Fertilization and Development of a Blastocyst-Stage Embryo After Selective Intracytoplasmic Sperm Injection of a Mature Oocyte from a Binovular Zona Pellucida: A Case Report

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Purpose: Our purpose is to describe the development of a blastocyst-stage embryo after the selective fertilization of a mature oocyte from a binovular zona pellucida by intracytoplasmic sperm injection (ICSI).

Method: A 34-year-old woman underwent intracytoplasmic sperm injection due to severe male-factor infertility. After oocyte retrieval, a binovular zona pellucida including one mature metaphase II oocyte and one immature oocyte at the germinal vesicle stage as well as nine metaphase II oocytes was injected with spermatozoa using a one-to-one approach.

Results: The injected mature oocyte of the binovular zona pellucida showed fertilization as evidenced by the presence of two pronuclei and cleaved to a four-cell embryo on the second day, while the uninjected oocyte showed signs of degeneration. On the third day, this embryo further cleaved to six blastomeres with slight fragmentation and it reached the blastocyst stage on the sixth day.

Conclusions: Selective fertilization of one oocyte from a binovular zona pellucida by ICSI may lead to the development of a morphologically normal blastocyst-stage embryo which can be used for embryo transfer in the presence of a limited number of embryos.

KEY WORDS: binovular zona pellucida; intracytoplasmic sperm injection.

INTRODUCTION

A binovular cumulus mass containing two oocytes, each surrounded by a separate zona pellucida, was first reported by Zeilmaker *et al.* (1), followed by other reports including only a few cases (2–4). Ben-Rafael

et al. (2) and Ron-El *et al.* (3) also reported that an oocyte from such a pair was successfully fertilized and subsequently developed into an embryo by standard in vitro fertilization (IVF). Recently, Safran *et al.* (4) described two cases in which intracytoplasmic sperm injection (ICSI) of a mature oocyte from a binovular zona pellucida allowed fertilization and development of a chromosomally balanced cleavage-stage embryo.

In this report, we further demonstrated that selective fertilization of a mature oocyte from a binovular zona pellucida by ICSI can lead to the development of a blastocyst-stage embryo, which can be used for transfer.

CASE REPORT

A couple was admitted to our Assisted Reproductive Technologies Unit because of severe male-factor infertility. The female partner, aged 34, had regular menstrual cycles and her medical history was unremarkable. Ovarian stimulation was carried out with the combination of gonadotropin-releasing hormone agonist (GnRH α ; Suprecur; Hoechst, Frankfurt, Germany) started on day 21 of the preceding cycle and the injection of follicular stimulating hormone (Metrodin; Serono, Rome, Italy) started on the third day of menstruation at a dose of 300 IU daily using a step-down protocol. Ovarian response was monitored using vaginal ultrasound and estradiol measurements. Oocytes were retrieved 36 hr after human chorionic gonadotropin (hCG) administration (10,000 U; Pregnyl; Organon, Turkey) by vaginal ultrasound-guided puncture of ovarian follicles. Of the 12 oocytes collected, following oocyte denudation with hyaluronidase, 9 were metaphase II, 2 were metaphase I, and 1 was a binovular zona pellucida including two adjacent

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oocytes. These two oocytes were of unequal size, the larger one being at metaphase II with an extruded polar body and the smaller oocyte appearing to be immature with a germinal vesicle (GV). Nine mature metaphase II oocytes and the mature oocyte from the binovular zona pellucida were injected with spermatozoa using a one-to-one approach (Fig. 1). Sixteen hours later, all nine morphologically normal mature oocytes and the injected mature oocyte of the pair showed fertilization as evidenced by the presence of two pronuclei. On the second day, the fertilized oocyte of the pair developed into a four-cell embryo and the second uninjected oocyte showed signs of degeneration. The other nine fertilized oocytes were also cleaved, two- to four-giving cell embryos. On the third day, three high-quality six- to eight-cell embryos obtained after the injection of normal-appearing oocytes were transferred into the uterine cavity and resulted in a clinical pregnancy with a single fetus. The embryo developing from injection of the mature oocyte from a binovular zona pellucida further cleaved to six blastomeres with a slight fragmentation, while the uninjected oocyte degenerated (Fig. 2). In vitro incubation of this embryo was extended and we observed that injected oocytes from a binovular zona pellucida reached the blastocyst stage on the sixth day (Fig. 3), while the adjacent noninjected oocytes degenerated.

DISCUSSION

The occurrence of polyovular follicles is more frequent in the immature ovary but the majority of them are lost due to atresia in late fetal and early neonatal life. Although they can also occur in adult females, it

Fig. 1. After denudation with hyaluronidase, the mature oocyte from a binovular zona pellucida was injected with spermatozoa.

Fig. 2. A six-cell cleaving-stage embryo was obtained after the injection of the mature oocyte from a binovular zona, while the uninjected oocyte degenerated on the third day.

was suggested that their incidence may increase with gonadotropic stimulation in the treatment cycles (4).

There are many hypotheses to explain the occurrence of two or more oocytes within a single zona pellucida (1,2,4) and the most convenient explanation accepted is that a binovular zona pellucida is probably created when granulosa cells fail to separate into two distinct oocytes during the early stages of follicular formation (4). The presence of two equal- or slightly unequal-sized cells does not support an improper division during the first meiosis producing an abnormal first polar body, which is claimed by Ben-Rafael *et al.* (2). The study by Safran *et al.* (4) also ruled out the possibility of the two cells being products of immediate

Fig. 3. The development of a blastocyst-stage embryo after ICSI of a mature oocyte from a binovular zona pellucida and a degenerated uninjected oocyte on the sixth day.

cleavage leading to the formation of two haploid cells. They reported two cases of a binovular zona pellucida and performed a cytogenetic analysis by fluorescent in situ hybridization (FISH) in one case. After selective application of the larger mature oocyte and its fertilization and cleavage, they analyzed the chromosomal status of both the smaller uninjected oocyte and the cleavage-stage embryo obtained. The FISH result of the smaller oocyte showed a chromosome X and 18 disomy, consistent with a diploid GV and FISH analysis of the embryo was consistent with a diploid female, suggesting that the larger cell was indeed a chromosomally balanced, mature metaphase II oocyte. All these reports as well as our case including a larger cell appearing as metaphase II and a smaller one at the GV stage favor the hypothesis that two oocytes from a binovular zona pellucida are actually two oocytes that failed to be separated during the early stage of folliculogenesis.

Ben-Rafael *et al.* (2) and Ron-El *et al.* (3) also showed that one or two oocytes of a binovular zona pellucida can successfully be fertilized and subsequently developed into an embryo by standard IVF. However, such embryonic development may lead to the development of dizygotic twins due to the fertilization of both oocytes or the two conceptuses can aggregate to form a diploid–triploid mosaic embryo (1,4). To avoid this possible undesired consequence, Safran *et al.* (4) recommended the selective fertilization of only one of the two oocytes using ICSI, and they have also proved that a chromosomally balanced embryo

can be obtained after such an approach. Our case also supports this report.

In conclusion, Safran *et al.* (4) were the first to demonstrate chromosomally normal embryo development from a binovular zona by selective fertilization of one oocyte by ICSI. As far as we know, our report shows blastocyst-stage embryo development for the first time. Our report also shows that a binovular zona pellucida representing a morphologically abnormal oocyte is not associated with a catastrophic outcome and selective fertilization of one oocyte of such a pair by ICSI may lead to the development of a morphologically normal blastocyst-stage embryo which can be used for embryo transfer in the presence of a limited number of embryos.

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