Experience of Testicular Sperm Aspiration in Intracytoplasmic Sperm Injection Cycles at Prince Rashed Bin Al-Hassan Hospital

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ABSTRACT

Objective: To determine the reproductive outcome after the application of intracytoplasmic sperm injection treatment with testicular sperms obtained by fine needle aspiration.

Methods: Between August 2005 and December 2007, a total of 55 infertile women (age 21-41 years) underwent intracytoplasmic sperm injection treatment due to male factor. All male partners (age 25-71 years) were azoospermic. Duration of infertility ranged from two to 17 years. The demographic, hormonal and treatment data of both male and female partner along with the reproductive outcome were analyzed.

Results: Of the 55 male partners, 12 (21.2%) men had obstructive azoospermia, and 43 (78.8%) had non-obstructive azoospermia. After intracytoplasmic sperm injection treatment, the fertilization and implantation rates were 68.8% and 21.1%, respectively. Twenty-eight of 55 (50.9%) women achieved pregnancy. Seven women of 28 (25%) had multiple pregnancy. Delivery took place in 23 women (41.8%) of which 16 (69.6%) were full-term and seven (30.4%) were preterm pregnancies (<37 weeks). Abortion occurred in five women (17.8%). No complications after the testicular fine needle aspiration procedure were encountered.

Conclusion: Intracytoplasmic sperm injection treatment is associated with high pregnancy rate after testicular fine needle aspiration of sperms from patients with obstructive and non-obstructive azoospermia. The procedure is simple, noninvasive, repeatable with no/or minimal complications.

Key words: Azoospermia, Fine needle aspiration, Intracutplasmic sperm injection, Pregnancy

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Introduction

The cause of infertility may be in the male partner in as many as 50% of infertile couples.[1] Azoospermia and oligospermia (<20 million sperm per milliliter) mandate detailed evaluation of the male partner both to determine the etiology and to plan therapy. Azoospermia defined as the absence of spermatozoa in ejaculated semen, is the most severe form of male factor infertility and is present in approximately 10% of all investigated infertile couples.[1] The introduction of intracytoplasmic sperm injection (ICSI) not only has improved significantly the prospects of fertility after assisted reproduction by using spermatozoa obtained from the seminal tract, but also has allowed extension of the spectrum of sperm recovery techniques.

Several procedures were performed to retrieve sperms from the infertile men with obstructive and non-obstructive azoospermia. These include: MESA (Microsurgical epididymal sperm aspiration), TESE (Testicular sperm extraction), TESA (Testicular sperm aspiration), PESA (Percutaneous epipidymal

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sperm aspiration) and PSFNA (Percutaneous sperm fine needle aspiration).[2-5]

Until recently, testicular biopsy was considered the method of choice to ascertain the presence and the quality of spermatogenesis. The invasiveness of the procedure limited its use, and therefore the less invasive alternative use of the fine needle aspiration (FNA) to recover testicular sperms was adopted.[6-9] An excellent agreement between testicular biopsy histology and the fine needle aspiration cytology has been reported.[8,10] Recovery of spermatozoa from azoospermic men with obstructive and non-obstructive azoospermia followed by intracytoplasmic sperm injection (ICSI) is a recent advance in the treatment of male infertility. It has been found that ICSI with testicular spermatozoa recovered by FNA yields results comparable to those obtained with spermatozoa recovered by open biopsy in azoospermic patients.[5] In this situation, the pregnancy rate was reported to range between 27% and 50%.[2,8,11,12] We conducted this retrospective analysis to determine the reproductive outcome after the application of ICSI treatment with testicular sperms obtained by FNA.

Methods

A total of 68 couples with male factor infertility were studied retrospectively at Prince Rashed Bin Al-Hassan Hospital between August 2005 and December 2007. All male partners were azoospermic (12 with obstructive and 56 with non-obstructive azoospermia). The mean age of the patients was 36.7 years (range 25-71). The mean duration of infertility was 5.3 years (range 2-17. They were evaluated with seminal fluid analysis (on two occasions), history (medical and surgical), hormonal assay, ultrasonographic examination and clinical examination to identify any testicular pathology (varicocele, epididymal cyst or enlargement). Their hormonal profile included follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), prolactin (PRL) and testosterone (T). Differentiation between obstructive and non-obstructive azoospermia was based on history, testicular volume, hormonal profile, ultrasonography and the FNA findings, as described elsewhere.[10,13] All female partners underwent infertility work-up including history, clinical examination, hormonal profile and hysterosalpingography. The mean age of women was 32.4 years (range 22-41). Of the 68 women, only 55 underwent embryo transfer. The other 13 women did not so because of the failed fertilization and the cell division arrest.

