Unexplained total abnormal fertilization of donor oocytes in ICSI with using spermatozoa from different patients

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**ABSTRACT**

In this report, we present a case of unexplained total triploidy of donor eggs fertilized by ICSI from four different male partners of different couples. Woman who served as a donor was 27 year old, had her own healthy child, and previously twice served as a donor with normal fertilizations and healthy baby born.

**KEYWORDS**

Triploidy; IVF; abnormal fertilization

**Introduction**

Until today over 8 million IVF children have been born since the first IVF reported in 1978 by Steptoe and Edwards \cite{1}. Over 2.5 million cycles are being performed every year, resulting in over 500,000 deliveries annually. During almost 40 years IVF techniques are developed and result into high fertilization rates. However, the development of human embryos \textit{in vitro} is still suboptimal, and in many IVF cycles embryos fail to implant and produce a viable pregnancy \cite{2}.

In \textit{in vitro} fertilization sometimes insemination of oocytes can result in abnormal fertilization like three or more pronuclei (>3PN) or only one pronuclei (1PN). Typically, triploidy results either from polyspermic fertilization or from oocyte-derived meiotic failure \cite{3-5}. Insemination of oocytes by direct intracytoplasmic sperm injection (ICSI) usually eliminates dispermic triploidy, but it does not prevent in all oocytes the formation of abnormally fertilized zygote showing 3PN.

Zygotes with triploid pronuclei (3PN) can cleave and some of them even can develop to blastocysts, which after the embryo transfer usually result in implantation failure or early pregnancy loss. The advent of assisted reproductive technology (ART) has provided an important insight into the mechanisms of normal and aberrant fertilization of human oocytes. Triploidy can appear either from dispermic fertilization or from retention of the second polar body extrusion. However, etiopathogenetic mechanisms, which lead to triploidy with digynic embryos (containing two maternal pronuclei) are not well understood.

In this report, we describe the case of abnormal fertilization with three pronuclei (3PN) after fertilization by ICSI using donor oocytes and spermatozoa from three different men. Triploid fertilization was unpredictable and unexplained by any obvious reason from the quality of semen sample or from the morphology of oocytes.

**Case**

Patient A. A 27 year old mother of a 5 years old healthy baby girl had previously served as egg donor in Fertility Center of Armenia. She had undergone two \textit{in-vitro} fertilization (IVF) cycles. One of this results in a pregnancy with delivery of healthy child. Two egg collections with 12 and 10 oocytes were retrieved and the majority of them were normally fertilized. In the first cycle one oocyte (1/12) displayed only one pronucleus and in the second cycle 2 oocytes (2/10) were immature at GV stage and were not injected. The remaining oocytes exhibited 2 PN and produced embryos with normal morphology. The pregnancy occurred in the first IVF cycle.

On current third IVF cycle, ovarian stimulation of donor was performed with using the same antagonist protocol as during previous two cycles.

Briefly, 225 IU of human gonadotropins (Gonal F, Serono Merck, Rome, Italy) were administrated for 6 d followed by Menopur (Ferring Pharmaceuticals, Saint-Prex, Switzerland). Ovarian stimulation was started from day 2 of the cycle and continued with the same dose until the trigger day. Stimulation days: 10. Total dose of gonadotrophins used: 2250 IU. Follicular monitoring was performed on day 2 of the cycle and then on days 7, 9, and 11. Once the dominant follicle reached 14 mm in size, Cetrotide at a dose of 0.25 s/c (Cetrotide, Serono Merck, Rome, Italy) was added. Oocyte maturation was triggered by using 0.2 mg of triptorelin (Decapeptyl, Ferring Pharmaceuticals, Saint-Prex, Switzerland) when 2 dominant follicles reached 18 mm in diameter. Transvaginal ultrasound-guided oocyte retrieval was conducted 35 h after the trigger administration.

A total of 16 oocytes were retrieved during oocyte ovum pick up of which 15 were mature (MII). All 15 mature oocytes were therefore inseminated for 4 different couples who wished to use donor eggs for their infertility treatment (3–4 eggs in each group). All oocytes were injected by ICSI with patients’ husband’s spermatozoa. No severe oligoasthenozoospermia was diagnosed in neither of the patients. All men underwent chromosomal karyotyping according to the protocols of our clinic and no abnormalities were detected. Fertilization check was routinely performed after 18 h post ICSI procedure, 14 out of 15 oocytes showed abnormal fertilization with 3 pronuclei (3PN) with one...
visible polar body. One oocyte showed abnormal fertilization with only 1 pronucleus.

