ANDROLOGY

The Outcome of Sperm Retrieval and Intracytoplasmic Sperm Injection in Patients with Obstructive Azoospermia: Impact of Previous Tuberculous Epididymitis¹

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Purpose: Our purpose was to investigate the influence of previous tuberculous epididymitis in patients with obstructive azoospermia on the outcome of sperm retrieval and intracytoplasmic sperm injection (ICSI).

Methods: Eighty-eight cycles of ICSI were performed in 44 patients with obstructive azoospermia; 16 cycles (7 patients) with tuberculous obstructive azoospermia and 72 cycles (37 patients) with nontuberculous obstructive azoospermia.

Results: The rates of fertilization and embryo cleavage were comparable, and there was no significant difference in the clinical pregnancy rate per fresh transfer between the two groups. The rates of embryo implantation and clinical miscarriage were also comparable.

Conclusions: Embryo quality and pregnancy outcome in sperm retrieval and ICSI were comparable in both the tuberculous and the nontuberculous obstructive azoospermia patients. Although there was a preponderance of testicular sperm used in the tuberculous obstructive azoospermia group, our results suggest that previous tuberculous epididymitis in patients with obstructive azoospermia does not affect the outcome of sperm retrieval and ICSI.

KEY WORDS: obstructive azoospermia; tuberculous epididymitis; sperm retrieval; intracytoplasmic sperm injection; in vitro fertilization-embryo transfer.

INTRODUCTION

Microsurgical operation has been performed successfully in some patients with obstructive azoospermia (1). In patients with congenital bilateral agenesis of the vas deferens (CBAVD) in whom reconstruction of the epididymis or vas deferens failed or was impossible, in vitro fertilization-embryo transfer (IVF-ET) has been attempted. This procedure has involved the use of epididymal sperms retrieved by microscopic aspiration (2,3), but due to the low fertilization rate, success has been very limited (4).

Intracytoplasmic sperm injection (ICSI) has recently been introduced in IVF-ET (5,6), and among patients with obstructive azoospermia, improved success in both fertilization and pregnancy has been reported using the retrieved sperms (7,8).

In an IVF-ET program, tuberculous infection in the female reproductive organs seriously affected the outcome of pregnancy (9). Tuberculous infection has also involved the male genitalia and resulted in tuberculous epididymitis or orchitis. Around the world, the incidence of male genital tuberculosis has been decreasing, but this condition is still prevalent in Korea, where approximately a quarter of patients with obstructive azoospermia have a history of tuberculous epididymitis (10); during sperm retrieval and ICSI, patients with

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previous tuberculous epididymitis are therefore often encountered.

To investigate the influence of previous tuberculous epididymitis on the outcome of sperm retrieval and ICSI in infertile males with obstructive azoospermia, we retrospectively compared the outcome in patients with tuberculous versus nontuberculous obstructive azoospermia.

MATERIALS AND METHODS

Patient Population

From October 1995 to August 1998, sperm retrieval and ICSI were performed in 44 patients with obstructive azoospermia at the Infertility Clinic, Seoul National University Hospital. Obstructive azoospermia was confirmed by physical examination, ultrasonography, vasography, and serum follicle stimulating hormone (FSH) levels. All men had undergone testicular biopsy, and in all cases, spermatogenesis had been found.

Seven patients had a history of tuberculous epididymitis, hence the incidence of genital tuberculosis in patients with obstructive azoospermia (Group A) was 15.9% (7/44). Two of seven patients with tuberculous obstructive azoospermia had a history of genital surgery due to tuberculous epididymitis; one had undergone a right epididymectomy and left partial orchiectomy, and the other, bilateral epididymectomy. The time interval from genital operation to sperm retrieval was 24 and 9 years, respectively. In these two cases, genital tuberculosis in males was confirmed by histologic examination of surgical specimens, and in the others, diagnosis was made by clinical impression and/or ultrasonographic finding (11). As the remaining 37 patients had no history of genital tuberculosis, they were considered to have non-tuberculous obstructive azoospermia (Group B). Among these, 15 patients had a history of genital surgery: failed epididymovasostomy ($n = 7$), failed vasovasostomy ($n = 5$), failed artificial spermatocele ($n = 1$), left orchiectomy ($n = 1$), and vasectomy ($n = 1$). Eight patients suffered from CBAVD. No female partner had a history of genital tuberculosis.

