PERCUTANEOUS EPIDIDYMAL SPERM ASPIRATION AND INTRACYTOPLASMIC SPERM INJECTION IN THE MANAGEMENT OF OBSTRUCTIVE AZOOSPERMIA.

FIRUZA R. PARIKH. ● SUDESH A. KAMAT. ● PERCY J. CHIBBER. ● NANDKISHOR NAIK
SACHIN MANJREKAR. ● SUPARNA NADKARNI ● RAJESH M PARIKH

SUMMARY

Between July 1996 and January 1997, five men with obstructive azoospermia due to congenital absence of the vas deferens underwent Percutaneous Epididymal Sperm Aspiration (PESA) to recover spermatozoa for Intracytoplasmic Sperm Injection (ICSI). A total of 35 oocytes were retrieved (7 oocytes per cycle) of which 25 oocytes were in the Metaphase II stage of maturity and were micromanipulated The fertilisation rate was 80% and the cleavage rate was 100% . An average of 4 embryos were transferred per cycle. The pregnancy rate per cycle was 60%. Intracytoplasmic sperm injection with spermatozoa retrieved by PESA offers a novel, simple, and effective treatment for men with obstructive azoospermia PESA involves minimal scrotal exploration, less morbidity and better patient compliance and is an acceptable alternative to Microsurgical Epididymal Sperm Aspiration (MESA). This paper reports the first pregnancy in India using the combined technique of Percutaneous Epididymal Sperm Aspiration and Intracytoplasmic Sperm Injection in men with obstructive azoospermia.

INTRODUCTION

In recent years, the application of intracytoplasmic sperm injection (ICSI) to clinical practice has revolutionized the

treatment of male factor infertility (Palermo et al., 1992). Recent developments in sperm retrieval, and micromanipulation techniques, have opened new horizons for potentially successful treatment, thereby drastically reducing the need to consider the potential use of donor spermatozoa. Advances in epididymal sperm retrieval techniques like microsurgical epididymal sperm aspiration (MESA) have enabled men with obstructive azoospermia to become biological fathers (Sliber et al., 1995). However, the technique of MESA involves surgical exploration of the testis and epididymal tubules with the attendant risk of hematoma, infection and surgical trauma. The recent introduction of Percutaneous Epididymal Sperm Aspiration (PESA) has offered an acceptable alternative to MESA (Craft et al, 1994; Craft et al, 1995). The technique of PESA is simple, short, requiring minimal scrotal exploration, under local anaesthesia, carries minimal risk of complications, and has better patient compliance. We present our data of 5 cycles where PESA was performed in combination with ICSI for men with obstructive azoospermia and pregnancies were achieved.

MATERIALS AND METHODS

Patients

Between July 1996 and January 1997, Percutaneous epididymal sperm aspiration was performed on 5 azoospermic men who had congenital absence of the vas deferens but normal spermatogenesis. The surgical technique was undertaken using sterile precautions under local anaesthesia. Sperm aspiration was achieved by directing a 21-guage butterfly needle connected to a syringe into either the head of the epididymis or, to the corpus, after immobilization of the testis by holding it stable beneath the thumb and index finger, which were placed immediately above it so that the epididymis was felt as a distinct structure overlying the proximal palmar aspect of the index digit. Suction was applied to the syringe (10 ml) and the needle was withdrawn gradually to a point where segments of fluid from the epididymis were seen entering the tubing of the microeffusion set attached to the butterfuly needle. Steady, gentle negative pressure was maintained until the segments of epididymal fluid ceased to flow and an occlusive artery forcep was then applied across the microtubing before the needle was withdrawn from the skin The aspirate then was washed out of the needle and tubing into a sterile falcon tube containing about 1 ml. of HEPES buffered flushing medium (Medi-Cult, Denmark). The procedure was repeated few more times until sperm suitable for ICSI was retrieved. Excess spermatozoa were frozen for use in later cycles.

Semen Evaluation and Preparation

Computer assisted semen analysis of the epididymal spermatozoa was carried out using the Hamilton Thorn Motility Analyzer (Danvers, MA). A 5ul aliquot to the epididymal fluid

Dept. of Infertility Management & Assisted Reproduction, Jaslok Hospital & Research Centre, Mumbai 400 026.

was placed in the Makler chamber and the sperm concentration and motility parameters were recorded. Smears of epididymal fluid were stained by the Papanicolaou technique and examined under the phase contrast microscope.

Epididymal spermatozoa were prepared with a routine mini-Percoll method. The epididymal sperm fractions were washed in Sperm-prep medium (Medi-cult, Denmark) by centrifuging at 1800 x g for 5 minutes. The pellet was put on a two-layer Percoll gradient (95% to 47.5%) and then was centrifuged at 300 x g for 20 minutes. The 95% Percoll fraction was aspirated and washed again with Sperm-prep medium for 5 minutes at 1800 x g and the pellet was recentrifuged in Sperm-prep medium just before microinjection.

