Successful fertilization and embryo development after spermatid injection: A hope for nonobstructive azoospermic patients

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ABSTRACT

Spermatids are the earliest male germ cells with haploid set of chromosomes. Spermatid injection was introduced in human assisted reproduction for the treatment of men with non-obstructive azoospermia. Spermatozoa can be recovered in half of patients with nonobstructive azoospermia. The use of spermatids for intracytoplasmic injection (ICSI) has been proposed for cases in which no spermatozoa can be retrieved. However, there are low pregnancy rates following ICSI using round spermatids from men with no elongated spermatids or spermatozoa in their testes. The in vitro culture of immature germ cells has been proposed as a means to improve this poor outcome. Oocyte activation rarely occurs when injected with a spermatid. Therefore, spermatid injection requires use of calcium ionophores for oocyte activation which is otherwise carried out by PLC zeta from mature sperms. This is the only option available for the nonobstructive azoospermic patients to have their own biological child.

KEY WORDS: Intracytoplasmic sperm injection, nonobstructive azoospermia, oocyte activation, round spermatids

INTRODUCTION

Earlier, treatment for severe male factor infertility was limited to intrauterine inseminations or *in vitro* fertilization using donor sperm. However, most infertile couples, and particularly men, are reluctant to use donor sperm because of various cultural and ethnic concerns. Today, advances in male infertility have introduced innovative therapeutic options, in particular, intracytoplasmic sperm injection (ICSI), which offers men, including those with azoospermia, a greatly improved chance to conceive their own biological offspring.[1] Azoospermia is present in about 1% of all men[2] and in 10–15% of infertile men.[3] The majority of cases are attributed to obstructive azoospermia (OA), in which spermatogenesis is normal, but patients with non-OA (NOA) are characterized by impaired spermatogenesis.[4]

Currently, due to advances in the science of reproduction, pregnancy may still be achieved despite severe male factor infertility. This is mainly attributed to the success of the ICSI technique coupled with advanced surgical testicular sperm retrieval technique testicular sperm aspiration and micro-testicular sperm extraction (m-TESE). Men with NOA can also be treated by using round spermatid injection (ROSI) or elongated spermatid injection (ELSI) into the ooplasm. Spermatids can be retrieved from the ejaculate or from the testicular biopsy. The nucleus of these cells contains a haploid number of the chromosomes, thus may be injected directly into oocytes. Since spermatids are immature germ cells, the fertilization potential is considered to be low. Low fertilization can be attributed to lack of activation in the oocytes in such cases.

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Oocyte activation is characterized by a sudden rise in intracellular calcium concentration, which occurs in the form of calcium oscillations[5,6] caused by phosphoinositide-specific phospholipase C (PLC), PLC-ξ.[7] Different methods have been proposed to overcome oocyte activation failure in ICSI cycles, including electrical,[8,9] mechanical,[10-12] and chemical oocyte activation using different calcium ionophores.[13,14] The fertilization rates in case of ICSI cycles have gone up dramatically by the use of these calcium ionophores.

CASE REPORTS

Case 1

This young couple had primary infertility with 5 years of marriage. The husband's ejaculated semen analysis report was found to be complete azoospermia, repeated many times. Follicle stimulating hormone (FSH) was 25 IU, LH was 11 IU, and testosterone was 0.9 ng/ml. On physical examination, testes were found to be very small in size and were a case of testicular failure. He had no relevant medical or surgical history. Karyotyping showed Klinefelter syndrome. The patient was advised Y-chromosome microdeletion (YCMD) assay but denied. The couple strictly wanted to use their own gametes despite counseled for the outcome of spermatid injection. Hence, we decided to do m-TESE and spermatid injection.

The wife was normal as far as her hormonal reports were concerned. She was stimulated using antagonist protocol. We got five metaphase II (M-II) oocytes. M-TESE was carried out on the day of oocyte retrieval. The seminiferous tubules were mechanically macerated using sterile needles. The resulting supernatant containing some extracted cells was centrifuged at 100–200 g for 10 min and resuspended in 50–200 μl of sperm medium. Red blood cells were lysed by using RBC lysis buffer. Ten microliter drops were made with this cell suspension alongside of PVP and GMOPS drops in ICSI dish under oil. The biopsy did not reveal even a single mature sperm, so the late round spermatids (stage sb1) were picked and used for oocyte injection.

