Thai Journal of Obstetrics and Gynaecology
June 2000, Vol. 12, pp. 151-155

CASE REPORT

Pregnancy Achieved by Intracytoplasmic Injection of Testicular Spermatozoa Retrieving by Testicular Sperm Aspiration (TESA)

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ABSTRACTS

Open testicular biopsy is a classic method of sperm retrieval in men with azoospermia. Recently, testicular sperm aspiration (TESA) of the testis has been used in attempts to obtain material for histopathological diagnosis and to retrieve spermatozoa for intracytoplasmic sperm injection (ICSI). We report a case of normogonadotrophic azoospermia failed to retrieve epididymal sperm by percutaneous epididymal sperm aspiration (PESA) technique. A successful outcome was achieved by TESA and ICSI.

Key words: Testicular sperm aspiration (TESA), Azoospermia, Intracytoplasmic sperm injection

Testicular spermatozoa have been used for intracytoplasmic sperm injection (ICSI) in azoospermic men since the first successful pregnancies.[1] Surgically open biopsy after failed sperm retrieval by epididymal aspiration has been used from the beginning.[2-5] It has also been proved to be useful in non-obstructive azoospermia.[6,7] A more simple fine needle aspiration method was then adopted,[8] and it has also been used successfully over the last few years.[9-11] Testicular sperm aspiration has been used to obtain testicular tissues for testicular histopathological evaluation[12,13] and to obtain testicular spermatozoa for ICSI.[14] We report a case of normogonadotrophic azoospermia who failed to retrieve epididymal sperm by percutaneous epididymal sperm aspiration (PESA) technique. A Successful outcome was then achieved by testicular sperm aspiration (TESA) and ICSI.

Case report

A couple was presented to our center with primary infertility of 2 years duration. The 33 year-old husband had two semen analyses performed using centrifugation at 1,800 g for 10 min failed to recover spermatozoa in our laboratory. Upon physical examination a normal male hibitus and normal

androgenization were observed. The left testis was small (12 ml.) and right testis was slightly small (15 ml.) His serum follicle stimulating hormone (FSH) concentration was 3 mIU/ml. The 25 year-old wife had had normal ovulatory cycles with normal hormonal profiles and normal gynecological status.

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. They chose the latter option and they were informed of the availability of donor spermatozoa for use in case that sperm retrieval was unsuccessful during the treatment cycle. Informed consent was also obtained after counseling.

Oocyte retrieval

Ovulation was induced using a long protocol. In the down regulation protocol, Buserelin acetate (Suprefact®; Hoechst AG, Frankfurt, Germany) 600 μg/day was administered by nasal spray, starting on day 21 of the cycle and then decreased to 400 μg/day on the first day of menses and continued until the day of hCG administration. Recombinant follicle stimulating hormone (Gonal-F®; Serono Laoratories, Aubourne, Switzerland) was administered 150 IU/day intramuscularly from day 3 with the monitoring of follicular development by serial transvaginal ultrasono graphy. Human chorionic gonadotrophin (hCG; Profasi®, Serono Laboratories) 10,000 IU was administered intramuscularly when leading follicles were 20 mm. Oocyte retrieval was performed 36 hours later with transvaginal ultrasound guidance under sedation analgesia.

Sperm collection and preparation

Immediately after oocyte retrieval, we proceeded with the percutaneous epididymal sperm aspiration (PESA) using intravenous sedation anesthesia with Propofol (Dripivan®; Zeneca Pharma, Wilmslow, UK) and local anesthesia with Bupivacaine injected at the skin and the underneath tissue. A comprehensive description of the procedure of PESA can be found in previous report.[15] In this case, we failed to obtained the epididymal sperm by PESA technique and proceeded to testicular sperm aspiration.

Testicular sperm aspiration (TESA) and testicular biopsy processing

Testicular sperm aspiration was done using 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 testis.

The testicular tissue was placed in a Petri dish containing HEPES-buffered Ham's F 10 medium 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 sperm. If no sperm was observed, another aspiration was done. Aspiration was stopped when the number of sperm was enough for intracytoplasmic injection. No pain, hematoma, infection or other side-effects were noted following the testicular and epididymal sperm aspiration procedure and the patient was discharged 2 hours later.

The morselized tissues were incubated in a Petri dish containing HEPES-buffered Ham's F 10 medium for approximately 2 hours. The contents then were mixed and allowed to settle for 1 minute and the deposited pieces of testicular tissue were removed. Two or three microdroplets were used from his suspension to be placed near the polyvinylpyrrolidone (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 PVP droplet and used for microinjection.

Oocyte preparation, oocyte handling and intracytoplasmic sperm injection procedure

A comprehensive description of the procedure for oocyte preparation, oocyte handling and intracytoplasmic sperm injection procedure can be found in our previous report.[15]

Embryo transfer and luteal phase support

At 18 hours after microinjection, the oocytes were microscopically examined under the inverted microscope (x 400 magnification). Sequential culture media were used.[16] A maximum of three embryos were transferred into the uterine cavity and the excess embryos were cryopreserved at two pronuclei stage. The luteal phase was supplemented with natural progesterone pessaries (Cyclogest® 400 mg, Hoechst, Hounslow, UK) given daily.

