Successful Pregnancy in a Case of Azoospermia from Congenital Bilateral Absence of Vas Deferens (CBAVD) Using Percutaneous Epididymal Sperm Aspiration (PESA) and Intracytoplasmic Sperm Injection (ICSI)

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Abstract

In men with obstructive azoospermia, bypass surgery would obviously be the most acceptable form of treatment as it gives the couple an opportunity to conceive naturally. However, when this has failed to restore patency or when surgery is not feasible (congenital absence of vas), fertility treatment using spermatozoa aspirated from the epididymis should be considered. Percutaneous epididymal sperm aspiration (PESA) is more acceptable to patients than micro epididymal sperm aspiration (MESA) because it eliminates the requirement for a general anaesthetic, post-operative pain, and the risk of haematoma formation, thus allowing a rapid return to normal activity of the husband.

To our knowledge, this is the first reported case in Thailand where a pregnancy resulted in a couple whose infertility was due to azoospermia from the congenital absence of vas deferens. Spermatozoa collected through PESA were used in ICSI to achieve fertilization. The PESA technique, due to its simplicity is the choice of treatment for obstructive azoospermia.

Recent advances in assisted reproductive technologies (ART) and micro-manipulation of human gametes, in particular intracytoplasmic sperm injection (ICSI)[1] have made it possible for men with severe male factor subfertility to become biological fathers. Azoospermia is found in 2 per cent of infertile men, of which obstructive azoospermia is diagnosed in about half of the cases[2]. However, azoospermia may make up to 20 per cent of the cases referred to an *in-vitro* fertilization (IVF) centre[3]. Obstructive azoospermia may be due to congenital bilateral absence of the vas deferens (CBAVD), post-inflammatory obstruction, or post vasectomy. The continuity of the male genital tract may be restored with microsurgical techniques when the obstruction lies in the vas deferens. However, attempts at bypassing blocks in the region of the epididymis have not been very successful. Patients with obstructive azoospermia due to CBAVD, or those who had suffered failure of

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reconstructive surgery, have been historically been considered hopelessly infertile.

More recently, assisted conception techniques have been used to achieve pregnancies with the spermatozoa being retrieved by epididymal micro-aspiration. Temple-Smith et al[4] reported the first such pregnancy following *in-vitro* fertilization (IVF) in 1985. The limitation of micro-epididymal sperm aspiration (MESA) is that an open surgical procedure under general anaesthesia is required which, given the poor success rates with assisted reproduction treatments, the procedure may need to be repeated. This is unacceptable to many couples. One solution is to create an artificial spermatocoele, but Belker et al[5] reported a low pregnancy rate with aspirated spermatozoa. Recently a new therapy involving surgical retrieval of spermatozoa combined with assisted fertilization using ICSI (intracytoplasmic sperm injection) has improved the results significantly[6,7], and high fertilization and pregnancy rates have been reported following percutaneous epididymal sperm aspiration (PESA)[8-10].

We describe here the technique of PESA from the caput epididymis from a case of azoospermia and the treatment of aspirated epididymal fluid to yield viable spermatozoa. The spermatozoa obtained were used for assisted reproduction treatment by ICSI and a successful pregnancy was achieved for this case of CBAVD which is the first such pregnancy reported in Thailand.

CASE REPORT

A husband aged 39 and his wife aged 35 with a 5 year history of infertility from azoospermia were referred to Chulalongkorn Hospital after two unsuccessful attempts for bypass surgery by urologists. The diagnosis of congenital bilateral absence of vas deferens (CBAVD) was given after the operation and testicular biopsy showed hypospermatogenesis. The serum levels of FSH and Testosterone of the husband were normal. The initial infertility investigations for the wife including general physical checkup, pelvic examination, transvaginal ultrasound, and basal hormonal level[11], were normal.

After counselling about the possibilty of cystic fibrosis (CF) carrier and karyotyping to exclude chromosomal abnormality, the couple agreed to undergo assisted reproduction combined with PESA for the treatment of infertility.

