Thai Journal of Obstetrics and Gynaecology
September 1996, Vol. 8, pp. 253-255

CASE REPORT

Pregnancy after Intracytoplasmic Injection of Sperm Obtained from Testicular Sperm Extraction : The First Case Report in Thailand

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ABSTRACT

Here we report a case in which fertilization and pregnancy was achieved after intracytoplasmic sperm injection (ICSI) of testicular sperm in an azoospermic patient with minimal spermatogenesis and maturation arrest. Eight oocytes fertilized normally out of 10 injected. Four pronuclear stage embryos were transferred into the fallopian tubes and the others were cryopreserved. Despite using nearly immotile sperm, pregnancy was still possible. So in association of ICSI and testicular sperm extraction (TESE) most cases of azoospermic patient can now be successfully treated.

Key words : intracytoplasmic sperm injection, testicular sperm, azoospermia

Intracytoplasmic sperm injection (ICSI) has been recommended as the method of choice in assisted fertilization for severe oligospermic patients.[1] The presence of only a few weakly motile sperm in a centrifuged ejaculate is sufficient to produce results equivalent to that of IVF in couples with normal semen.[1,2] However, in patients with secretory azoospermia, a few sperm can occasionally be recovered by testicular sperm extraction (TESE) for subsequent ICSI.

ICSI with epididymal and testicular extracted sperm gives fertilization and pregnancy rates similar to results using ejaculated spermatozoa.[3-5] Here we report a case in which fertilization and pregnancy was obtained from ICSI of testicular sperm in an azoospermic patient with severely impaired spermatogenesis and maturation arrest. This is, to our knowledge, the first pregnancy reported by the combined TESE/ICSI in Thailand.

Case Report

A couple with 5 years primary infertility was studied. The husband was 30 years of age and was found to be azoospermia from mump orchitis. His testicular biopsy result showed minimal spermatogenesis with maturation arrest and Leydig's cells were of usual appearance. Serum FSH and testosterone were normal. His wife was also 30 years of age and had normal menstrual cycles and normal tubal patency (both). After counseling the couple entered our IVF programme to try ICSI with testicular sperm.

A testicular biopsy was performed after local anaesthesia. A small piece of extruding testicular tissue was excised and put in 1-2 ml of HEPES-buffered Medicult medium in a small test tube. Content of the test tube was poured into a petri dish and the tissues were dissected thoroughly with 27 gauge needles under a dissecting microscope. After dissecting, the testicular tissue was checked under an inverted microscope for the presence of sperm cells. The dissection medium was aspirated carefully into a 1.5 ml Eppendorf tube. Any of sperm preparation method would have been unnecessary to be attempted since the number of spermatozoa was so few and motility was so weak. We had no choice but to pick the individual spermatozoon out of the field of debris, red blood cells and Sertoli cells for ICSI. So without further treatment, the testicular tissue solution was kept in an incubator at 37°C, 5% CO_2 until the moment of the injection procedure.

The female patient was prepared for egg retrieval after GnRH agonist suppression and hMG ovarian stimulation. Transvaginal egg retrieval was performed thirty-six hours after hCG administration. The cumulus and corona cells were removed by incubation for 30 sec in HEPES-buffered Medicult medium containing 80 IU/ ml of Hyaluronidase (type VIII) and by mechanical aspiration. Afterwards, the oocytes were observed under the inverted microscope and nuclear maturation was recorded. Until the moment of the injection procedure, oocytes were kept in 25 µl droplets of Medicult IVF medium covered by light-weight paraffin oil in an incubator at 37°C, 5% CO_2. ICSI was carried out on all oocytes that had extruded the first polar body (at metaphase II).

Just before the injection procedure, testicular suspension was centrifuge at 1,800 g for 5 min in Eppendorf tube. The supernatant was removed with a Pasteur pipette, after adding 0.2 ml of HEPES-buffered Medicult medium the pellet was gently resuspended.

Intracytoplasmic sperm injection was performed according to Palermo et al.[6] A single almost immotile spermatozoon was aspirated into the injection pipette and transferred to a clean droplet of 10% PVP solution. Debris and contaminated red blood cells were removed by repeated pipetting of the sperm in several clean area of the PVP droplet. The sperm cell was aspirated, tail first, into the injection pipette. The oocyte was secured by the holding pipette with the polar body positioned at 6 o'clock, then a single spermatozoon was injected into the ooplasm. The injection procedure was repeated

until all metaphase II oocytes were injected. The injected oocytes were washed in several drops of IVF Medicult medium and incubated in 25 µl of IVF Medicult medium under light-weight paraffin oil overnight at 37°C, 5% CO_2.

About 16 hours after the microinjection, the oocytes were observed for intactness and for the presence of pronuclei and second polar bodies. Fertilization was considered normal when two clearly distinct pronuclei were present. The pronuclear stage embryos were transferred into the fallopian tubes by laparoscopic procedure.

Results

Of the 10 metaphase II oocytes injected, 8 fertilized normally. Four pronuclear stage embryos were transferred into the fallopian tubes (2 embryos for each tube) and the other three were cryopreserved. A positive serum βhCG was detected on day 12. Five weeks after transferred, an ultrasound scan confirmed a twin pregnancy with positive fetal heart beat.

Discussion

The present report demonstrated that fertilization and pregnancy can be achieved by intracytoplasmic sperm injection of testicular sperm from an azoospermic man resulting from mump orchitis. Eventhough testicular sperm retrieved in this case were either immotile or, at the most, displayed shaking movements, a high fertilization rate was still possible. Epididymal transit may not necessary for testicular spermatozoa to acquire fertilizing ability since with intracytoplasmic injection we bypassed the zona pellucida and oolemma of the oocyte, thus allowing a testicular sperm with deficient motility or acrosome to fertilize an oocyte. Since the number and motility of testicular spermatozoa are so low, thus cryopreservation has not yet been feasible. Therefore, the husband has to undergo multiple biopsy procedures if subsequent cycles are needed. However, many testicular biopsies can be taken without major inconvenience.[3] In summary, the present case report shows that intracytoplasmic sperm injection of testicular sperm extraction can be attempted for azoospermic male with severely decreased spermatogenesis and can result in fertilization and pregnancy.

References

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