

Live birth after calcium ionophore oocyte activation and ICSI of MACS selected sperm from oligoasthenoteratozoospermic man with disrupted sperm DNA integrity: A case report

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Abstract

Purpose

To report a successful delivery of healthy baby after transfer of frozen thawed day - 3 embryos derived from artificial oocyte activation and Magnetic Activated Sperm Sorting (MACS) treated sperm.

Design

Case report

Setting

In Vitro Fertilization Center

Patient

A male patient whose sperm exhibited low concentration, motility and morphology as per WHO criteria, and also had disrupted sperm DNA integrity.

Intervention(s)

Advance sperm preparation method Magnetic Activated Sperm Sorting was used for functional sperm selection. Sibling oocytes were activated by calcium ionophore after ICSI.

Main Outcome Measure(s)

Fertilization rate and cleavage rate in oocytes treated with and without calcium ionophore.

Result(s)

The fertilization rate of oocytes activated with calcium ionophore (4 of 5, 80.0%) was higher than that of the non-activated oocytes (2 of 7, 28.57%). Four embryos derived from the activated oocytes were frozen on day 3, whereas two embryos with unequal blastomeres were subjected to freezing after the patient's consent. Two thawed embryos transfer resulted in a single pregnancy in the first attempt.

Conclusion

Artificial oocyte activation with calcium ionophore improved the fertilization rate and quality of the embryos in an oligoasthenoteratozoospermic man with disrupted sperm DNA integrity.

Disclaimer: The authors have no conflicts of interest.

J Reprod Biotechnol Fertil 9:41-44

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Language Compliance: This article was edited by the Australian Editorial Services (www.nativeenglisheditor.com)

Acknowledgment: Published in collaboration with the International Human Embryology Research Academy (IHERA; www.ihera.org). This manuscript won a prize, under the category 'Free Communications', at the EMBART Online Conference of the IHERA, 3 October 2020

Keywords: Calcium ionophore, oocyte activation, Magnetic Activated Cell Sorting, MACS

Introduction

The implementation of intracytoplasmic sperm injection (ICSI) in 1992 and advanced sperm selection methods like Magnetic Activated Cell Sorting (MACS) gave hope to couples where male partners were diagnosed as

oligoasthenoteratozoospermia [Palermo et al., 1992] and with sperm DNA fragmentation [Monica, 2013]. Although the reported fertilization rate after ICSI is 80- 90%, 1-3 % of cases observed reduced or failed fertilization after ICSI.

Most cases of fertilization failure following ICSI can be traced back to a lack of oocyte activation [Yanagida, 2004].

Deficient cytosolic sperm-associated oocyte activating factor (SAOAF) is the reason for this lack of oocyte activation [Dozortsev et al.,1995]. The first case of live healthy birth with artificial oocyte activation with calcium ionophore was reported in 1997 [Rybouchkin et al., 1997]. Since then, it is used for various sperm abnormalities [Kim et al., 2001; Elder-Geva et al., 2003].

Case Report

Patient

A 27-year-old woman and her 30-year-old husband presented at our institute for consultation. Primary diagnostic tests were performed for both partners. The female partner had endometriosis but a normal uterine cavity while the male partner's sperm concentration, motility and morphology were less than standard values as per WHO criteria. Based on the male partner's lifestyle and profession, sperm DNA fragmentation test was prescribed and was found to be positive. Before an ICSI cycle, the male partner was advised to take a course of oral antioxidant medicines for three months. The male partner was counseled for testicular sperm aspiration and advanced sperm selection methods like MACS. Patient opted for MACS.

Material and methods

Antagonist protocol was the choice for controlled ovarian hyper stimulation. 225 IU/day HMG was administered for 9 days. Final oocyte maturation and the induction of ovulation were performed with administration of 10,000 IU hCG soon after observation of leading follicles between 17mm and 18mm. Thirty-six hours after the hCG injection, oocyte retrieval took place using transvaginal ultrasound guided follicle aspiration. Thirteen cumulus-oophorus complexes were recovered, out of which twelve were at the metaphase II (MII) stage.

