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Successful case of Embryo formation with Round Spermatid Injection: A Case Report

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Abstract

Round cell Spermatids are the earliest immature haploid set chromosomal germ cell that can be seen after testicular biopsy in some of the infertile male. These spermatids can be used for the treatment of nonobstructive azoospermic male who does not want to use donor semen and want their own biological child by assisted reproductive technology. Although the successful pregnancy rate following intracytoplasmic sperm injection of round spermatids are low, yet round spermatid injection can be used as a last hope for the azoospermic infertile couple to have their own genetic offspring

Introduction

The new reproductive technologies, such as IVF ICSI, are becoming increasingly common, enabling infertile couples to become parents and create families. Among many causes of male infertility, azoospermia is one in which there are no spermatozoa in the ejaculate and it is affecting about 1% of men in the general population. [1] Azoospermia is usually detected during the course of an infertility investigation and for such males; testicular sperm extraction (TESE) has been advised to retrieve the testicular spermatozoa. But when neither mature spermatozoa nor late-stage spermatids are found in the testes, the round spermatids are injection into the oocytes to make the embryos is the option to get their own genetic offspring.

ROSI (round spermatid injection) is a method of assisted in vitro fertilization (IVF) in which precursors of mature sperm obtained by ejaculated specimens or TESE are injected directly into oocytes as treatment for male infertility. [2] Round spermatid are the young haploid cell that undergoes a series of structural and biochemical modification in testis and epididymis to become mature and functional spermatozoa.

In the present case, there is a successful embryo development in the patient after injecting round spermatids extracted by TESE into oocytes.

Case Report

A 23year old female came to our center with primary infertility. We had advised the hormonal profile and the ultrasound pelvis. Serum hormonal measurements were AMH: 5.88 ng/ml, Prolactin: 11.3 ng/ml, thyroid stimulating: 1.39 pg/ml. Ultrasound showed bilateral polycystic ovaries (PCO) with normal uterus. The husband's semen analysis showed azoospermia, with

hormonal profiles - FSH: 13.0 IU/ml, LH: 6.5 IU/ml, Total Testosterone 450 ng/dL, Prolactin: 4.0 ng/ml, TSH: 2.6 p g/ml.

In view of the above diagnosis, we recommended IVF with ICSI with TESE to the couple. As PCO was diagnosed, we stimulated the ovaries of patient with the recombinant human FSH 150 IU (rFSH, Folisuge; Intas Pharmaceuticals Ltd, India). After six days of stimulation, transvaginal scan showed 10 good follicles of 14mm size in both ovaries. After that daily subcutaneous injection of GnRH antagonist, 0.25 mg Cetrorelix (Cetrotide, Merck Serono S.p.A, Italy), was added. When follicles reached 18 mm, 500 mcg recombinant hCG (rhCG, Ovitrelle; Merck Serono S.p.A, Italy) was given to trigger ovulation. Transvaginal oocyte aspiration was performed before 36 h, under ultrasound guidance, using Wallace OPU needle and Cooks gamete buffer media. We retrieved 14 oocytes from both ovaries. The husband had undergone a TESE before the stimulation of the wife to check the presence of spermatozoa in the biopsy tissue. Biopsy was performed under general anesthesia. Reasonable amount of tissue was extracted. The biopsy tissue was teased with the help of 27G needles and fluid was observed under inverted microscope to check for the presence of spermatozoa. Few round primary and secondary spermatids were identified by their size and morphology, in the initial examination of the tissue. The tissue was teased thoroughly and then washed with Double density gradient method and was immediately cryopreserved.

Two hours post retrieval, oocytes were denuded with the help of 80IU/ml hyluronidase, and 12 mature oocytes were obtained. The cryopreserved sperm sample was thawed and given a simple wash with COOK gamete buffer. The pellet was resuspended in 0.2 ml gamete buffer. An ICSI dish was prepared using two PVP drops in the center with elongated drops of gamete buffer on the left to hold the sperm sample and round drops of buffer on the right to hold the eggs. Intra Cytoplasmic Sperm Injection (ICSI) was performed in the laboratory in Cooks gamete media, in 12 mature oocytes. The round spermatids were again identified by their size and morphology and ease of cytoplasm separation from the nucleus when aspirated in the injection pipette. These round spermatids were injected in the mature oocytes.

ROSI very often needs artificial oocytes activation to achieve successful fertilization. Hence, in this case it was performed using commercially available artificial activation medium, post ICSI. The injected oocytes were then cultured in COOK cleavage medium for 2 days. Four 2 cells embryos each of grade A were formed and all four embryos were transferred to the patient under USG guidance, using COOK K-7019 catheter. After 14 days of luteal support, serum beta HCG was investigated, which reported a negative outcome.

Discussion

Azoospermia affects 1% of general population and 10- 15 % of infertile couple. It can be obstructive azoospermia (OA) or non-obstructive azoospermia (NOA), each having very different etiologies and treatments modalities. On histological examination spermatogenesis is complete in OA, while in nonobstructive azoospermia germ cell aplasia (Sertoli cell only pattern), maturation arrest and tubular sclerosis and atrophy is seen on histological examination with or without focal spermatogenesis. At the end of spermatogenesis, the most mature stage of male gamete, the elongated spermatids undergo various structural and functional modifications in tubular lumen of epididymis to become functional spermatozoa. In obstructive azoospermia, testicular sperm retrieval for ICSI and/or cryopreservation is possible by microsurgical

epididymal or testicular sperm aspiration while this is not possible in 50% of NOA. [3] The only resort for these couples are donor semen or if they want to be father of their own genetic children then to use immature haploid germ cell the elongated spermatids or round spermatids for ICSI. ICSI with immature haploid germ cells, including elongating and round spermatids, has yielded conflicting results and despite reported deliveries of healthy offspring, the method has very low efficiency. [4]

This microsurgical injection of a round spermatid into an oocyte as a substitute is commonly referred to as round spermatid injection (ROSI). The idea of using spermatids for infertile couple in human was firstly proposed by Edward et al.[5] The first human live birth with round spermatids microinjection (ROSI) was reported by Tesarik et.al (1995). [6-7]). There was a report from Japan of the birth and development of 14 children born to 12 women following ROSI of 734 oocytes previously activated by an electric current.[8] In a another study from japan nighty babies including previous 14 babies born by ROSI was followed for 2 years for physical and cognitive development and found comparable to babies born naturally.[9] In India we have come across only one case report in which ROSI was used resulting in successful pregnancy . [10]

Although we did not get a clinical pregnancy in the present case and the overall results are variable and unpredictable with ROSI till now , these spermatids might be a competent male gamete for some hopeless azoospermic couples. Proper identification of round spermatids by shape , size, forms and nucleus with confirmation with FISH and karyotyping prior to oocytes injection, improved oocytes activation techniques like electrical stimulation , proper assessment of fertilization embryo culture, transferring frozen embryo at 4 cell or blastocyte stage etc are the few key elements to improve the outcome of ROSI,as used in study in japan.

With better laboratory facilities, doing more and more such new cases or doing repeat cycle with these old patients we hope more positive results in our country too. Thus ROSI can give a hope for azoospermic couple whose germ cell fails to develop beyond the round spermatid stage, who refuse donor semen, as a last resort to have their own genetic offspring.

Abbreviations and symbols

IVF - in vitro fertilization

ICSI - intracytoplasmic sperm injection

OA - obstructive azoospermia

NOA - non-obstructive azoospermia

TESE - testicular sperm extraction

ROSI - round spermatid injection

PCO - polycystic ovaries

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