Timing of Second Polar Body Extrusion and Pronuclear Formation After Intracytoplasmic Sperm Injection (ICSI)

SUWIT SUPPINYOPONG, M.D.*,
ROUNGSIN CHOAVARATANA, M.D.*,
CHARUNEE KARAVAKUL, B.Ed.*

Abstract

To determine timing of second polar body extrusion and pronuclear formation to find out when the intracytoplasmic sperm injected (ICSI) oocytes should be checked for successful fertilization.148 oocytes from 15 patients from January to May 1998 were analyzed. Metaphase II oocytes were followed by ICSI. Fertilization was checked at 2, 4, 6, 8, 16 and 18 hours later for observation of second polar body extrusion and pronuclear formation. Normal fertilization was achieved in 73.6 per cent of the oocytes (109/148). After ICSI at 2, 4, 6, 8, 16 and 18 hours, extrusion of second polar body was 8.3 per cent, 49.5 per cent, 84.4 per cent, 98.2 per cent, 98.2 per cent, 98.2 per cent and pronuclear formation was 0 per cent, 0 per cent, 8.3 per cent, 30.3 per cent, 96.3 per cent, 100 per cent respectively. The earliest extrusion of second polar body and pronuclear formation were at 2 and 6 hours after ICSI respectively. This study suggests that the appropriate time to determine fertilization is at 2, 6 and 18 hours after ICSI.

Key word : Second Polar Body Extrusion, Pronuclear Formation, Intracytoplasmic Sperm Injection

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Micromanipulation of the gametes by intracytoplasmic sperm injection (ICSI) has been widely used since 1991 after the first human ICSI pregnancy was reported(1). In recent years, ICSI has become the first treatment of choice for severe male factor infertility and fertilization failure in previous conventional IVF procedures(2). ICSI offers not only a high fertilization and pregnancy rate(3) but also an opportunity to observe consecutive stages of fertilization, since ICSI requires

* Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

cleaning of oocytes from their surrounding cumulus cells within a few hours after retrieval creating clearly to study in oocyte morphology and fertilization. In conventional *in vitro* fertilization (IVF), there were some disadvantages to check early fertilization because intact cumulus and corona cells and oocytes couldn't be visualized directly without destroying them. This problem with oocyte visualization was overcome by removing the cumulus and corona cells and submitting the oocytes either to subzonal injection (SUZI)[4] or to ICSI[5] and early nuclear changes in oocyte at fertilization could be periodically observed, e.g. the extrusion of second polar body and pronuclear formation. Determining the time period of the different fertilization steps and especially the time range when all or most of the oocytes exhibit two pronuclei is also important for practical reasons. In this report, we studied timing of second polar body extrusion and pronuclear formation to find out when the ICSI oocytes should be checked for successful fertilization.

MATERIAL AND METHOD

A total of 148 ICSI oocytes from 15 patients were carried out. At the moment of injection, the status of the first polar body was assessed and fragmented clearly noticed. All injected oocytes were checked 2, 4, 6, 8, 16 and 18 hours after ICSI for the presence of a second polar body and two pronuclei.

Ovarian stimulation

Ovarian stimulation was initiated by the regimen of a combination of GnRH analogue with human menopausal gonadotropin (hMG). Ovarian response was monitored by ultrasonography. When at least two leading follicles reached 18 mm in diameter, 10,000 units of human chorionic gonadotropin (hCG) were administered. Oocyte retrieval was performed by transvaginal ultrasound-guided aspiration 36 hours later.

Oocyte preparation

The cumulus cells surrounding oocytes were removed by needle dissection and pipetting, then treated with 80 U/ml hyaluronidase for 30-60 seconds to complete removal of cumulus cells. The oocytes were washed 4 times and incubated in culture medium. Only the mature oocytes (metaphase II) were selected for further processing.

Sperm preparation

Fresh semen was obtained just after oocyte retrieval. Motile spermatozoa were separated by the discontinuous Percoll gradient centrifugation method.

Intracytoplasmic sperm injection

ICSI was performed with the aid of two commercial pipettes under an inverted microscope. Grossly normal spermatozoa were chosen and immobilized by touching the midpiece, then aspirated into the injection pipette. The metaphase II oocyte was held by a suction holding a pipette and injecting one spermatozoa after aspirating some ooplasm. The injected oocytes were washed and incubated in culture medium at 37°C in an atmosphere of 5 per cent CO_2.

Assessment of polar body extrusion and fertilization

At the moment of injection, the status of the first polar body was assessed and when fragmented clearly noticed. All injected oocytes were checked on an inverted microscope 2, 4, 6, 8, 16 and 18 hours after ICSI for the presence of a second polar body and two pronuclei. In case of fragmented first polar body, the presence of a second polar body was admitted only when it was clearly and separately distinguished from the fragments as a well-shaped glob.

RESULTS

Patients' background and fertilization outcome are shown in Table 1 and Table 2. A total of 178 oocytes from 15 patients were collected. Of these, 148 oocytes (83.1%) were mature in metaphase II and could be injected. 24 oocytes (16.2%) degenerated following ICSI procedure. The remaining 124 injected oocytes were further observed for fertilization and we found that 13 oocytes (8.8%) were non fertilized and 2 oocytes (1.4%) were abnormal fertilization (3PN). Normal fertilization rate was 73.6 per cent (100/148 oocytes) Table 3 shows the time course of fertilization after ICSI. The earliest time that oocytes extruded a second polar body and pronuclear formation was 2 and 6 hours respectively (8.3%, 8.3%) Almost all oocytes extruded a second polar body and pronuclear formation at 8 and 18 hours respectively (98.2%, 100%).

