The Outcome of Sperm Retrieval and Intracytoplasmic Sperm Injection for Obstructive Azoospermia

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Abstract

Objectives : To study the outcome of sperm retrieval and intracytoplasmic sperm injection (ICSI) from obstructive azoospermic men.

Method : Overall, 50 sperm retrieval procedures were performed in 45 obstructive azoospermic men, followed by 57 ICSI procedures with fresh epididymal spermatozoa (n=40), fresh testicular spermatozoa (n=4) or frozen-thawed epididymal spermatozoa (n=13).

Results : Sperm retrieval was accomplished *via* percutaneous epididymal sperm aspiration (PESA) in 42 cases, testicular sperm aspiration (TESA) in 1 case and testicular sperm extraction (TESE) in 2 cases. TESA and TESE were only applied when PESA failed to produce enough spermatozoa for simultaneous ICSI. PESA was successful in 92 per cent of cases. Fertilization rate after ICSI was 79.6 per cent of the metaphase II oocytes. Seventy one embryo transfers were performed using both fresh and frozen thawed embryos resulting in clinical pregnancy in 39.4 per cent. Ongoing pregnancy was achieved in 35.2 per cent.

Conclusion : ICSI has been shown to give a high fertilization and pregnancy rate with epididymal and testicular spermatozoa retrieved from obstructive azoospermic men. PESA is a noninvasive and simple technique for retrieving spermatozoa from obstructive azoospermic men. Therefore, it is suitable as the primary sperm recovery technique in patients with obstructive azoospermia.

Key word : Obstructive Azoospermia, Sperm Retrieval, Intracytoplasmic Sperm Injection, ICSI PESA, TESA, TESE

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Azoospermia, the most severe form of male infertility, is caused by obstructions in the genital tract or by testicular failure. In obstructive azoospermia, the process of spermatogenesis is not impaired. The most common causes of obstruction are epididymovasal occlusions caused by infections, congenital bilateral absence of the vas deferens (CBAVD) and ligation or resection of the vas deferens during hernia repair, prostatic and vesicle surgery or vasectomy. These patients are characterized by normal testis volume and normal concentration of follicular stimulating hormone (FSH). Several microsurgical techniques are available for surgical correction of these obstructions. These surgical treatments can result in spontaneous pregnancies in 27-56 per cent of cases[1,2] The results are determined by several factors, including site and duration of the obstruction, epididymal function, recurrent genital tract infections and sperm antibodies. In cases of poor sperm quality after the operation, assisted reproductive techniques (ART) have been applied successfully.

Recently, intracytoplasmic sperm injection (ICSI) has become available for treatment of severe male factor infertility[3]. This technique has also been applied to treat azoospermia if viable spermatozoa could be retrieved from the epididymis or the testis, giving results similar to those using ejaculated spermatozoa from oligozoospermia[4]. With the new advances in assisted reproductive techniques, patients with poor surgical prognosis such as failed vasectomy reversal and long period vasectomy can now be referred for treatment with combination of sperm retrieval and ICSI[5].

Different methods for recovering epididymal or testicular spermatozoa have been introduced, such as, microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA), testicular sperm extraction (TESE), testicular sperm aspiration (TESA) or fine needle aspiration (FNA). Each sperm retrieval technique has its drawbacks and advantages[6]. Combination of sperm retrieval and ICSI has been shown to give a high fertilization and pregnancy rate[7,8]. However, there has been no data on the outcome of sperm retrieval and intracytoplasmic sperm injection for obstructive azoospermia in Thailand. Therefore, we present the results of 57 ICSI procedures for obstructive azoospermia in combination with percutaneous epididymal sperm aspiration (PESA), testicular sperm aspiration (TESA) or testicular sperm extraction (TESE).

