICSI; nonobstructive azoospermia; TESE.

INTRODUCTION

The perception and treatment mode of male factor infertility have undergone radical changes in recent years, especially with the introduction of intracytoplasmic sperm injection (ICSI), using fresh and frozen–thawed spermatozoa. The use of frozen–thawed sperm obtained by testicular extraction showed similar results when compared with fresh testicular extracted sperm (1–3). This mode of treatment provides several advantages for the team conducting assisted reproduction, as well as the patients.

Flexibility in timing of testicular sperm extraction (TESE) is very useful in busy IVF centers, where personnel and operating room facilities for the purpose of TESE are not available daily, and where freezing facilities exist. However, only scant reports, mainly uncontrolled retrospective studies (4–6) are available concerning the impact on IVF–ICSI outcomes when performing sperm extraction on the day of ovum pickup (OPU), or on the previous day.

This study randomly, and in a controlled manner, analyzed the outcome of IVF–ICSI in patients suffering from nonobstructive azoospermia, who were undergoing TESE and/or cryopreservation on the same day, versus patients undergoing these procedures the day before OPU. We compared the outcome of fresh and frozen–thawed spermatozoa of TESE in both groups of patients.

¹ IVF Units, Carmel Medical Center, Technion, The Rappaport School of Medicine, 34362, Haifa, Israel.

² Department of Obstetrics and Gynecology, The Chaim Sheba Medical Center, 52621, Tel Hashomer, Israel.

³ Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

⁴ To whom correspondence should be addressed.

MATERIAL AND METHODS

Ninety consecutive TESE-IVF-ICSI cycles in 54 patients who presented with nonobstructive azoospermia were evaluated over a 3-year period. Patients were allocated to this study randomly, depending only on their scheduled day of OPU. Because of operating room and staff availability, TESE procedures were performed only on Sundays, Tuesdays, or Thursdays. When oocyte retrieval was scheduled for these days, the TESE procedure and cryopreservation of extra spermatozoa was performed on the same day and served as a control group. When retrievals were scheduled for other days, the TESE procedure and cryopreservation of extra spermatozoa was performed 24 prior to OPU, and served as the study group.

Four types of treatment cycles were evaluated: 1) 33 IVF-ICSI cycles performed with fresh TESE sperm retrieved on the same day of OPU; 2) 12 IVF-ICSI cycles using frozen-thawed sperm previously cryopreserved from TESE performed on the day of OPU; 3) 33 IVF-ICSI cycles performed with fresh TESE sperm collected 24 h before OPU; 4) 12 cycles using frozen-thawed sperm cryopreserved 24 h before OPU.

This study involved only therapeutic interventions that would normally be carried out in the course of our IVF-ICSI-TESE routine. The study analyzed a well-established, clinical therapeutic procedure that is not experimental. Consequently, no Institutional Review Board Committee approval was requested. However, written informed consent was obtained from each couple when entering our TESE-ICSI program.

Testicular Biopsy

Samples of testicular biopsies were sent for histological examination. The specimens were placed in a petri dish with human tubal fluid (HTF) + HEPES (10 μ L/mL) at 37°C. The seminiferous tubules were teased apart using two glass slides, and the contents were squeezed into the surrounding media. The tissue was then transferred to a 15-ml conical tube and incubated at 37°C for 2 h. The suspension was centrifuged at 1800 rpm for 10 min.

After centrifugation, the supernatant was discarded, 100 μ l of fresh HTF medium, supplemented with 50% synthetic serum supplement (SSS, Irvine Scientific, Santa Ana, CA), was added, and the tube was incubated for 1–2 h. Drops of 5 μ l were then used for the micromanipulation procedure. Before freez-

ing, an aliquot was removed to assess sperm quality. At cryopreservation, multiple aliquots of sperm were frozen whenever possible. The cell suspension was slowly diluted 1:1 with TEST-citrate-yolk buffer and 12% glycerol, so that the final concentration of glycerol was 6%. The samples were packaged in 1-ml cryovials (Nunc, Denmark) and cooled slowly at a rate of 1.7°C/min to –6°C. The vials were frozen at a rate of 5°C/min to –100°C and stored in liquid nitrogen at –196°C.