Results

Of the 16 oocytes recovered, eleven were at methaphase II stage with an extruded polar body. Live motile sperms with normal morphology were selected from the sperm preparation. A single spermatozoon was injected into each of the eleven metaphase II oocytes. All of the oocytes were undamaged by injection. Upon examination, 18 hours after injection, all oocytes were fertilized as shown by the presence of two pronuclei, as well as the presence of two polar bodies. Two early blastocysts were transferred 96 hours after egg retrieval and the patient conceived. The excess nine embryos were cryopreserved at 2PN stage.

Discussion

The ability to use only a few spermatozoa from the ejaculate for intracytoplasmic sperm injection (ICSI) in order to achieve fertilization and pregnancies[1] has revolutionized the potential to treat patients suffering from azoospermia. The patients with obstructive azoospermia were firstly treated using viable spermatozoa aspirated from the epididymis[17] or testis[18] and permitted the achievement of high pregnancy rates following ICSI.[3] The surgically sperm retrieval methods include open surgery aimed at the epididymis, by microsurgical epididymal sperm aspiration (MESA)[19-21] and testicular biopsy with testicular sperm extraction (TESE).[1-3,5,22] Following the remarkable results of these techniques in patients with obstructive azoospermia, success has been reported using a less invasive method of sperm retrieval, the percutaneous aspiration by fine needle, aiming at the epididymis (percutaneous epididymal sperm aspiration, PESA)[23-25] or testis (testicular sperm aspiration, TESA).[26] Alternatively, in obstructive azoospermia, efficient retrieval of testicular spermatozoa by closed percutaneous testicular biopsy was also reported, using a modified 20-gauge Menghini testicular biopsy needle[9] or a Biopty gun.[13]

Traditionally, open testis biopsy has been the standard procedure for obtaining testicular spermatozoa for ICSI treatment. Recently, percutaneous needle biopsy has been used as a diagnostic tool[12,27] and also for testicular extraction of spermatozoa for ICSI.[9,14] The open excisional biopsy technique is an invasive procedure which may cause discomfort for the patient, even if only taken through a small incision with meticulous hemostasis.[28] Testicular damage after biopsy has been described. There were avascular areas in the testes after biopsy, probably resulting from rupture of the arteries during the operation. Smaller arteries are not clearly visible and they often lie so close to the tunica that they are cut when the tunica is opened. Knowing the anatomy of the testicular artery, it is easy to avoid the main artery under the tunica by taking a needle biopsy sample from either side adjacent to the epididymis. The medial and lateral parts of the upper pole are less likely to contain major branches of the testicular artery than other regions and it has therefore been recommended that percutaneous biopsies be performed close to these areas.[29] Because the area penetrated is very small, when compared with a knife cut, the risk of hitting an artery accidentally is lower when a needle is used. The occurrence of intratesticular bleeding, observed sonographically as a hypoechoic region in the testicular parenchyma, within 30 minutes after the fine

needle biopsy was 7%. In contrast, the proportion of open testis biopsies resulting in signs of intratesticular bleeding was 29%.[30] It appears that the needle biopsy is associated with fewer complications than the open biopsy.

In a recent study, the patients after open testicular biopsy were evaluated with serial scrotal sonography and histopathological examination. At 3 months after the open biopsy, 82% of the patients had ultrasonographic abnormalities in the testis suggesting resolving inflammation or hematoma at the biopsy site. By 6 months, the acute changes had resolved leaving linear scars or calcifications. Repeat testicular biopsies were more likely to retrieve spermatozoa if the second attempt was performed more than 6 months after the initial biopsy, relative to those performed within 6 months, suggesting transient adverse physiological effects after testicular biopsies. Permanent devascularization of the testis may occur after testicular biopsies.[28]

Testicular sperm aspiration using a fine 21-gauge needle is a less invasive procedure which has been used successfully to recover testicular samples for diagnostic purposes. It has also been reported to recover testicular spermatozoa for ICSI, and pregnancies have been obtained.[9] Therefore, testicular sperm aspiration (TESA) has several advantages over open biopsy : the method using different punctured sites may provide a more representative sample than the single piece of tissue obtained probable by open biopsy.[8]

As a procedure, needle biopsy is technically more simple and cheaper than open biopsy. It can be easily carried out in an IVF unit before or after oocyte retrieval. Because it can be carried out under general or local anesthesia, the patient can leave the clinic soon after the procedure. Follow up of patients who had undergone testicular sperm aspiration (TESA) to obtain spermatozoa for ICSI has not shown long-term post-operative complications.[26]

As shown in this case report, testicular sperm aspiration with a 21 G needle yields a good quality specimen for obtaining testicular spermatozoa and the results regarding fertilization rate were at least as good as those we achieved using open surgical biopsy. There was no post aspiration complication in this case.

In conclusion, TESA with a 21G needle under local and general anaesthesia is a safe and reliable testicular sperm retrieval technique for normogonadotrophic azoospermic men who failed to retrieval epididymal sperm by PESA technique. It has been shown to result in acceptable fertilization rate in our first case treated by TESA technique. Therefore, it is an attractive alternative to testicular excision biopsy.

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