In Vitro Fertilization Treatment for Severe Male Factor: A Comparative Study of Intracytoplasmic Sperm Injection with Testicular Sperm Extraction and with Spermatozoa from Ejaculate

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Purpose: Our purpose was to evaluate whether the source of spermatozoa influences the results of intracytoplasmic sperm injection (ICSI) treatment in couples with severe male-factor infertility.

Methods: A retrospective analysis of 40 cases of ICSI with testicular-retrieved spermatozoa, matched with 40 cases of ICSI with ejaculated spermatozoa, was performed. We included only couples with normoovulatory females younger than 37 years who were matched according to the day of ovum pickup with the patients in the study group.

Results: Eighty cycles were analyzed: 40 cycles using testicular spermatozoa and 40 cycles using ejaculated spermatozoa. In 32 (80%) of the 40 ICSI transcuteaneous needle aspiration cycles, we obtained enough spermatozoa to inject all the mature oocytes retrieved. In eight (20%) cases there were not enough spermatozoa to inject all the oocytes. Only 76 (54%) of 141 available oocytes were injected in these eight patients. The oocyte fertilization rates were 42% for the study group and 55.5% for the controls ($P < 0.005$). Thirty-six (90%) patients in the group with nonobstructive azoospermia (NOA) and 37 (92.5%) patients in the oligoteratoasthenospermia (OTA) group had embryos for replacement. The mean cleavage rates per cycle (96% with testicular and 93% with ejaculated spermatozoa), the mean number of embryos per transfer (3.72 ± 1.6 in the NOA group and 4.24 ± 1.5 in the OTA group), the embryo quality (cumulative embryo scoring = 34.03 ± 22.62 in the testicular sperm group and 36.08 ± 19.28 in the ejaculated sperm group), and the clinical pregnancy rates (22.5% in the NOA

patients and 20% in the ejaculate group) were not significantly different between groups.

Conclusions: High fertilization, cleavage, and pregnancy rates can be achieved with intracytoplasmic testicular sperm injection from patients with NOA, reaching levels comparable with those of ICSI using ejaculated spermatozoa.

KEY WORDS: intracytoplasmic sperm injection; infertility; male factor.

INTRODUCTION

In 1991, the first pregnancy after the transfer of embryos obtained after injection of a single spermatozoon into the ooplasm of a metaphase II oocyte was established (1). Initially, intracytoplasmic sperm injection (ICSI) was applied in couples with repeated failed fertilization following standard in vitro fertilization (IVF). It soon became clear that ICSI could also be used in couples whose male partners have a very limited number of spermatozoa in the ejaculate. Moreover, it became clear that none of the conventional semen parameters have any influence on the results of ICSI, except when total immotile spermatozoa are available for the microinjection procedure (2).

In many patients with nonobstructive azoospermia (NOA), it may be possible to find enough testicular spermatozoa in one or several testicular biopsy specimens to carry out ICSI in metaphase II oocytes (3). The question of whether the origin of the spermatozoa influences the outcome of IVF treatment was partially answered only by limited experience (4–6).

In this study, we evaluated whether the source of spermatozoa influences the results of ICSI. We compared the results of ICSI with testicular spermatozoa (from infertile males with NOA) with those of ICSI with ejaculated spermatozoa.

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MATERIALS AND METHODS

ICSI was introduced into our IVF program in April 1993, and we have used ICSI from testicular biopsies of NOA since 1994. In this study, we included all the testicular-retrieved spermatozoa (TRS) procedures from NOA starting from January 1995, because by this date the laboratory procedures were well established and were beyond "the learning curve of the procedure." We included all cases of TRS between January 1, 1995, and April 1, 1996, where at least one spermatozoon was found in the testicular prepare, the female partner was younger than 37 years, and the baseline hormonal profile [follicle-stimulating hormone (FSH), luteinizing hormone, estradiol, thyroid-stimulating hormone; human prolactin; and androgens] was within normal limits. We matched every case with an ICSI procedure with sperm obtained from ejaculate; the control cases were matched for female age, female hormonal status, and close proximity to the study group's procedure (most of the cases on the same day or, maximum, the next day).

The control group required IVF with the ICSI procedure either because of oligoteratoasthenospermia (OTA) (less than 5×10^6 total motile spermatozoa per ejaculate in 31 cases) or because of previous failure of fertilization in regular IVF (9 cases).

The NOA group comprised patients with normal or high FSH levels and/or those with a previous testicular biopsy showing maturation arrest or Sertoli-only prepares. The majority of patients presented with medium- to small-sized testes (8–15 ml) with a history of bilateral cryptorchidism. The female patients received controlled ovarian stimulation with a gonadotropin releasing hormone analogue (GnRH-a; Decapeptyl Depot, 3.75 mg CR; Ferring, Malmo, Sweden) long protocol (injected either at midluteal phase or on the first day of the menstrual cycle) and human menopausal gonadotropin (hMG; Pergonal, 225 IU; Teva Pharmaceuticals Ltd., Petach Tikva, Israel); FSH (Teva Pharmaceuticals Ltd., Petach Tikva, Israel), and luteinizing hormone (Teva Pharmaceuticals Ltd.), commencing 15 days after GnRH administration (8). The cycle monitoring was described elsewhere (8).