Ovarian stimulation and oocyte preparation for microinjection

The ovarian stimulation protocol involved luteal phase suppresion with GnRh agonist analogues at a dose of 900 ug subcutaneously daily until ovarian suppression was achieved followed by decreasing the dose of GnRh agonist analogues to 500 ug daily with the addition of ovarian stimulation with gonadotropins. Once follicular maturity was reached, oocyte retrieval was scheduled 34-36 hours after hCG injection.

The cells of the cumulus and corona radiata were removed by incubation of the cumulus-corona-oocyte complexes for < 1 minute in HEPES-buffered Earle's medium, containing 80 IU/mL hyaluronidase (type VIII, Sigma Chemical Co, St. Louis, MO) and by aspiration of the cumulus complexes in and out of glass pipettes (having diameters of 250 um and 200 um). Nuclear maturity of the oocytes was judged under an inverted microscope at a x200 magnification.

Intracytoplasmic sperm injection (ICSI)

ICSI was carried out on all morphologically intact oocytes that had extruded the first polar body (Metaphae II oocytes). The injection dish contained microdroplets of 5 uL HEPES buffered culture medium. A 5 uL droplet of 10% polyvinylpyrroldi done (PVP) was placed in the centre of the dish and a 1 2 uL sperm droplet was placed in the 10% PVP droplet. The ICSI procedure was carried out on the heated stage of an inverted microscope (labovert FS) at x 400 magnification. The spermatozoa swimming out into the PVP droplet were immobilized and then picked up with the injection pipette (5-7 um diameter). The oocyte was fixed on the holding pipette (60-80 um outer diameter and 10-20 um inner diameter) with the polar body at the 6 'O' clock position. The injection pipette was pushed through the zona pellucida at the 3 'O' clock position and into the cytoplasm, and the sperm was delivered at the far end of the cytoplasm The oocytes were then transferred into IVF medium (medi Cult) and incubated overnight in the CO2 incubator. The oocytes were

observed for survival and fertilization 16-18 hours after microinjection. Embryo cleavage and quality were evaluated 40-44 hours after ICSI. Intrauterine embryo transfer was performed on day 3, on 6-8 cell embryos.

RESULTS

Between July 1996 to January 1997, five azoospermic men with congenital absence of the vas deferens underwent the procedure of percutaneous epididymal sperm aspiration to recover spermatozoa for intracytoplasmic sperm injection. The mean age of the male patients was 37 years (range 33 to 41 years(, and of the female partners was 31 years (range 28 to 37 years). Computer assisted semen analysis (CASA) of the percutaneously aspirated epididymal spermatozoa was carried out using the Hamilton Thorn Motility Analyser. The total count, motility, progressive motility, path velocity, type A motility and other motion parameters of the epididymal spermatozoa such as curvilinear velocity, straightline velocity, linearity, straightness, amplitude of lateral head displacement and beatcross frequency is shown in Table I. The mean percentage of the normal epididymal spermatozoal forms and the different morphological

TABLE I
SPERM COUNT AND MOTILITY PARAMETERS OF PERCUTANEOUSLY ASPIRATED EPIDIDYMAL SPERMATOZOA ANALYSED USING THE HAMILTON THORN MOTILITY ANALYSER

Parameters (n=5)	Mean ± S.D.
Total count (million / ml)	36 3 ± 15.9
Motility (%)	3.0 ± 2.60
Path velocity (microns / sec)	20.4 ± 22.9
Type A motility (%)	0.6 ± 0.90
Curvilinear velocity	21.4 ± 23.1
Straight-line velocity	15.6 ± 19.4
Linearity	40.8 ± 38.6
Straightness	44.2 ± 41.0
Amplitude of lateral head displacement	0.2 ± 0.30
Beat cross frequency	0.8 ± 1.80

TABLE II
SPERM MORPHOLOGICAL CHARACTERISTICS OF EPIDIDYMAL SPERMATOZOA IN MEN UNDERGOING THE PESA PROCEDURE

Parameters (n=5)	Mean ± S.D.
Normal forms	28.0 ± 16.2
Acrosome deficient	11.6 ± 2.90
Small head	16.8 ± 9.20
Large head	10.8 ± 7.00
Round head	6.8 ± 4.40
Amorphous forms	2.8 ± 3.90
Pyriform	3.2 ± 2.90
Post acrosomal elongation	6.8 ± 2.70
Bent neck	4.4 ± 0.90
Thick midpiece	0.8 ± 1.10
Cytoplasmic droplet	0.8 ± 1.80
Coiled tail	6 0 ± 3.20

abnormalities observed in epididymal spermatozoa is shown in Table II.