MATERIAL AND METHOD

Study population

From January 1997 to December 2000, a total of 45 couples underwent surgical sperm retrieval followed by ICSI because of obstructive azoospermia. The causes of obstructive azoospermia were failed vasectomy reversal (35.6%, n=16), long-period vasectomy (24.4%, n=11), congenital bilateral absence of vas deferens (CBAVD) (2.2%, n=1), radical surgery for genitourinary tract malignancy (2.2%, n=1) and unknown etiology (35.6%, n=16).

At the initial consultation, both partners were fully assessed. The patients underwent history taking and physical examination of their genitalia. All patients underwent at least two semen analyses using centrifugation at 1,800 g for 10 minutes and with failure to recover spermatozoa in our laboratory prior to their surgical sperm retrieval. Following clinical evaluation of patients, the treatment strategy including further investigation for obstructive azoospermia and corrective surgery was discussed with the couples. The alternative option including sperm retrieval and intracytoplasmic sperm injection (ICSI) was also offered. When the latter option was chosen, informed consent was obtained after counseling. In CBAVD couples, cystic fibrosis carrier status was evaluated in both partners[9].

Ovarian stimulation

All female partners received ovarian stimulation treatment by a combination of gonadotrophin-releasing hormone agonist (GnRHa) and human menopausal gonadotrophins (hMG) or recombinant follicle stimulating hormone (rFSH). When the patient had at least three follicles with a diameter of 18 mm, ovulation was induced with 10,000 IU human chorionic gonadotrophin (hCG). All patients had a transvaginal ultrasound-guided ovum aspiration ~36 h after hCG injection under intravenous anesthesia. A comprehensive description of ovarian stimulation can be found in a previous report[4].

Percutaneous sperm aspiration (PESA)

A comprehensive description of the procedure for PESA can be found in a previous report [4]. Briefly, PESA was carried out in the operating room after oocyte retrieval using local anesthesia with Bupivacaine injected at the skin and underneath the epididymal caput and/or intravenous sedation anesthesia with Propofol. The epididymal caput was identified and held firmly between thumb and

index finger. A small needle (26 gauge) was connected to a 1 ml disposable tuberculin syringe. The proximal part of the epididymal caput was punctured. Suction was applied to the syringe and the needle was withdrawn gradually to the point where segments of fluid from the epididymis were seen entering the syringe. The aspirate was then flushed out of the syringe into a sterile petri dish using HEPES-buffered Ham's F10. This procedure can be performed as many times as necessary on that same side or on the other side until sufficient sperms are recovered. Testicular sperm retrieval was prepared as a back up procedure in case PESA failed.

Epididymal sperm processing

The epididymal sample was washed with HEPES and centrifuged for 7 minutes at 400 x g. The pellet was resuspended in 0.2 ml media and incubated until the time of injection.

Testicular sperm aspiration (TESA)

A comprehensive description of the procedure for TESA can be found in a previous report (10). Briefly, testicular sperm aspiration was done using a 21-gauge needle attached to a 10 ml plastic syringe serving as an aspiration device. The needle was passed directly through the scrotal skin into the testis. Once the needle was in the testicular tissue, strong negative pressure was exerted. The needle was then slowly withdrawn from the testis through the scrotal skin and a core of attached testicular tissue was cut off, on withdrawal from the skin surface. The procedure was undertaken at different sites on the skin.

Testicular sperm extraction (TESE)

A comprehensive description of the procedure for TESE can be found in a previous report (4). Briefly, open excision testicular biopsies were taken under general and local anesthesia as described above. The testicular tissue was placed in a Petri dish containing HEPES-buffered Ham's F10 medium supplemented with 10 percent patient serum and then was teased apart with two needles. Under an inverted microscope (x 400 magnification) the minced tissue was then checked for the presence of sperms. If no sperms were observed, another biopsy specimens was taken. Surgery was stopped when sperms were found.