MATERIAL AND METHOD

Sperm retrieval and preparation

PESA was carried out with a combination of local anaesthesia (using 1% lignocaine infiltrated circumferentially around the anterior half of the root of the scrotum) and intravenous sedation, using Midazolam (Dormicum, Roche, Germany) 5mg and Pethidine 75 mg. An assistant stabilized the testis and the distended caput epididymis was held between the surgeon's thumb and forefinger. For aspiration of sperm, a standard 25 gauge needle attached to a 1 ml tuberculin syringe was used. The needle was inserted into the caput epididymis and suction was applied while the needle was withdrawn slowly to a point where epididymal fluid could be seen to enter the syringe. Steady and gently, negative pressure was maintained in the syringe until the flow of epididymal aspirate stopped. At this time the needle was withdrawn, taking care not to release the negative pressure in order to reduce blood contamination. The aspirate was then dispensed onto a cavity dish containing human tubal fluid (HTF) medium with 10 per cent synthetic serum substitute. The sperm numbers recovered were assessed and the sperm aspirate mixed with HTF medium was centrifuged at 400 g for 10 min. The sperm pellet was overlaid with fresh HTF medium and the motile sperm recovered in the supernatant after incubation for one to one and a half hours.

Ovarian stimulation

Ovarian stimulation was performed as previously described[11] using a GnRH agonist 600 μg (Suprefact nasal spray, Hoechst AG, Germany) on a pituitary down-regulation protocol in combination with hMG 150 IU (Pergonal, Sereno, U.S.A.), and FSH 150 IU (Metrodin, Sereno, U.S.A.). Monitoring was performed using serum estradiol and transvaginal ultrasound evaluation of follicular development. Human chorionic gonadotrophin 10,000 unit (Profasi, Sereno, U.S.A.) was administered when three follicles were more than 18 mm. Transvaginal ultrasonically guided oocyte retrieval was performed 35 hours following the trigger injection.

Microinjection Procedure

Oocytes had the cumulus and coronal cells removed by brief exposure to 80 IU/ml hyaluronidase followed by aspiration through a fine bore

pipette. Microinjection was performed using an inverted microscope (Nikon Diaphot, Tokyo, Japan) fitted with hydraulic course control and joystick micromanipulators (ML188 and IM6; Narishige, Tokyo, Japan) and heated stage. The sperm suspension was scanned and a single motile sperm was aspirated into the injection pipette (outer diameter 7 to 9 μm) and transfered to the clean HEPES-buffered HTF drop containing an oocyte. The sperm was release into the fresh medium and then aspirated in and out of the injection pipette a number of times to dislodge any debris attached to the sperm.

Before injection, the motile sperm was immobilized by crushing the tail between the tip of the injection pipette and the petridish. Polyvinyl pyrolidone was not used for immobilization of sperm. The immobilized sperm was aspirated and brought to the tip of the injection pipette. The injection pipette was then pushed through the zona pellucida towards the center of a mature oocyte held with the polar body directed at either 12 or 6 o'clock position by gentle suction to a round-ended holding pipette (inner diameter 10 to 20 μm). Before releasing the sperm, a portion of the oocyte membrane was aspirated into the injection pipette until the ooplasm flowed freely to confirm that the oolemma had been breached. Then, the sperm was pushed out of the injection pipette using a slight positive pressure and the injection pipette was removed gently. Oocytes were incubated and assessed for the presence of pronuclei after 18 hours.

RESULTS

The first aspirate from the right side diluted with HTF medium measured 0.2 ml and contained a few motile sperm, grade 1 progression, contaminated with red blood cell. Three aspirates of 0.3, 0.2 and 0.2 ml (after dilution with the HTF culture medium used to flush the needle) were obtained from the left side. The spermatozoa was allowed to swim-up and finally 0.3 $x10^6$/ml with 30 per cent motility. Forward progression was graded 2.

Ten oocytes were retrieved and were at metaphase II stage. Only four oocytes fertilized after ICSI. All embryos were transferred to the uterine cavity at 4-8 cell-stage approximately 72 hours of incubation.

A blood test performed after 16 days of embryo transfer gave a ßhCG level of 136 mIU/ml and an transvaginal ultrasound scan at 7 weeks of gestation confirmed a singleton pregnancy. At present the patient is at 28 weeks of gestation.

Fig. 1. Percutaneous sperm aspiration done under intravenous sedation and local anesthesia. A 25-g needle attached to 1 ml syringe filled with culture media was used for aspiration of sperm.