A semen sample was collected post oocyte pickup. The sperm revealed a count of 8 Million per ml with 15% motility, and 2% normal morphology according to the criteria of the World Health Organization [WHO,1999]. As his

previous report had high sperm DNA fragmentation, double density gradient method MACS separation was done with the patient's consent in order to obtain functional sperm.

Oocyte denuding and ICSI was performed according to the Istanbul consensus workshop on embryo assessment [Istanbul consensus, 2011]. Twelve oocytes were injected with morphologically normal sperm.

A ready to use calcium ionophore (Sigma Chemical) was used to artificially activate the oocytes after ICSI. Thirty minutes after ICSI, the oocytes were exposed to 10 μ mol calcium ionophore for 5 minutes and subsequently washed thoroughly in IVF medium. Only five injected oocytes were artificially activated with calcium ionophore because of lack of evidence in the literature.

Fertilization was assessed 18 hours after ICSI by the appearance of two distinct pronuclei and two polar bodies. The zygotes were cultured in an atmosphere of 6 % CO₂, 5 % O₂ and 90 %N₂. Four clear pronuclei were observed out of five calcium ionophore treated oocytes, whereas only two oocytes fertilized out of seven in the non-treatment group.

Embryo quality was evaluated on day 3 using an inverted microscope. The following parameters were recorded: [1] number of blastomeres, [2] fragmentation percentage, [3] variation in blastomere symmetry, [4] presence of multinucleation, and [5] defects in the zona pellucida and cytoplasm. Four timely divided embryos were vitrified in the treatment group, whereas two slow growing embryos with unequal blastomere were also vitrified with the patient's consent.

After a gap of two months, two frozen thawed day 3 embryos of treated group were transferred. Initial quantitative serum beta- HCG test was conducted on day 15 after embryo transfer to screen for a pregnancy. Pregnancy was confirmed by the presence of a heartbeat in a gestational sac.

Results

The patient delivered a healthy girl (3,270 g) by Caesarean section in pregnancy week 36.

Discussion

Fertilization, cleavage, implantation, pregnancy and live birth rate can be improved for oligoasthenoteratozoospermic men with disrupted sperm DNA integrity with ICSI, advanced sperm selection methods like MACS, and artificial oocyte activation.

Artificial oocyte activation with calcium ionophore treatment, in particular, is the most commonly applied method in clinical trials. (Swann and Ozil, 1994) showed that when sperm activates eggs at fertilization, the signal for activation involves oscillations in the intracellular free calcium concentration. This is known as a 'trigger'. The proposed causative agent of the calcium oscillations is PLC-zeta - phosphoinositide-specific phospholipase C, which is a soluble sperm protein that is delivered into the egg after membrane fusion [Saunders et al., 2002]. Activation of PLC-zeta depends upon interactions with factors present in the ooplasm [Dozortsev et al., 1997]. Ooplasm Deficiency in cytosolic sperm-associated oocyte activating factor (SAOAF) results in a partial or complete inability of the sperm to activate the oocyte, or the inability of the oocyte to decondense the sperm.

Men with low expressions of PLC-zeta at the RNA and protein levels have an absence or deficiency in the acrosome (Heytens et al., 2009). Therefore, ICSI followed by artificial oocyte activation has been shown to improve fertilization and pregnancy outcomes in these types of infertile couples. Few studies confirm that oligoasthenoteratozoospermic man with disrupted DNA integrity may have low expressions of PLC-zeta [Nahid et al., 2018, Marziyeh et al., 2017], and have shown significant negative correlations with embryo cleavage, implantation rates, miscarriage rates, and pregnancy loss after ICSI [Morris et al., 2002, Brahem et al., 2011].

Embryos from infertile couples with low levels of damaged DNA in sperm who achieved pregnancy, grew faster compared to infertile couples with high levels of sperm DNA damaged who did not achieve pregnancy [Wdowiak et al., 2011]. However, there are indications that the oocyte is able to repair sperm DNA damage; but it is strongly related to extent of sperm DNA

fragmentation, age, and quality of the oocyte [Akten et al., 2013].

Conclusion

The results of this sibling oocyte study clearly revealed that the main cause of low fertilization was failure in oocyte activation due to the sperm factors involved in oocyte activation. Calcium ionophore activation is a useful method to improve ICSI outcome.

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