The testicular sperm retrievals were always performed on the day of ovum pickup under general anesthesia, anticipating the female procedure, and were performed by two techniques: (a) testicular sperm extraction (TESE) or (b) transcutaneous needle aspiration (TESA). The surgical technique for TESE was performed as described by Silber *et al.* (9). A horizontal incision measuring 2 cm was made in the scrotal skin and carried through the peritoneal tunica vaginalis. The testis was withdrawn and a small incision albugi-

nea was made. A small piece of extruding testicular tissue was excised and placed in a petri dish containing IVF medium. If no sperm was found on the first specimen of testicular tissue, a biopsy was repeated in other areas of the testis. A maximum of four biopsies was performed on each testis to prevent vascular damage.

Testicular sperm aspiration was performed as described by Madjar *et al.* (4) by directing a 21-gauge butterfly needle connected to a 20-ml syringe into the immobilized testis. Suction was applied and the needle withdrawn gradually, to the point where testicular fluid or tissue was seen entering the microinfusion set tubing attached to the butterfly needle. Steady, gentle, negative pressure was maintained until the segments of testicular tissue ceased to flow, and occlusive artery forceps were then applied to the microtubing before the needle was withdrawn from the skin. Any testicular tissue extracted with the syringe was immediately cut at skin level. The aspirate was then flushed from the needle into a sterile petri dish, using IVF culture medium. The specimen was examined microscopically and the procedure repeated as many times as necessary. Specimens obtained by testicular sperm retrieval (TRS) (open biopsy or needle biopsy) were gently crushed under direct microscopy.

Initially, we performed testicular sperm retrieval by open biopsy only. By mid-1995, we generally used transcutaneous testicular sperm aspiration, and only if no sperm was found did we continue with open biopsy. The ICSI procedure was performed according to the method described by Palermo *et al.* (1). The state of oocyte fertilization was assessed 16–18 hr after the injection. Fertilization was considered normal when two pronuclei were present. Up to five embryos were placed in the uterine cavity 44–48 hr after the microinjection procedure. The embryonic numbers of blastomeres and morphological quality were noted, and in addition, the cumulative embryo score (CES) was calculated (7). Any additional embryos of good morphological quality were cryopreserved for possible later use. Pregnancy was confirmed by detecting rising α -human chorionic gonadotropin (hcg) concentrations at least twice and at least 12 days after embryo replacement. Clinical pregnancies were diagnosed only when a gestational sac and fetal pole with a beating heart were noted at ultrasonography.

Statistical Analysis

All data were analyzed by the SPSS program. Statistical significance was tested using Student's *t*-test, χ^2

Table I. Baseline Parameters of the Couples from the Two Groups^a

	NOA group	OTA group	P value ^b
Women's ages (yr)	30.97 ± 3.79	28.73 ± 4.34	NS ^c
Women's FSH levels (IU/L)	6.28 ± 2.26	6.22 ± 2.26	NS
Women's LH levels (IU/L)	4.92 ± 2.57	5.21 ± 2.69	NS
Husbands' ages (yr)	33.88 ± 4.83	33.80 ± 6.25	NS
Husbands' FSH levels (IU/L)	12.55 ± 8.24	5.88 ± 3.27	0.03

^a Values expressed as mean ± SD. LH, luteinizing hormone.^b Student's *t*-test.^c Not significant.

test, and Fisher's exact test as appropriate. Statistically significant differences were determined at $P < 0.05$.

RESULTS

The study comprised a total of 77 patients from 80 treatment cycles; 40 cycles of couples diagnosed suffering from NOA and 40 from OTA. Previous histologic results of testicular biopsy in idiopathic azoospermia were available for 29 patients. Late maturation arrest was found in 21 (52.5%) patients, 6 (15%) patients were diagnosed as Sertoli cells only, and 2 patients had tubular sclerosis. The other eight (20%) patients had suffered from childhood cryptorchidism. Needle aspiration was the only procedure used in 4 (10%) patients, and open biopsy in 36 (90%). In 30 (75%) patients, sperm were recovered immediately, and in 10 (25%) cycles, sperm were found only after incubation. In 22 (55%) cases, sperm were immotile at the time of recovery.