Testicular biopsy processing

A comprehensive description of the procedure for testicular biopsy processing can be found in a previous report(4). Briefly, the morselized tissues were incubated in a Petri dish containing HEPES-buffered Ham's F 10 medium supplemented with 10 percent patient serum for approximately 2 hours. The contents were then mixed and allowed to settle for 1 minute and the deposited pieces of testicular tissue were removed. Two or three microdroplets were used from this suspension to be put near the PVP microdroplet in the injection dish. Using the injection micropipette, a search was done for a motile sperm, which was aspirated from among Sertoli cells, red blood cells and debris and transferred to the polyvinylpyrrolidone (PVP) droplet and used for microinjection later.

Epididymal sperm cryopreservation

Following ICSI using fresh epididymal spermatozoa or diagnostic PESA, the remaining epididymal spermatozoa were cryopreserved using a freezing protocol with Test Yolk Buffer freezing medium (Irvine Scientific). The spermatozoa-containing extract was diluted dropwise 1:1 with the freezing medium and sealed in freezing straws. A simple two-step cryopreservation protocol was used. The straws were dropped in a nitrogen vapor chamber stabilized at -80°C for 20 min (cooling rate of -10°C/min) prior to immersion into liquid nitrogen for sperm storage at -196°C. Following removal of the straws from the liquid nitrogen, rapid thawing occurred at room temperature. Then the frozen-thawed sperm mixture containing cryoprotectant was diluted with insemination medium, centrifuged at 300 g for 7 min and the pellet was resuspended in 0.2 ml media and incubated until the time of injection.

Oocyte preparation, oocyte handling and intracytoplasmic sperm injection (ICSI)

After recovery, oocytes were denuded from the surrounding granulosa cells and metaphase-II oocytes were microinjected. A comprehensive description of the procedure for oocyte preparation, oocyte handling and intracytoplasmic sperm injection procedure can be found in previous report(4). Injected oocytes were cultured under oil.

Assessment of fertilization and pregnancy outcome

At 16-18 h after microinjection, the oocytes were microscopically examined under the inverted microscope (x 200 or x 400 magnification). Normal fertilization was defined by the presence of two pronuclei and a second polar body. After an additional 24-30 h of *in vitro* culture, embryos were examined under the microscope to assess their developmental stage and quality on the basis of their morphological aspects. Depending on the day of embryo transfer, embryo cleavage was judged 3, 4 and 5 days after the ICSI procedure. A maximum of three cleaving embryos were then transferred into the uterine cavity. Supernumerary embryos were cryopreserved at the two pronuclei stage using dimethylsulphoxide as a cryoprotectant(11). The luteal phase was supplemented daily with natural progesterone pessaries (Cyclogest® 400 mg, Hoechst, Hounslow, UK). Pregnancy was confirmed 14 days after embryo transfer by serum β-hCG. A clinical pregnancy was defined as the pre-sence of at least one gestational sac with a fetal heart beat by transvaginal sonography performed 3-4 weeks after detection of β-hCG. Implantation rate was defined as the ratio of the number of gestational sacs containing a fetus with heart activity and the number of transferred embryos. Clinical pregnancies reaching 20 weeks of gestation were considered ongoing.

Statistical analysis

All results were expressed as range and percentage. All statistical analyses were performed with the SPSS for Windows v. 10.05 (SPSS Inc., Chicago, IL) on an IBM compatible microcomputer.

RESULTS

A total of 45 obstructive azoospermic patients underwent 57 consecutive ICSI cycles. The spermatozoa were retrieved from the epididymis by percutaneous sperm aspiration (PESA) in 53 cycles (fresh epididymal sperm, n=40; frozen-thawed epididymal sperm, n=13). TESA and TESE were done for 1 patient and 2 patients, respectively. A total of 46 PESA, 1 TESA and 3 TESE procedures were performed. Six PESA procedures were carried out for diagnostic purposes with cryopreservation of the epididymal spermatozoa and 40 PESA procedures were carried out on the day of ovum retrieval for ICSI. PESA was the initial sperm retrieval

Table 1. Results of 57 ICSI cycles with fresh epididymal spermatozoa obtained from PESA, fresh testicular spermatozoa obtained by TESA and TESE and frozen thawed epididymal spermatozoa after initial PESA procedure.