A total of 35 oocytes were retrieved (7.0 oocytes per cycle) out of which 25 oocytes (92%) were in the metaphase II stage (extrusion of the first polar body) and were subjected to the Intracytoplasmic sperm injection procedure. The remaining 10 oocytes (8%) were either in the metaphase I or germinal vesicle stage and hence were not micromanipulated. Of the 25 micromanipulated oocytes, 20 oocytes showed the presence of two pronuclei 16 to 18 hours after the ICSI procedure giving a fertilization rate of 80%. All the 20 fertilized oocytes cleaved into embryos giving an embryo cleavage rate of 100%.

Embryo transfer was carried out in all the 5 cycles. On an average 4.0 embryos were transferred per cycle. Three pregnancies were achieved which were detected by increasing serum HCG concentrations 14 days after embryo replacement Clinical pregnancies were confirmed by observing the gestational sac by echographic screening at 7 weeks of pregnancy. All the three pregnancies are currently ongoing uneventfully. The pregnancy rate per cycle was 60% (Table III).

TABLE III
RESULTS OF INTRACYTOPLASMIC SPERM INJECTION USING PERCUTANEOUSLY ASPIRATED EPIDIDYMAL SPERMATOZOA

Parameters (n=5)	PESA
No. of cycles	5
Oocytes retrieved	35
Oocytes / cycle	7
Oocytes micromanipulated (Metaphase II)	25
Metaphase I/Germinal vesicle oocytes	10
Fertilisation rate (%)	80
Cleavage rate (%)	100
Embryos transferred / cycle	4
Clinical pregnancies	3
Pregnancy rate / cycle	60 (3/5)

DISCUSSION

It is clear from our results that high fertilization and cleavage rates can be obtained with intracytoplasmic sperm injection using percutaneously aspirated epididymal spermatozoa. Nonmotile spermatozoa obtained from the epididymis are also capable of achieving high fertilization and cleavage rates when the correct technique is applied. In our study, the fertilization and cleavage rates of 80% and 100% obtained using percutaneously aspirated epididymal spermatozoa are comparable to those obtained using either ejaculated spermatozoa or spermatozoa obtained by MESA. Using PESA, it was possible to recover enough spermatozoa for microinjection on all occasions. Although a high sperm count was obtained following PESA, the motility of the spermatozoa was low (Table I). In 4 patients, the epididymal spermatozoa had sluggish motility whereas 1 patient had completely nonmotile spermatozoa The progressive motility, path velocity and the type A motility were also lower in epi didymal spermatozoa. Nevertheless high fertilisation and cleavage rates could be obtained

All the 5 patients in our series had embryo transfers (4.0 embryos per cycle). Three pregnancies were determined after 14 days of embryo transfer with serum HCG and

were confirmed clinically by observing the gestational sac after 7 weeks. A pregnancy rate of 60% per cycle was obtained using PESA in combination with ICSI Our results suggest that PESA in combination with ICSI is the treatment of choice for azoospermic men with congenital absence of vas deferens or other obstructive azoospermic conditions.

PESA is a simple, effective and less traumatic technique for retrieval of epididymal spermatoza compared with an open microsurgical operation like MESA Microepididymal sperm aspiration with scrotal exploration under general anaesthesia has been used to retrieve spermatozoa for use in assisted fertilisation cycles like ICSI with encouraging results (Silber et al, 1995). Nevertheless, the technique involves a certain amount of trauma and postoperative morbidity pain, hematoma formation, and infection may occur, and any subsequent surgery may be more complex because of post operative adhesions and fibrosis The high fertilisation, cleavage and pregnancy rates obtained in our study combined with better patient compliance clearly suggest that PESA is the preferred method of sperm retrieval over the formal open MESA procedures. The presence of contaminating red blood cells in the open microepididymal aspiration which can affect the epididymal spermatozoal preparation can be avoided using the PESA technique. The high patient acceptability and limited cost and repeat ability of the procedure, without the prospect of having associated fibrosis and other complications after open surgery, are advantages over the MESA procedure

CONCLUSION

Percutaneous epididymal sperm aspiration can be used successfully to retrieve spermatozoa for intracytoplasmic sperm injection, in men with obstructive azoospermia By virtue of is ease and simplicity, minimal scrotal exploration, less morbidity, minimal risk of complications, better patient compliance along and retrieval of sufficient spermatozoa for intracytoplasmic sperm injection PESA scores over the open microepididymal sperm aspiration procedure. This paper reports the first pregnancy in India by the combined technique of Percutaneous Epididymal Sperm Aspiration and Intracytoplasmic Sperm Injection in men with obstructive azoospermia.

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