The baseline parameters of the two groups are summarized in Table I. As expected, the only significant difference is the higher FSH levels among the azoospermic males ($P < 0.05$). No significant differences were noted between the two groups with respect to ovarian stimulation and number of eggs recovered (Table II). We achieved a significantly higher fertilization rate in the ejaculatory group ($P < 0.005$) than in

Table II. Cycle Characteristics^a

	NOA group	OTA group
E ₂ at hCG (pg/ml)	2112 ± 922	2165 ± 1024
No. of eggs retrieved/cycle	16.86 ± 12.09	14.86 ± 8.31
No. of MII eggs/cycle	11.76 ± 6.91	10.54 ± 5.82

^a Values expressed as mean ± SD. E₂, estradiol; MII, metaphase II. Student's *t*-test: no significant differences.**Table III.** ICSI Procedure Results^a

	NOA group	OTA group	P value ^b
No. of fertilized eggs/cycle	4.14 ± 2.86	5.69 ± 3.5	0.005
Mean embryos/ET	3.72 ± 1.6	4.24 ± 1.5	NS ^c
CES/cycle	34.03 ± 22.62	36.08 ± 19.28	NS

^a Values expressed as mean ± SD.^b Wilcoxon scores; Kruskal-Wallis test (χ^2 approximation).^c Not significant.

the NOA group, but transferred a comparable number of embryos in both groups (Table III). Furthermore, the transferred embryos were of comparable morphology.

The clinical pregnancy rates were 25% per transferred cycle for the azoospermic group of patients and 21.62% for the OTA group (Table IV). Thirteen (10%) embryos were implanted in the biopsy group and 11 (7%) in the controls. In the NOA group, the fertilization rate was higher when the sperm were found during the initial screening, 46.6%, compared with 27.9% when sperm were found only after the incubation period ($P < 0.005$). Interestingly, when motile sperm were injected, the fertilization rate was 49.2%, compared with 39.8% when immotile sperm were injected (NS).

For the TRS group, a direct correlation was found between the fertilization rate per cycle ($P = 0.0131$), the number of embryos available for replacement ($P = 0.025$), and the embryo morphology ($P = 0.0138$) and the ability to achieve a pregnancy.

DISCUSSION

ICSI has now been accepted by many clinicians as the method of choice to overcome the infertility of patients with severe male factor. Since Devroey *et al.* (3) demonstrated the presence of sperm in patients with Sertoli cells only, or tubal sclerosis, it is logical

Table IV. Cycle Results^a

	NOA group	OTA group
No. of ET cycles	36 (90%)	37 (92.5%)
Clinical pregnancy (PR/ET)	9 (25%)	8 (21.62%)
Ongoing ^b /delivered pregnancy	7 (19.4%)	6 (16.21%)
Implantation/embryo	10%	7%

^a PR, pregnancy rate; ET, embryo transfer. Wilcoxon scores, Kruskal-Wallis test (χ^2 approximation): no significant differences.^b More than 20 weeks of gestation.

to attempt sperm retrieval in patients with germinal failure and azoospermia, even with high FSH levels. The purpose of this study was to evaluate the fertilization and the implantation potential of the embryo resulting from nonobstructive spermatozoa which had only recently been released from the Sertoli cells. The study tried to define sperm characteristics that can predict treatment outcome and compare the results of these cycles with those of treatment cycles in patients with OTA. Based on previous reports that showed a negative impact of advanced female age and aging oocytes on the cycle outcome after IVF, we deliberately enrolled patients younger than 37 years old with normal hormonal profiles, hoping to minimize the impact of the additional ovarian factor on the study results. Like those of Nagy *et al.* (6) our results showed that although the fertilization rate was higher for ejaculated spermatozoa, the number of embryos available for transfer and the implantation potential of the resulting embryos did not differ significantly between the two groups. The similar implantation rates per embryo in both groups and the quality of embryos as assessed by the embryo scoring system indicated that testicular spermatozoa could be used successfully compared with mature spermatozoa retrieved from the ejaculate.

The fertilization rate was significantly higher for the testicular spermatozoa when the sperm was found during the initial screening compared with those treatments where spermatozoa were recovered after a long incubation period ($P < 0.005$). This was the only sperm characteristic to be related to the cycle outcome for the TRS procedure. An interesting finding of our study was the fertilization potential of the immotile spermatozoa extracted by biopsy. Recently Nijs *et al.* (10) reported on pregnancies after ICSI with totally immotile spermatozoa. Although they obtained fertilization with all kinds of immotile spermatozoa, ongoing pregnancies were obtained only with immotile sperm originating from the testis, and not with sperm from the ejaculate. In our study, although the injection of motile spermatozoa showed a trend for a higher fertilization rate, this was not of statistical significance. The relatively small number of cycles analyzed may be a possible explanation for this lack of significance. Like Nagy *et al.* (2), we found no correlation between the sperm parameters in the OTA group and the fertilization, cleavage, and/or pregnancy rates.

We conclude that testicular spermatozoa, which have only recently been released from the Sertoli cells, can successfully fertilize mature oocytes, resulting in a normal pregnancy. The similarity of the results when testicular spermatozoa and ejaculate spermatozoa were compared indicate that when ICSI was applied the physiological maturation of the spermatozoa was not dependent on the histologic appearance of the testis or the contribution of the epididymis and was different for final maturation.

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