Sperm sources	ICSI (cycles)	No. of oocytes	No. of MII oocytes	Fertilization	%	No. of embryo transfer	Pregnancy	%	Ongoing pregnancy	%
Fresh epididymal sperm from PESA	40	486 (3-33)	389 (2-25)	308/389	79.2	49	20/49	40.8	18/49	36.7
Fresh testicular sperm from TESA or TESE	4	96 (16-27)	81 (12-24)	64/81	79.0	7	2/7	28.6	2/7	28.6
Frozen thawed epididymal sperm	13	139 (2-22)	105 (2-16)	86/105	81.9	15	6/15	40	5/15	33.3
Total	57	721	575	458/575	79.6	71	28/71	39.4	25/71	35.2

ICSI = intracytoplasmic sperm injection, PESA = percutaneous epididymal sperm aspiration, TESA = testicular sperm aspiration, TESE = testicular sperm extraction

procedure. Testicular sperm retrieval (TESA and TESE) was prepared as a back up procedure in case PESA failed. PESA was successful in 46/50 (92%) procedures. There were 3 cases of failed epididymal sperm retrieval because of severe fibrosis after vasectomy reversal for 2 times and congenital absence of vas deferens, TESA or TESE was performed in the same procedure, and viable spermatozoa were found in 4/4 (100%) procedures.

Table 1 summarizes the results of the 57 treatment cycles. Oocyte retrieval resulted in a total of 721 oocytes, of which 575/721 (79.8%) had extruded the first polar body (metaphase II oocytes). Fertilization occurred in 458/575 (79.6%) of the metaphase II oocytes after the ICSI procedure. The fertilization rate using epididymal spermatozoa and testicular spermatozoa was similar. In 14 cycles, supernumerary embryos of good morphological quality were cryopreserved and frozen embryos were thawed and transferred later. Both fresh and frozen thawed embryo transfer resulted in a clinical pregnancy in 28/71 (39.4%). Fresh and frozen thawed epididymal spermatozoa produced 36.7 per cent and 33.3 per cent ongoing pregnancies, respectively and fresh testicular spermatozoa from TESA and TESE produced 28.6 per cent ongoing pregnancies. Cumulative pregnancy rates were 25/45 couples (56%).

DISCUSSION

We performed the ICSI procedure with percutaneously retrieved spermatozoa from the epididymis (PESA) or the testis (TESA or TESE) in 57 treatment cycles. PESA was introduced as a minimal invasive technique of sperm retrieval, and was successful in 92 per cent of cases. Several reports have shown PESA to be successful in the majority of cases of obstructive azoospermia[12-14]. The advantages of PESA are: minimal discomfort for the patient, low complication rate compared to open surgery, repeatability and the production of clear aspirated fluid with usually minimal blood contamination and less debris. PESA does not require microsurgical skills, is easy to learn and can be performed as an outpatient clinic procedure. In a recent report, PESA was shown to be as effective as MESA with comparable pregnancy rates[15].

Testicular biopsy may be an alternative to PESA as a source of spermatozoa. Successful harvesting of spermatozoa has been reported with TESE using both the open biopsy technique and more recently the testicular sperm aspiration (TESA), although needle aspirations were less effective compared to open biopsies[16]. Late complications have been described after testicular sperm retrieval techniques, including inflammation, haematoma and even devascularization of the testis[17]. Therefore, we believe that PESA should be the first technique performed in cases of obstructive azoospermia. In this study, PESA was unsuccessful in 3 cases and a subsequent TESA or TESE procedure was performed, which gave viable spermatozoa.