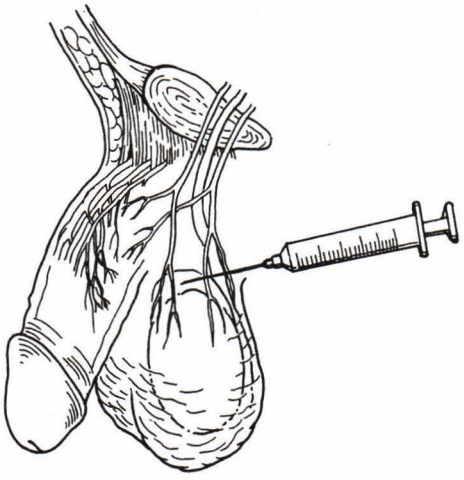

Fig. 2. Diagram shows the needle aspiration at caput epididymis.

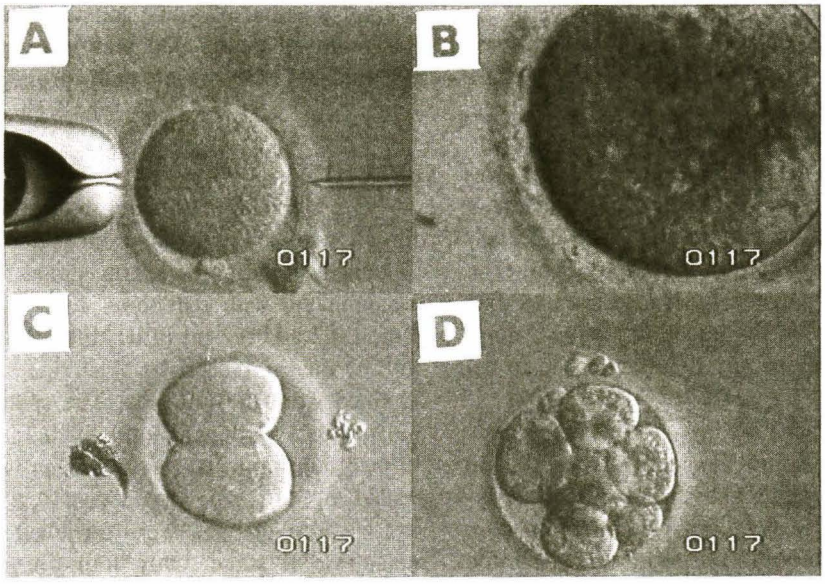

Fig. 3. Sequences show step of ICSI with epididymal sperm and the result.
A) Single spermatozoa was injected into the oocyte
B) Fertilization took place, showing 2 PN
C) Two-cell stage embryo developed
D) Eight-cell stage embryo ready for transfer.

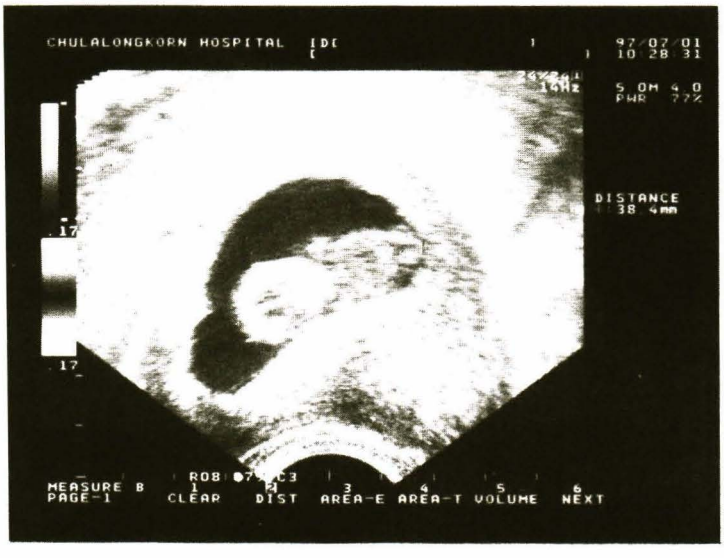

Fig. 4. Transvaginal ultrasound shows single intrauterine ongoing pregnancy. The fetal heart beat has been demonstrated.