All males underwent testicular FNA before commencing the ICSI cycle to confirm the presence of spermatozoa. Testicular sperm FNA was performed as described elsewhere.[10] Briefly, by using 21- gauge butterfly needles attached to a 20 ml plastic syringe serving as an aspiration device. While holding the testicle between the index finger and thumb, 3-5 different entries were made in each testicle after passing directly through the scrotal skin into the testis, moved up and down at various sites. Following each aspiration, the aspirate was deposited in tubes containing special media with heparin. Then the aspirates were immediately examined under the microscope at X200 and X400 magnification to detect the presence of sperms. The aspirate was collected and transferred to 6ml conical tubes and centrifuged at 300g for 10min.

All female partners did undergo a standardized pituitary down-regulation protocol as described elsewhere.[14] Briefly, the long-luteal pituitary down-regulation using the GnRH analogue triptorelin (Decapeptyl: Ipsen, Paris; France) was commenced on day 21 of the current menstrual cycle. Ovarian stimulation with human menopausal gonadotropins (HMG) was started in all patients on the third day of menses of the second menstrual cycle. Transvaginal ultrasound follow up for follicular growth was commenced on day eight of ovarian stimulation and repeated every 3-4 days thereafter. When at least 3 follicles reached a mean diameter of 17 mm, a single dose of 10.000 IU of hCG was administered. After 36 hours, transvaginal-guided oocyte retrieval was performed under general anesthesia. Fertilization was considered successful after noting the presence of two pronuclei and second polar body 20-24 hours after the ICSI procedure. Embryos were graded as previously described by Coskun *et al*.[15] Good embryos included those with even-sized blastomeres and no obvious fragmentations or even-sized with <10% fragmentations or uneven-sized with no obvious or <10% fragmentations. Fair embryos included those with 10-30% fragmentation. Embryos with >30% fragmentations were considered poor.

The best 1-3 embryos were transferred 72 hours following oocyte retrieval by using Wallace catheter (Marlow Surgical Technology, Willoughby, OH; USA). Pregnancy rate was calculated considering only clinical pregnancies, defined as the visualization of intrauterine gestational sac with positive fetal heart activity by transvaginal

Table I. The demographic, clinical and hormonal data of the 55 azoospermic men

Age (year)	36.7 (25-71)
Duration of infertility (year)	5.3 (2-17)
Type of azoospermia	
- Obstructive	12 (21.2%)
- Nonobstructive	43 (78.8%)
Medical history	
- Drug ingestion	5 (9.1%)
- Diabetes mellitus	2 (3.6%)
- Genitourinary tract infection	3 (5.5%)
Surgical history	
- Varicocelectomy	2 (3.6%)
- Orchidopexy	1 (1.8%)
- Herniorrahpy	2 (3.6%)
Testicular volume (ml)	17.3 (12-24)
Hormonal assay	
- FSH (mIU/ml)	7.8 (4.2-15.7)
- LH (mIU/ml)	7.5 (3.1-12.3)
- TSH (mIU/ml)	3.9 (1.5-4.7)
- PRL (ng/ml)	16.4 (8-27)
- Testosterone (ng/ml)	388.6 (199-769)

FSH= Follicle stimulating hormone LH= Luteinizing hormone TSH= Thyroid stimulating hormone PRL= Prolactin

Table II. Demographic, hormonal and treatment data and reproductive outcome of 55 women who underwent embryo transfer.

No of patients underwent ET	55
Age (year)	32.4 (22-41)
Day 3 FSH (mIU/ml)	5.2 (3.8-13)
No of oocytes retrieved	708
No of oocytes fertilized	487
No of embryos transferred	179
Implantation rate (%)	36/179 (20.1)
Pregnancy rate / ET (%)	28/55 (50.9)
- Multiple PR	7/28 (25)
- Abortion rate	5/28 (17.8)
Delivery of alive baby rate (%)	23/55 (41.8)
- Full-term delivery	16/23 (69.6)
- Premature delivery (<37 weeks)	7/23 (30.4)

ET= Embryo transfer FSH=Follicle stimulating hormone

ultrasound 4-5 weeks after embryo transfer. Early pregnancy loss was defined as pregnancy loss before 12 weeks gestation. Statistical analysis was presented as means, SD and percentages. Hormonal assays including FSH, LH, TSH, PRL and testosterone were performed in all patients by standard radioimmunoassay (RIA) kits (Diagnostic Product Corporation, USA). Informed consent was obtained from all women. The study was approved from the scientific and ethical committee of the Royal Medical Services

Data are expressed as percentages and mean SD. Clinical characteristics were analyzed using Student's t-test. All other analyses were performed using Chi-Square test and Fisher's exact test. A p-value < 0.05 was considered statistically significant.