In conclusion, ICSI has been shown to give a high fertilization rate with epididymal and testicular spermatozoa obtained by PESA, TESA and TESE techniques. We combined ICSI with sperm retrieval (PESA, TESA and TESE) in obstructive azoospermic men, which resulted in an ongoing pregnancy rate of 35.2 per cent per treatment cycle which is similar to an ongoing pregnancy rate of 31-34 per cent per treatment using the combination of ICSI with the MESA procedure[18]. Therefore, PESA has been proposed as the primary sperm recovery technique in patients with obstructive azoospermia.

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ผลการเก็บอสุจิและการฉีดอสุจิเข้าในไข่ในผู้ป่วยที่ตรวจไม่พบอสุจิในน้ำอสุจิจากการอุดตันของทางเดินอสุจิ

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วัตถุประสงค์ : ศึกษาผลของการเก็บอสุจิและการฉีดอสุจิเข้าในไข่ (intracytoplasmic sperm injection : ICSI) ในผู้ชายที่ตรวจไม่พบอสุจิในน้ำอสุจิจากการอุดตันของทางเดินอสุจิ

วิธีการ : ได้ทำการเก็บอสุจิจำนวน 50 ครั้ง จากผู้ชายที่ตรวจไม่พบอสุจิในน้ำอสุจิจากการอุดตันของทางเดินอสุจิ มาทำการฉีดเข้าในไข่ จำนวน 57 รอบการรักษา โดยใช้อสุจิสดจากท่อนำอสุจิ (epididymis) (จำนวน 40 รอบการรักษา), อสุจิสดจากอัณฑะ (จำนวน 4 รอบการรักษา) หรือ อสุจิแช่แข็งจากท่อนำอสุจิ (จำนวน 13 รอบการรักษา)

ผลการรักษา : การเก็บอสุจิทำโดยวิธีการดูดอสุจิจากท่อนำอสุจิผ่านทางผิวหนัง (percutaneous epididymal sperm aspiration : PESA) จำนวน 42 ราย, การดูดอสุจิจากอัณฑะ (testicular sperm aspiration : TESA) จำนวน 1 ราย และการผ่าตัดแยกอสุจิจากอัณฑะ (testicular sperm extraction : TESE) จำนวน 2 ราย จะทำเก็บอสุจิจากลูกอัณฑะเฉพาะในรายที่ไม่สามารถเก็บอสุจิจากท่อนำอสุจิได้เพียงพอสำหรับฉีดเข้าในไข่ในรอบการรักษานั้นเท่านั้น การดูดอสุจิจากท่อนำอสุจิผ่านทางผิวหนังประสบความสำเร็จ 92% อัตราการปฏิสนธิภายหลังฉีดอสุจิเข้าในไข่เป็น 79.6% ของไข่ในระยะเมตาเฟส II ทำการย้ายฝากทั้งตัวอ่อนสดและตัวอ่อนแช่แข็งทั้งหมด 71 รอบ ได้ผลการตั้งครรภ์ 39.4% มีการตั้งครรภ์ต่อ 35.2%

สรุป : การฉีดอสุจิที่ได้จากการเก็บอสุจิจากท่อนำอสุจิและอัณฑะในรายที่ตรวจไม่พบอสุจิในน้ำอสุจิจากการอุดตันของทางเดินอสุจิได้อัตราการปฏิสนธิและการตั้งครรภ์สูง การเก็บอสุจิจากท่อนำอสุจิผ่านทางผิวหนังเป็นวิธีที่ไม่อันตรายและสะดวกในการเก็บอสุจิจากผู้ป่วยที่ตรวจไม่พบอสุจิในน้ำอสุจิจากการอุดตันของทางเดินอสุจิ ดังนั้นการเก็บอสุจิจากท่อนำอสุจิผ่านทางผิวหนังจึงเป็นวิธีที่เหมาะสมลำดับแรกในการเก็บอสุจิในรายที่มีการอุดตันของทางเดินอสุจิ

คำสำคัญ : การตรวจไม่พบอสุจิจากการอุดตันของทางเดินอสุจิ, การเก็บอสุจิ, การฉีดอสุจิเข้าในไข่

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