In addition, all TESE procedures were divided into three categories according to the presence of sperm and freezing availability: 1) cycles without sperm retrieval (defined as “no retrieval”); 2) cycles with very few sperm retrieved without any freezing possibility (defined as “poor retrieval”); and 3) cycles with sperm retrieval and freezing availability (defined as “good retrieval”).

Protocol of Ovarian Stimulation and Oocyte Retrieval

Down-regulation using gonadotropin-releasing hormone analogue (GnRH-a) was performed in all patients, using Buserelin (Suprefact[®] nasal spray, Hoechst, Frankfurt, Germany) 1000 μ g/day, and continued up to human chorionic gonadotrophin (hCG) administration. When laboratory testing indicated pituitary suppression (estradiol [E₂] <40 pg/ml), three ampoules/day of human menopausal gonadotropin (hMG; Teva, Tel Aviv, Israel) were administered, until three or more follicles of >18-mm mean diameter were present on transvaginal ultrasound (TVS).

Follicular monitoring by serum E₂, luteinizing hormone (LH), progesterone (P), and serial TVS scans were performed as previously described (7,8). Patients then received 5000 IU of hCG i.m. 35–36 h before the scheduled TVS-guided follicular aspiration.

Insemination and Fertilization

Oocytes were scored for maturity at recovery and incubated for 3–4 h before fertilization in HTF medium, with 2.5% SSS (Irvine Scientific, Santa Ana, CA). Sperm recovery was carried out as mentioned previously, and the ICSI procedure as previously described (8). Observation of two pronuclei (PN) was performed in both groups 16–20 h after the ICSI procedure. Cleavage was assessed after 48–50 h and embryo quality was evaluated using the modified criteria of Cummins and Breen (9). Up to three embryos were selected for transfer into the uterine cavity. In women

aged 38 years or above and/or in cases of repeated failures, up to four embryos were replaced. Luteal support was performed using *P* in oil, 100 mg/day i.m. (Gestone, Paines, & Byrne, Greenford, U.K.). All data were compiled on a relational database (Excel, Microsoft Corp., Redmond, WA) and statistical analysis was performed using SPSS for Windows. Chi-square and Student's *t* tests were applied for statistical calculations where applicable. A *P* value of < 0.05 was considered statistically significant.

RESULTS

The mean women's age, male serum FSH, number and mean of retrieved oocytes, histological examination findings of testicular specimen, and freezing availability were similar in the group of patients that underwent ICSI with fresh TESE on the day of OPU and the day before (Table I). Statistically, no significant differences were observed between the groups in terms of "poor retrievals," "no retrievals," or "good retrievals." Fertilization rate, mean number of embryos transferred, number of cycles with embryo transfer (ET), and pregnancy rate (PR)/ET were similar in both groups. However, a trend existed for a lower pregnancy rate per ET for TESE performed on day -1.

When comparing the same aforementioned variables in an additional 24 cycles in which sperm cryopreservation was performed on the day of OPU, or on the previous day, no statistically significant differences were noted in any parameters (Table II).

Table III depicts the overall results of all TESE cycles with fresh versus frozen-thawed embryos regardless of the day of OPU. Fertilization rate was found to be significantly higher in the fresh TESE cycles than in the frozen-thawed TESE cycles. However, despite the fact that there was no statistical significant difference noted between the groups in terms of mean number of embryos transferred, number of cycles with ET and PR/ET, it certainly appears that there may be an important trend here towards an actual difference in fresh versus frozen-thawed PR/ET.