Ovarian Stimulation

Ovarian stimulation was performed in 88 cycles, using FSH (Metrodin; Serono, Aubonne, Switzerland) after pituitary desensitization with gonadotropin

releasing hormone (GnRH) agonist (Decapeptyl; Ferring, Malmo, Sweden). In Group A, a long protocol was used in 10 cycles and a flare-up protocol in six. In Group B, the corresponding figures were 62 and 10. Ovarian response was monitored by transvaginal ultrasonography (Combison 320; Kretztechnik, Austria), and 10,000 IU of human chorionic gonadotropin (hCG; Profasi; Serono, Aubonne, Switzerland) was administered when the leading follicle was 18 mm or more in diameter. Transvaginal oocyte aspiration was performed 36 hr after hCG injection (12,13).

Preparation of Oocytes

After 1–2 hr of incubation of oocytes retrieved, the cumulus and corona radiata cells were removed by exposure for 30 sec in phosphate-buffered saline (PBS) containing 80 IU/ml of hyaluronidase (Sigma H-4272, USA) and complexes were aspirated in and out of a hand-drawn glass pipette. Each oocyte was rinsed in several drops of medium to remove any remaining enzyme and to assess the morphology and nuclear maturity, examined under an inverted microscope ($\times 200$), and then cultured for 3 hr. Immediately prior to micromanipulation, the nuclear maturity of oocytes was assessed, and only metaphase II oocytes were microinjected.

Preparation of Sperm

In Group A, two cycles of microsurgical epididymal sperm aspiration (MESA), five of percutaneous epididymal sperm aspiration (PESA), and nine of testicular sperm extraction (TESE) were performed. In Group B, 13 cycles of MESA, 40 of PESA, and 19 of TESE were performed.

All sperm retrieval procedures were undertaken using sterile precautions after shaving scrotal hair. MESA involved a longitudinal incision in the most proximal epididymal tubules using an operating microscope under general anesthesia. Blood-free epididymal fluid was aspirated with a plastic pipette, then sperm were promptly assessed and prepared using IVF culture medium (Medicult, Copenhagen). The epididymal tubules were closed with 10–0 nylon, and the peridiverticular tissue with 9–0 nylon. An alternative approach was PESA, undertaken when it was difficult to time MESA to coincide with oocyte retrieval. Percutaneous sperm aspiration was achieved by directing a 23-gauge butterfly needle connected to a 10-ml syringe into either the head or the corpus of the epididymis, after holding the testis immobile beneath the thumb

and index fingers. Gentle suction was applied to the syringe and the needle was withdrawn gradually to a point where segments of fluid from the epididymis were aspirated. The aspirate was washed out of the needle using IVF culture medium. The specimen was examined microscopically, and the procedure was repeated on the other side until sufficient sperm were retrieved. If sperms could not be obtained by MESA or PESA, needle aspiration or open biopsy was attempted directly in both testes.

Aspirate containing epididymal sperm was used for ICSI after centrifugation in 90% Percoll, followed by two washes in Ham's F-10 medium. If available, surplus epididymal or testicular sperm were cryopreserved for future use. Testicular tissue, suspended in Ham's F-10 medium, was torn apart and minced, using a 25-gauge needle, then washed and incubated for 1–2 hr at 37°C in 5% CO₂. After sedimentation, the supernatant was collected, then added to IVF culture medium, thus allowing sperm to swim out from the cells.

ICSI Procedure

Microinjection tools were prepared, and ICSI was performed as described by Van Steirteghem *et al.* (6). A single sperm was then aspirated tail first into the tip of an injection pipette, and the oocyte was immobilized by slight negative pressure exerted on the holding pipette to hold the polar body at 12 or 6 o'clock. The micropipette was pushed firmly through the zona pellucida and the oolemma deep into the ooplasm at 3 o'clock. A small amount of ooplasm was drawn into the injection pipette and then expelled along with the sperm. Injected oocytes were then transferred into 30- μ l droplets of IVF medium under lightweight paraffin oil and incubated at 37°C in 5% CO₂ incubator.