In this case oocyte, activation was carried out injecting calcium chloride ($CaCl_2$, 5 mm) along with the spermatid during ICSI. After injection, the oocyte was very quickly rinsed in 8% ethanol, washed in GMOPS Plus buffer and transferred to the culture drop.

Fertilization was assessed 18 h after ICSI, and normal fertilization was declared, when two clearly distinct pronuclei were present. Two out of 8 oocytes were fertilized. Both of them cleaved to good 4-celled embryos and embryo transfer was carried out.

Case 2

Another couple presented to us with 7 years of marriage and primary infertility. The husband had a history of surgery done for bilateral hydrocele 10 years back. He was detected with azoospermia post 2 years of marriage. We repeated the semen analysis at our center also and confirmed azoospermia. The testicular volume was normal. FSH was 19 IU, LH 12 IU, and testosterone 2.89 ng/ml. Karyotype was normal. YCMD assay was not done. Again, the husband was not willing for the use of donor sperms. The wife had all parameters normal, and her ovaries were stimulated using agonist protocol. We retrieved 13 M-II oocytes. M-TESE was carried out using the same protocol as discussed in case 1. We did not find any mature sperm or elongated spermatids in the biopsied sample. Therefore, ROSI was carried out.

Oocyte activation was carried out in Ionomycin (5 μmol/l) (MP Biomedicals) for 30 min post-ICSI. The injected oocytes were then washed in GMOPS Plus and then transferred to culture medium.

Fertilization was assessed 18 h after ICSI, and normal fertilization was declared when two clearly distinct pronuclei were present. Totally, 8 fertilized out of 13 oocytes, and all of them cleaved. Finally, 4 of them were grade A embryos which were transferred.

DISCUSSION

In humans, the injection of spermatids leading to fertilization and early cleavage was reported by Vanderzwalmen *et al*.[15] The first reported successful births were by Tesarik *et al*.[16] using round spermatids from the ejaculate and by Fishel *et al*.,[17] using elongated spermatids extracted from the testis. Most clinical studies have successfully used an injection of whole spermatids into the oocytes, that is, ROSI and ELSI as described by Tesarik and Mendoza.[18] These data clearly indicated that viable embryos can be obtained by fertilizing oocytes with spermatids.

In the mid-nineties, several IVF centers used testicular spermatids for ICSI, and most of the reported pregnancies were achieved using late spermatids. When round spermatids were used, the pregnancy rate was much lower. More than 15 years after the first live birth achieved with ROSI, fewer than ten children have been born worldwide using this technique.

In India, we have come across only one report in which round spermatid nucleus injection was used, and successful pregnancy was achieved.[19]

The cases attempted in this study, with spermatid injection gave us different experiences. In the first case, we got 25% fertilization (2 fertilized out of 8). In our experience, ionomycin gave us almost 63% fertilization (8 fertilized out of 13) and was considered the best. Fertilization rates more than 90% have been reported using calcium ionophore for

oocyte activation.[16] All the fertilized oocytes underwent cleavage and became embryos. We confirmed 2PN status to exclude any parthenogenetic activation of the oocytes.

Review of reports of ICSI with round spermatids by Sousa *et al.*,[20] showed that the success rates of ROSI are dramatically lower when compared with ELSI. ROSI was found to be clinically inefficient, with a 21.8% fertilization rate and a 2.8% clinical pregnancy rate.[21] Since 2002, no clinical pregnancies have been reported following ROSI.[22]

Though we did not get any clinical pregnancy in these cases but hope to succeed if we do more such cases or do repeat cycles with these patients. We plan to culture round spermatids to the elongated stage *in vitro* and use as the success rates of clinical pregnancy are higher with ELSI as discussed earlier.

The testicular sperm retrieval is successful in almost all the patients with OA, and sufficient numbers of spermatozoa can be obtained for ICSI and/or cryopreservation. However, it is difficult to get mature spermatozoa in case of NOA patients. The only hope for these patients to father their own genetic children is the use of more of immature germ cells for ICSI.

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Conflicts of interest

There are no conflicts of interest.

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