Results

Table I summarizes the demographic, clinical and hormonal data of the 55 azoospermic men whom female partners underwent embryo transfer. Their age ranged from 25 to 71 years (mean 36.7) with duration of infertility ranging from two to 17 years (mean 5.3). Forty-three men of 55 (78.8%) suffered from nonobstructive azoospermia and 12 (21.2%) were diagnosed to have obstructive azoospermia. Ten patients had positive medical history (5 with drug ingestion, 2 diabetics, and 3 with genitourinary tract infection) and five previous surgical histories (varicocelectomy 3, orchidopexy 1 and herniorrhaphy 2). Their testicular volume ranged between 12 and 24 mls (mean 17.3) and serum hormonal levels (FSH, LH, TSH, PRL, Testosterone) were within normal ranges. The demographic data, treatment characteristics and reproductive outcome are shown in Table II.

The women's age ranged between 22 and 41 years (mean 32.4), and their basal FSH levels ranged between 3.8 and 13 mIU/ml. A total of 708 oocytes were retrieved of which 487 fertilized (68.8%) and 179 embryos were transferred (mean 3.3). The implantation and pregnancy rate were 20.1% and 50.9%, respectively. The pregnancy was achieved in 19 out of 43 (44.2%) women of the nonobstructive azoospermia-group, and in nine out of 12 (75%) of the obstructive group. Of the 28 women who achieved pregnancy, seven had multiple pregnancies (one set triplet and 6 set twins), five aborted, 23 delivered (7 premature deliveries of which 6 were multiple pregnancies and 16 full-term deliveries).

No complications were encountered in the male patients during and after the testicular FNA.

Discussion

Different methods for recovering epididymal or testicular spermatozoa have been described and each has its drawbacks and advantages. Recovery of testicular spermatozoa from infertile men with obstructive and non-obstructive azoospermia for ICSI treatment is a recent advance in the treatment of male infertility. Until recently, testicular biopsy was considered the method of choice to ascertain the presence and the quality of spermatogenesis. Recently, testicular fine needle aspiration was introduced. It is a simple and less invasive procedure compared to microsurgical intervention on the testis. Comparable results to those obtained with spermatozoa recovered by open biopsy in azoospermic patients were reported by performing the testicular sperm fine needle aspiration.[5]

The results of this study show testicular sperm fine needle aspiration from infertile men with both obstructive and non-obstructive azoopsermia and the application of ICSI treatment is associated with a high fertilization, implantation and pregnancy rates (68.8%, 20.1% and 50.9%, respectively). These results are comparable with those reported previously by other investigators.[9,12]

In a prospective study, Mercan *et al.*[12] evaluated 63 infertile men with non-obstructive azoospermia and reported an implantation rate of 20.7%, fertilization and pregnancy rates of 69.5% and 46%, respectively. In their series, 50 out of 63 (79.4%) patients had previous TEFNA before commencing the ICSI treatment. One limitation of their study was the lack of female demographic data, which might explain the slightly lower pregnancy rate observed in our study. In a more recent study, Levine *et al.*[9] performed 56 procedures for men with non-obstructive azoospermia who underwent testicular sperm aspiration for ICSI treatment. An adequate number of viable sperm was obtained in 82% of cases, with fertilization and pregnancy rates of 55% and 44%, respectively. Similar to the previous study, no female demographic data were mentioned.

In contrast to our results, previous experiences showed that testicular spermatozoa obtained by FNA for ICSI treatment were associated with lower rates of fertilization, implantation and pregnancy. Friedler *et al.*[11] in 37 rigorously selected patients with non-obstructive azoopsermia, reported a fertilization rate of 49%, implantation and pregnancy rates of 13% and 29%, respectively. Moreover, Tournaye *et al.*[5] conducted a retrospective controlled study that compared the efficacy of FNA and the open biopsy for testicular sperm recovery in a group of patients with obstructive azoospermia. In the TEFNA group, despite the sufficient sperm recovery in 96% of patients, low rates of fertilization, implantation and pregnancy were reported in their series (65.6%, 7.8% and 27.5%, respectively). In these studies, the low fertilization, implantation and pregnancy rates were attributed to the rigorous preselection and genetic potential of these cases.

The results of this study showed that the pregnancy rate was higher among cases with obstructive azoospermia when compared to non-obstructive azoospermia (75% Vs 44.2%). This finding is in agreement with previous reports.[16,17] It has been reported that spermatozoa from men with non-obstructive azoospermia may have chromatin defects and DNA abnormalities as compared to spermatozoa obtained from men with obstructive azoospermia.[18] This may explain the lower fertilization capacity, implantation and pregnancy rates in cases with non-obstructive azoospermia.

Several complications have been described after testicular sperm retrieval techniques. These include inflammation, hematoma and even testicular devascularization.[19] Fortunately, no such complications were encountered in our patients, since butterfly 21-gauge needle was used to aspirates the sperms with experienced hands.

Conclusion

The results of this study showed that ICSI treatment is associated with high pregnancy rate after testicular FNA aspiration of sperms from patients with obstructive and non-obstructive azoospermia. The application of TEFNA shortly before commencing treatment to confirm the presence of sperms is helpful to increase the chances of success of the ICSI procedure The procedure is simple, noninvasive, repeatable with no/or minimal complications.

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