About 16–18 hr after the microinjection, the oocytes were observed under an inverted microscope ($\times 200$, $\times 400$) for any sign of damage. Fertilization was confirmed 16 hr later by determining the presence and number of pronuclei. If a single pronucleus was observed, the second evaluation was carried out 4 hr later, and then oocytes with one pronucleus or three or more pronuclei were discarded.

At the time of ET, the embryo grading was assessed, and embryos were scored for quality using the cumulative embryo score (CES) (13). Embryos were replaced transcervically into the uterus 48 hr after ICSI, and excess embryos were cryopreserved. Luteal support was provided by administering 50 mg of progesterone in oil (Progest; Samil Pharmacy, Seoul, Korea) daily

from the day of ET. Clinical pregnancy was defined by the presence of an intrauterine gestational sac, and clinical miscarriage was defined by spontaneous abortion before 20 gestational weeks.

Statistical Analysis

For data analysis, chi-square and Student's *t* test with SPSS for Windows (version 7.0) were used. A *P* value below 0.05 was considered statistically significant.

RESULTS

In both groups, male patients and female partners were of a similar age [36.3 ± 1.6 vs 36.7 ± 1.1 and 34.3 ± 1.2 vs 33.2 ± 0.5 years (mean $\pm$ SM), respectively], and basal serum LH and FSH levels in female partners were also comparable (5.5 ± 0.8 vs 8.5 ± 1.4 and 13.7 ± 2.1 vs 10.4 ± 0.6 mIU/ml, respectively).

Both the serum estradiol (E₂) level on hCG day (1681 ± 350 vs 2957 ± 212 pg/ml; *P* < 0.05) (conversion factor to SI units, 3.671) and the number of oocytes aspirated were significantly lower in Group A, but the number of mature, metaphase II oocytes suitable for ICSI was comparable in the two groups (Table I). The percentage of these mature oocytes among the total retrieved oocytes was thus higher in Group A (86.9 vs 77.2%, *P* < 0.05).

The fertilization rate in Group A was 62.5%, not significantly different from that of 69.3% in Group B. Six embryos from one cycle were cryopreserved in Group A, and a total of 70 excess embryos from 11 cycles was cryopreserved in Group B.

Table I. Outcome of Ovarian Hyperstimulation and ICSI in Patients with Tuberculous and Nontuberculous Obstructive Azoospermia^a

	Group A tuberculous	Group B nontuberculous
No. oocytes aspirated*	7.9 $\pm$ 2.1	12.8 $\pm$ 0.8
No. oocytes suitable for ICSI (metaphase II)	6.9 $\pm$ 1.9	9.5 $\pm$ 0.6
No. oocytes fertilized with 2PN*	4.0 $\pm$ 1.0	6.4 $\pm$ 0.4
Fertilization rate (%)	62.5 $\pm$ 7.8	69.3 $\pm$ 2.5
No. embryos transferred*	3.7 $\pm$ 0.5	4.9 $\pm$ 0.2

^a Values are means $\pm$ SE.

* *P* < 0.05.

In Group A, fresh embryo transfer was performed in 14 cycles and canceled in two due to unfertilization of all oocytes after ICSI. In Group B, one cycle was canceled due to unfertilization; 71 cycles of fresh embryo transfer were therefore performed. The number of fresh embryos transferred was significantly lower in Group A (Table I). However, the embryo cleavage rate in both groups was comparable (92.7 vs 90.9%).

The clinical pregnancy rate (PR) per fresh embryo transfer was not significantly different: 28.6% (4/14) in Group A and 26.8% (19/71) in Group B (Table II). The rates of embryo implantation, clinical miscarriage, and multifetal pregnancy were all similar in both groups.

DISCUSSION

In IVF-ET programs, tuberculous infection in female reproductive organs has seriously affected the outcome of pregnancy. It has been reported that, compared with other tubal factor patients, patients with genital tuberculosis had higher basal serum FSH levels, a poorer response to ovarian hyperstimulation, a lower clinical PR per cycle, and a higher clinical miscarriage rate (9), but IVF-ET offers the only realistic treatment for tuberculous female infertility (14,15).

Tuberculous infection has also affected male genitalia, especially the epididymis, and often resulted in obstructive azoospermia. The incidence of male genital tuberculosis has been decreasing in the world, but this condition is still prevalent in Korea; one study (10) reported a previous history of tuberculous epididymitis in approximately 22% of patients with obstructive azoospermia, a prevalence which is slightly higher than that in our series (15.9%).