DISCUSSION

Although flexibility of sperm retrieval and the use of cryopreserved testicular sperm for ICSI has several advantages for the reproductive team, data concerning IVF-ICSI outcomes using frozen-thawed testicular sperm, or spermatozoa retrieved 24 h before the ICSI procedure, are limited and inconclusive. When

Table I. Cycle Parameters and Outcome of TESE Performed on the Same Day (0) and 24 h Prior to OPU (-1) Using Fresh Sperm

Variable	Day -1 (%)	Day 0 (%)
No. of TESE cycles	33	33
Female patient age (years) ^a	28.5 ± 5.36	30.6 ± 5.6
Serum FSH (men) ^a	15.11 ± 13.8	18.07 ± 13.82
Patients with Sertoli-only cell syndrome	3/21 (14.28)	4/24 (16.66)
Patients with hypospermatogenesis	12/21 (57.14)	12/24 (50)
Patients with maturation arrest	6/21 (28.57)	8/24 (33.33)
No. (%) of cycles with no sperm retrieval	5/33 (15.15)	9/33 (27.3)
No. (%) of cycles with poor retrieval	15/33 (45.45)	13/33 (39.4)
No. (%) of cycles with good retrieval	13/33 (39.4)	11/33 (33.3)
No. of retrieved oocytes	397	428
Mean (±SD) No. of retrieved oocytes ^a	12 ± 5.13	13 ± 6.25
No. of cycles with fertilization failure	3	3
No. of MII oocytes ^a	7.16 ± 4.32	6.6 ± 5.12
Fertilization rate	128/236 (54)	114/233 (48.9)
Embryo transferred	23	21
Mean (±SD) number of embryo transferred ^a	2.061	1.727
No. of cycles with ET/No. of cycles	23/33 (69.7)	21/33 (63.63)
Pregnancy rate/ET	5/23 (21.74)	8/21 (38.1)

Note. No significant differences were found between groups. MII = Metaphase II-stage oocytes; TESE = Testicular Sperm Extraction; ET = Embryo transfer; FSH = Follicle stimulating hormone. Poor retrieval = cycles with sperm retrieval but without freezing possibility; Good retrieval = cycles with sperm retrieval and freezing possibility.

^a Values are mean ± SD.

Table II. Cycle Parameters and Outcome of TESE with Sperm Cryopreserved on the Day of OPU (0) or 24 h Prior to OPU (-1)

Variable	Day -1 (%)	Day 0 (%)
No. of frozen-thawed TESE cycles	12	12
Female patient age (years) ^a	28.9 ± 5.46	30.25 ± 6.6
Serum FSH (men) ^a	5.27 ± 3.758	10.93 ± 8.8
No. of retrieved oocytes	153	144
Mean (±SD) No. of retrieved oocytes ^a	12.8 ± 8.26	12 ± 6.44
No. of cycles with fertilization failure	2	1
No. of MII oocytes ^a	9.44 ± 4.64	7.09 ± 3.48
Fertilization rate	31/107 (28.97)	24/83 (28.91)
No. of cycles with ET	9	11
Mean (±SD) number of embryo transferred ^a	1.91	1.833
No. of cycles with ET/No. of cycles	9/12 (75)	11/12 (91.6)
Pregnancy rate/ET	1/9 (11.1)	2/11 (18.18)

Note. No significant differences were found between groups. MII = Metaphase II-stage oocytes; TESE = Testicular sperm extraction; ET = Embryo transfer; FSH = Follicle stimulating hormone.

^a Values are mean ± SD.

Table III. Cycle Parameters and Outcome of Total TESE Cycles with Fresh and Frozen–Thawed Sperm

Variable	Fresh TESE (%)	Frozen–thawed TESE (%)
No. of TESE cycles	66	24
Female patient age (years) ^{a,b}	29.53 ± 5.55	29.58 ± 5.96
Patients with Sertoli-only cell syndrome ^b	7/45 (15.55)	2/17 (11.8)
Patients with hypospermatogenesis ^b	24/45 (53.33)	11/17 (64.7)
Patients with maturation arrest ^b	14/45 (31.11)	4/17 (23.5)
No. of retrieved oocytes	825	297
Mean (±SD) No. of retrieved oocytes ^{a,b}	12.5 ± 5.7	12.38 ± 7.25
No. of cycles with fertilization failure	6	3
No. of MII oocytes ^{a,b}	6.89 ± 4.71	7.92 ± 3.80
Fertilization rate ^{***}	242/469 (51.6)	55/190 (28.95)
No of Cycles with ET	44	20
Mean (±SD) No. of embryos transferred ^{a,b}	1.89 ± 1.67	1.88 ± 1.33
No. of cycles with ET/No. of cycles ^b	44/66 (66.7)	20/24 (83.3)
Pregnancy rate/ET ^b	13/44 (29.54)	3/20 (15)

Note. M II = Metaphase II-stage oocytes; TESE = Testicular Sperm Extraction; ET = Embryo transfer; FSH = Follicle stimulating hormone.