On the same day, another egg retrieval from other patient was performed. Collected oocytes were inseminated by ICSI using donor sperm, carried out by the same embryologist, using same culture medium and the same equipment (ICSI microscope and incubator for embryo culture). Normal fertilization was observed in all oocytes in this case.

ICSI procedure

Approximately 3–4 h after the egg collection, oocytes were treated by hyaluronidase (80 mIU/mL) for 45–60 s in order to remove surrounding cumulus cells. Spermatozoa with normal morphology and progressive motility were selected for injection by using ICSI procedure under Olympus inverted microscope. Oocytes were fixated by holding micropipette at the 9-O’clock position and the polar body was oriented at the 6- or 12-O’clock position. The injecting pipette then was gently advanced through the zona pellucida and the oolemma until the pipette was beyond the center of the oocyte, and the sperm was gently injected into the cytoplasm of the oocyte.

Discussion

After the introduction of ICSI by Palermo et al. [6] several studies have been performed to clarify practical and theoretical issues related to this procedure. The ICSI technique states that only a single sperm cell is injected in the cytoplasm of each oocyte, but sometimes, when fertilization check is performed 16–19 h after ICSI, some zygotes show three pronuclei (3PN) instead of the normal fertilization with 2 pronuclei (2PN). Zygotes morphologically classified as triplo-nucleus (3PN) after ICSI, are typically thought to be digynic in their origin via retention of the second polar body [7]. Retention of the second polar body during meiosis II is the most common cause of digynic triploid embryos but they may less frequently result from the retention of the first polar body during meiosis I. Insemination of a binucleate oocyte by a single spermatozoon will result in a digynic, triploid embryo. The underlying etiology for triploidy following conventional IVF or ICSI remains unknown.

Some investigators have suggested that the incidence of triploid fertilization after IVF is a result of advanced maternal age or severe sperm abnormalities [8]. Although these inherent patient attributes may predispose them to triploid fertilization, other investigators have suggested that the propensity toward triploidy is a function of ovarian stimulation, as indicated by high peak E2 levels, large oocyte yields, high gonadotropin doses, and lengthy stimulations and advanced maternal age [9,10].

Different levels of 3PN zygotes obtained after ICSI has been described in several published studies. Van Steirteghem et al. [11] and Nagy et al. [12] reported 5.1 and 2.4%, respectively. Another study published by Macas et al. [13] reported a rate of 3PN at 6.2%. He suggested that the incidence of this abnormality could be increased by the hydrostatic pressure exerted during the ICSI procedure, thus disrupting the microtubules of the oocyte spindle.

A Spanish group Escribá et al. [14] described a technique that improves identification and removal of the extra paternal pronuclei. They microsurgically removed the pronucleus located furthest from the second polar body in 3PN human zygotes using cytoskeletal relaxing agents. The resulting embryos were diploid and developed to blastocyst stage, with the majority being heteroparental. The authors concluded that microsurgical removal of one pronucleus located at the farthest position to the second polar body from 3PN zygotes is feasible and can result, in vitro, into a morphologically normal, heteroparental diploid blastocysts. This technique of embryo recycling could be useful for reproductive purposes or human embryonic stem cell research. These could potentially be used to derive patient-specific embryonic stem cell lines, instead of using viable embryos [14]. In our case, the origin of 3PN fertilization remains unexplained. The case is more interesting because ICSI with using four different patients spermatozoa resulted in the same abnormal fertilization, which suggests rather into the reason, which derives from oocytes than from spermatozoa.

Conclusion

IVF outcome is highly dependent on oocyte quality, a high incidence of triploidy in ICSI cycles may suggest an occult oocyte factor related to abnormal oocyte maturation competence. High level of triploid zygotes after ICSI indicated as a negative prognostic indicator for IVF cycle outcome. According to the literature, the most reasonable explanation of the incidence of triploid fertilization after ICSI is related to the advanced maternal age or to ovarian stimulation with relatively high gonadotropin doses and longtime stimulation protocol.

Disclosure statement

No potential conflict of interest was reported by the authors.

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