Although micro- and macrosurgical reconstruction of the epididymis has been performed successfully in

some patients with obstructive azoospermia, Lee (10) reported a poor surgical outcome in the tuberculous obstructive azoospermia group; both the anatomical and the functional success rates were significantly lower in that series.

With the introduction of sperm retrieval and ICSI, successful pregnancies have been reported in patients with obstructive azoospermia. In our study, overall clinical PRs per fresh transfer and per patient were 27.1 and 52.3%, respectively, and these figures were comparable to those reported elsewhere (16,17). We obtained similar clinical PRs in both the tuberculous and the nontuberculous obstructive azoospermia groups. It is well known that marked fibrosis and scar formation prevent successful epididymovasostomy in patients with tuberculous obstructive azoospermia, but in a sperm retrieval and ICSI program, it can be said that there is no limit to the sperm which may be obtained. Indeed, we obtained sperm suitable for ICSI from all patients with tuberculous obstructive azoospermia.

MESA, PESA, and TESE were performed in 2, 5, and 9 cycles in Group A, and in 13, 40, and 19 cycles in Group B. The distribution of three procedures was not different in the two groups ($p = 0.082$), but there was a preponderance of testicular sperm used in the tuberculous obstructive azoospermia group (56.3 vs 26.4%, $P = 0.045$). This indicates that in patients with previous epididymal tuberculosis, sperms can be retrieved more successfully from testis than from epididymis. Damaged or destructed epididymis due to previous tuberculous epididymitis seems to impede successful retrieval of epididymal sperms, or there may be a possible bias due to the small sample size.

It has been reported that in an ICSI program, the parameters of retrieved sperm were of no value in predicting fertilization or clinical PR and that there was no correlation between the outcomes of PESA/ICSI and the etiology of epididymal obstruction. Craft *et al.* (18) reported a similar fertilization rate and PR in three subgroups; failed vasectomy reversal, CBAVD, and inflammatory obstruction.

With conventional IVF with epididymal sperms, significantly lower fertilization and overall success rates have been observed in patients with CBAVD, compared to those with acquired obstruction such as vasectomy reversal failure, epididymal blockages, postsurgical ejaculatory failure, and vas deferens scarring (19). Among patients with CBAVD, the worst IVF results were seen in those with the more severe type of cystic fibrosis gene mutation. With epididymal sperm, the chance of successful fertilization was

Table II. Pregnancy Outcome in Patients with Tuberculous and Nontuberculous Obstructive Azoospermia

	Group A tuberculous	Group B nontuberculous
No. clinical pregnancies	4	19
Per couple (%)	57.1	51.4
Per sperm retrieval or oocyte aspiration (%)	25.0	26.4
Per fresh embryo transfer (%)	28.6	26.8
No. multifetal pregnancies	2 (50.0) ^a	7 (36.8)
No. clinical miscarriages	1 (25.0)	4 (21.1)
Embryo implantation rate (%)	11.5	9.6

^a Values in parentheses are percentages.

directly correlated with the total number of motile sperm retrieved. Since the introduction of ICSI, however, the absence of a correlation between the etiology of azoospermia and ICSI outcome has been clearly demonstrated in other publications (18,20). Our results consistently support this concept.

We did not find that oocyte quality was significantly different between the two groups. Although the serum E₂ levels on hCG day and the number of oocytes retrieved were lower in the tuberculous obstructive azoospermia group, a similar number of metaphase II oocytes suitable for ICSI was obtained. Embryo quality was also found to be similar between the two groups, and their rates of fertilization, embryo cleavage, and embryo implantation were comparable. The mean CES per embryo transferred was not, moreover, significantly different between the two groups (10.6 ± 1.8 vs 12.2 ± 0.6).

The potential risk of using tuberculous patients' sperm relative to transfection of a resulting embryo was a special concern, but it was unlikely since the disease state was inactive, and the embryo quality and rates of fertilization, embryo cleavage, and embryo implantation were comparable in the two groups. Moreover, three healthy babies were born in the tuberculous group.

In conclusion, although the number of cases was rather small, this study suggests that in IVF-ET after sperm retrieval and ICSI, a history of epididymal tuberculosis has no impact on the pregnancy outcome in patients with obstructive azoospermia. Further study based on a larger population is required.

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