Normal pregnancy resulting from a non-pronuclear oocyte at the time of examination for fertilization

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Summary

Purpose: To report the case of a patient undergoing in vitro fertilization (IVF) in which a non-pronuclear (0PN) oocyte resulted in a normal pregnancy. *Methods:* A 36-year-old woman underwent an IVF-embryo transfer treatment cycle. *Results:* Four oocytes were retrieved for insemination by IVF. Examination for fertilization revealed two polypronuclearpolygynic and two non-pronuclear oocytes. The non-pronuclear oocytes were observed further for development. One embryo developed from the non-pronuclear cohort and was transferred at the 8-cell stage on day 3. Subsequently, a pregnancy developed, and resulted in the delivery of a healthy term infant. *Conclusions:* Non-pronuclear oocytes may represent a source of developmentally competent embryos, and further observation of this cohort should be considered, particularly in situations involving a low yield of oocytes at retrieval.

Key words: Fertilization; Infertility; Non-pronuclear.

Introduction

During the process of in vitro fertilization, the assessment of fertilization is typically performed from 16 to 20 hours after insemination or intracytoplasmic sperm injection [1]. Normal fertilization is characterized by the presence of two pronuclei, and these zygotes are observed further for cleavage.

The finding of non-pronuclear (0PN) oocytes at the time of examination for fertilization is not uncommon. In one series, the incidence of 0PN oocytes after intracytoplasmic sperm injection (ICSI) was reportedly 24% [2]. The approach to non-pronuclear oocytes among IVF centers is variable. While some adhere to a policy of continued observation of these oocytes, other centers discard them after the evaluation. We present a case of a normal pregnancy resulting from an oocyte which was non-pronuclear at the time of fertilization evaluation.

Case Report

A 36-year-old Caucasian nulligravida presented with a two-year history of infertility, refractory to three cycles of clomiphene citrate and two cycles of IVF. Hormonal evaluation revealed a day 3 FSH of 5.0 mIU/ml and estradiol of 29 pg/ml. A clomiphene citrate challenge test was performed with a day 10 FSH of 8.6. The patient's history was notable for hyperprolactinemia, for which she was maintained on bromocriptine, 2.5 mg daily, with subsequent normalization of the serum prolactin level. The hysterosalpingogram was normal. Her husband's medical history was unremarkable, and his semen analysis was within normal limits.

The patient proceeded with a third IVF treatment cycle. Ovarian stimulation was conducted with 450 IU/day for eight days of recombinant FSH and human menopausal gonadotropins. Once daily antagonist (ganirelix) injection was initiated when the lead follicle reached a mean diameter of 14 mm. At retrieval timed 35 hours after human chorionic gonadotropin (hCG) injection, four metaphase II oocytes were aspirated from five mature follicles. These were inseminated with 100,000 motile spermatozoa per ml and cultured in IVF medium. At fertilization evaluation 20 hours post insemination, two polypolypronucleargynic oocytes and two non-pronuclear oocytes were documented. Our laboratory policy is to discard digynic/polypolypronucleargynic oocytes and to monitor non-pronuclear oocytes for cleavage potential. On day 2 post retrieval, a four-cell embryo was observed. On day 3, the embryo had developed to the 8-cell stage and assisted hatching was conducted prior to uterine transfer. The patient conceived with a single intrauterine pregnancy confirmed by ultrasound at six weeks of gestational age. She proceeded to deliver a healthy, 3,690 g female infant at 40 weeks of gestation.

Discussion

The series of events that occurs after fertilization of the oocyte are well described. After fertilization, decondensation of the sperm head is followed by extrusion of the second polar body and appearance of the pronuclei. Time-lapse recording of events involved in pronuclear formation after ICSI demonstrate that only 63% of oocytes evidence simultaneous appearance of male and female pronuclei [2]. Asynchrony of pronuclear formation presumably occurs secondary to accelerated formation of either male or female pronuclei. Asynchrony is more frequently observed in IVF than in ICSI [3]. The process of syngamy involves the breakdown of pronuclear membranes and the reorganization of maternal and

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paternal chromosomes to form a zygote. In contrast to their formation, the disappearance of pronuclei is nearly always synchronous. It is possible that in cases of non-pronuclear oocytes, the assessment may be timed such that the pronuclei are missed due to their collectively early or late formation and disappearance.

The finding of 0PN oocytes at the time of fertilization evaluation may be considered evidence of failed fertilization. Yet, the validity of this conclusion is based on accurate identification of organelles and on the allowance of adequate time for the assessment of the pronuclei. Pronuclear formation can be seen as early as six hours post insemination and as late as 20 hours [1]. The concurrent finding of polypronuclearpolygynic oocytes with the 0PN oocytes at the time of the fertilitzation check is significant, suggesting that the window for visualization of pronuclei was not missed.

We conducted a survey of IVF centers in our region. Three of seven (42%) centers reported discarding 0PN oocytes. The remaining centers reported continued observation of these oocytes for cleavage potential. The developmental potential of fertilized oocytes with less than two pronuclei at the time of fertilization check is evidenced by our case.

An extensive search of the international literature on this subject (PubMed, Ovid, Science Direct) shows that this is the first case report of term delivery of a healthy female infant after the transfer of a cleavage stage embryo derived from a non-pronuclear oocyte at fertilization check. We report transfer of a cleavage stage embryo derived from a non-pronuclear oocyte at fertilization check, which resulted in the term delivery of a healthy female infant. As described for 1PN oocytes, the finding of 0PN oocytes at fertilization check is not necessarily indicative of their developmental capacity. Our survey of IVF center practices indicates that monitoring of non-pronuclear oocytes is not universal. The possibility for embryo development from non-pronuclear oocytes may provide an under-recognized source of embryos for subsequent transfer. Surveillance of these oocytes beyond the fertilization check is therefore advisable, particularly for the couple with a low number of oocytes at retrieval.

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