DISCUSSION

Obstructive azoospermia in whom bypass surgical procedures have either failed or not feasible as in this case are suitable candidates for epididymal sperm retrieval. Two surgical techniques have been described in the medical literature. The first is known by the acronym MESA for micro-epididymal sperm aspiration[4]. The procedure involves surgical exploration of the testis and epididymal tubules which usually involves the risk of haematoma formation, infection and surgical trauma to the organs. The second most recent technique is known by the acronym PESA for percutaneous epididymal sperm aspiration[9,10]. This technique is simple, and requires minimal surgical equipment. Furthermore, it requires shorter training periods and above all carries minimal risk of complications.

The spermatozoa should be obtained from the distal most patent epididymal tubules whenever possible. Mathieu et al[8] have shown that under physiological conditions, spermatozoa from the caput epididymis were immotile or non-progressive, and progressive motility appeared in the corpus epididymis. However, Silber et al[7] have reported two pregnancies from motile spermatozoa retrieved from the caput epididymis in men with congenital absence of the vas. When PESA is attempted the caput is the most accessible region and can be felt as a tense, globular structure above the superior pole of the testis. It can be held firmly between the operator's finger and thumb and causes the patient only minimal discomfort. The corpus may also be aspirated but this requires different positioning of the surgeon's fingers so that the testis is held in the hand with the forefinger positioned behind the corpus and with the thumb stabilizing the testis itself.

When micro-aspiration is performed under optical amplification during open surgery, epididymal fluid relatively uncontaminated by blood can be obtained. This is not possible during the percutaneous technique as it is impossible to avoid a small amount of bleeding when the needle is being withdrawn from the epididymis and the skin. However, the majority of the RBC can be excluded from the final sperm preparation if the swim-up technique is used.

Due to the avoidance of an 'open' surgical procedure, PESA has a significant advantage over MESA and is more acceptable to the husband. The procedure may be performed on an out-patient basis under intravenous sedation and a local anaesthetic; as a consequence the cost of treatment is significantly reduced. Using spermatozoa obtained by this method, one successful clinical pregnancy has been achieved.

In cases of CBAVD, genetic analysis for the presence of the cystic fibrosis mutation should be performed[12], especially on patients with chronic bronchitis, pancreatic insufficiency, and sweat chloride abnormalities. The couple in this case report did not have any of these symptoms and their chromosome studies were normal.

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การตั้งครรภ์ในคู่สมรสที่สามีเป็นหมันเนื่องจากการไม่มีท่ออสุจิแต่กำเนิด โดยการเจาะดูดอสุจิร่วมกับการใช้เทคโนโลยีช่วยการเจริญพันธุ์ ฉีดอสุจิเข้าไปในไข่

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ภาวะการมีบุตรยากเนื่องจากสามีไม่มีตัวอสุจิในน้ำอสุจิ เป็นภาวะที่พบได้บ่อยในคู่สมรสที่มาขอรับการรักษา สาเหตุอาจเกิดจากปัจจัยต่างๆ เช่น การอุดตันจากการอักเสบ การทำหมันชาย หรือการไม่มีท่ออสุจิแต่กำเนิด ผู้ป่วยจะได้รับการรักษาโดยการผ่าตัดต่อท่ออสุจิ แต่ในผู้ป่วยที่ไม่สามารถต่อท่อได้ หรือ ผู้ป่วยที่ไม่มีท่ออสุจิแต่กำเนิด ในอดีตคู่สมรสจะหมดหวังที่จะมีบุตรของตนเอง ในปัจจุบันความก้าวหน้าของเทคโนโลยีช่วยการเจริญพันธุ์ ช่วยให้ผู้ป่วยกลุ่มนี้มีความหวังขึ้นมาบ้าง

รายงานนี้เป็นรายงานแรกในประเทศไทยที่ประสพความสำเร็จเป็นการตั้งครรภ์ ในคู่สมรสที่สามีไม่มีท่ออสุจิมาแต่กำเนิด (congenital absence of vas deferens) โดยการใช้วิธีเจาะผ่านผิวหนังดูดอสุจิ (percutaneous epididymal sperm aspiration – PESA) ร่วมกับการฉีดอสุจิเข้าไปในไข่ (intracytoplasmic sperm injection – ICSI)

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