^a Values are mean ± SD.

^b No significant differences were found between groups.

*** $P < 0.0001$.

applying sample-size requirement analysis, we estimated that to detect a 5% change in ongoing PR/ET with a power of 80% and with a 5% α error, 474 TESE cycles would have been required in each study group, which is beyond the reasonable abilities of our individual institute.

Our study is unique, since it is a randomized, controlled investigation reporting different aspects of handling fresh and cryopreserved spermatozoa in a unique population of nonobstructive, azoospermic males. This research compared IVF–ICSI results in the aforementioned population when the TESE procedure was undertaken on the same day of OPU or 24 h previously. In addition, we cryopreserved TESE sperm obtained during these time periods, and then used the sperm for ICSI procedures.

In the past, some authors (1,4,6) showed the possibility of using in vitro cultured testicular spermatozoa obtained prior to OPU. Zhu *et al.* (10) noted that testicular sperm attained motility after incubation for 22–48 h. Hu *et al.* (5) reported the possibility of using in vitro cultured testicular spermatozoa obtained the day before OPU. Jaroudi *et al.* (6) showed that TESE can be safely performed 48 h before ICSI without an effect on fertilization and PR.

Our study supports the observations of Urman *et al.* (4) and Hu *et al.* (5), who reported that a testicular biopsy procedure can be performed a day before OPU. Therefore, patients with nonobstructive azoospermia can avoid costly ovum oocyte retrievals when no sperm can be obtained from testicular specimens (11,12).

Our data also elaborates on cryopreservation possibilities by concluding that testicular sperm freezing is not compromised if performed 24 h after sperm retrieval. Previous studies demonstrated that TESE sperm quality before and after cryopreservation are comparable, and that postthaw recovery of viable sperm is adequate for subsequent use with IVF–ICSI (1–3,13–15). Although the main issue of our study was not to compare fresh and frozen–thawed TESE sperm results, we summarized (Table III) the outcome of TESE–ICSI in our study population. Our data are consistent with those of previous studies (3,13) and show no significant differences in terms of mean number of transferred embryos and PR. However, fertilization rate was found to be significantly higher in the fresh TESE cycles than in frozen–thawed TESE cycles. Moreover, it is important to note that a trend may exist for a lower PR/ET for TESE done at day –1. In addition, there may be a trend towards the actual difference in fresh versus frozen–thawed PR/ET, as demonstrated in Tables I and III.

More studies with a larger sample may provide a definite solution to this issue. Even if results will be proven to be less favorable for frozen–thawed sperm, it seems that cryopreservation of TESE sperm would be worthwhile.

In an era when reproductive medicine aims at minimal invasive procedures, and we are aware of the fact that TESE has a long-term effect on the testis, avoiding unnecessary procedures is crucial to avert associated complications (16,17). Therefore, by performing only a minimal number of TESE procedures

per patient in the knowledge that flexibility of sperm retrieval and incubation, as well as cryopreservation, will not reduce fertilization and PR, we can improve treatment and reduce complications. Furthermore, the coordination of two surgical procedures, testicular sperm and oocyte retrieval, for concomitant IVF–ICSI, is unnecessary.

In conclusion, our study shows that sperm retrieval and/or cryopreservation 24 h prior to OPU is as effective as sperm retrieval and/or cryopreservation on the day of OPU. The TESE procedure can be carried out at the convenience of the local, operating room schedule and staff of the IVF units. Clinics with freezing capabilities can cryopreserve testicular tissue obtained by TESE on the day of OPU or 24 h later, without being concerned about reduction in IVF outcome, and consequently unnecessary complications can be avoided.

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