Table 1. Background details.

	Mean ± SD	Range
Patient's age (years)	34.0 ± 4.0	24-39
Husband's age (years)	39.4 ± 5.7	30-50
Infertility period (years)	5.7 ± 2.5	2-10
Doses of hMG (ampoules)	23.7 ± 6.0	12-36
Number of oocytes retrieved	9.9 ± 4.9	3-20

Table 2. Fertilization outcome.

Total oocytes retrieved	178
Metaphase II oocytes	148 (83.1%)
Degenerated oocytes	24 (16.2%)
Non-fertilized oocytes	13 (8.8%)
Abnormal fertilized oocytes	2 (1.4%)
Normal fertilized oocytes	109 (73.6%)

Table 3. Time course of fertilization after ICSI.

Hours after ICSI	Second polar body	2 pronuclei
2	9 (8.3%)	0
4	54 (49.5%)	0
6	92 (84.4%)	9 (8.3%)
8	107 (98.2%)	33 (30.3%)
16	107 (98.2%)	105 (96.3%)
18	107 (98.2%)	109 (100%)

DISCUSSION

After ICSI procedure, the consecutive stages of fertilization can be clearly observed. Oocytes can complete second meiotic division within 3 hours proved by extrusion of the second polar body[6]. In 1997, Payne[5] used time-lapse video cinematography for observation of human oocytes fertilization and they found that there was a 20-53 minutes periodic cytoplasmic wave within the ooplasm. The sperm head decondensed then the second polar body was extruded and the cytoplasmic wave ceased. The earliest time of extrusion was 1 hour 24 minutes. There was cytoplasmic flare before a male pronucleus centrally appeared, accompanied, simultaneously or shortly thereafter by the appearance of a female pronucleus which was smaller than the male pronucleus (22.4 and 24.1 micron, respectively) and contained fewer nucleoli (4.2 and 7.0 respectively)[7] Using any two of three criteria (diameter, nucleoli and location), we were able to correctly identify pronucleus as male or female 95 per cent of the time. Cytoplasmic flare might be related to the Mazia cell body, which was an area of cytoplasm organized by the centrosome and included microtubule motor proteins drawing the female to the male pronucleus[8]. Pronuclei could appear as early as 6 hours[9] but most were between 9-20 hours[10].

Time course of second polar body extrusion was probably dependent on the maturity of the ooplasma on the ability of decondensation of the sperm chromatin. The extrusion of the second polar body at 2 hours and appearance of earliest pronuclei at 6 hours indicated that about 4 hours was needed for pronuclear formation from the end of meiosis II.

In ICSI process, the time of incorporation of the spermatozoon in the cytoplasm is always known precisely, therefore, the time course of fertilization will depend only on the sperm cell and oocyte. Determining the fertilization periods and exhibition of two pronuclei are important for practical reasons to check for successful fertilization after ICSI procedure.

In conclusion, our study suggests that oocyte activation (extrusion of the second polar body) after ICSI occurs as early as 2 hours, the first visible pronuclear development is observed at 6 hours and all pronuclei appear at 18 hours after ICSI, so the appropriate time to determine fertilization is at 2, 6 and 18 hours after ICSI.

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ระยะเวลาการปรากฏของ second polar body และ pronuclear ภายหลังการฉีดอสุจิเข้าภายในไข่

สุวิทย์ ศุภภิญโญพงศ์, พ.บ.,*
เรืองศิลป์ เชาวรัตน์, พ.บ., จารุณี คารวะกุล, ค.บ.**

ทำการศึกษาหาระยะเวลาการปรากฏของ second polar body และ pronuclear ภายหลังการฉีดอสุจิเข้าไปในไข่เพื่อหาระยะเวลาที่เหมาะสมในการตรวจสอบการปฏิสนธิ โดยทำการศึกษาในไข่ 148 ฟองจากคู่สมรสที่มีบุตรยาก 15 ราย ทำการฉีดอสุจิเข้าภายในไข่ซึ่งอยู่ในระยะ metaphase II ตรวจสอบการปฏิสนธิโดยสังเกตการปรากฏของ second polar body และ pronuclear ที่เวลา 2, 4, 6, 8, 16, 18 ชั่วโมงภายหลังการฉีดอสุจิเข้าภายในไข่ พบการปฏิสนธิปกติ 73.6% ที่เวลา 2, 4, 6, 8, 16, 18 ชั่วโมงภายหลังการฉีดอสุจิเข้าภายในไข่ ตรวจพบการปรากฏของ second polar body 8.3%, 49.5%, 84.4%, 98.2%, 98.2%, 98.2% และการปรากฏของ pronuclear 0%, 0%, 8.3%, 30.3%, 96.3%, 100% ตามลำดับ ระยะเวลาที่เร็วที่สุดในตรวจพบการปรากฏของ second polar body และ pronuclear ภายหลังการฉีดอสุจิเข้าภายในไข่ คือ ที่ 2 และ 6 ชั่วโมงตามลำดับ ดังนั้น ระยะเวลาที่เหมาะสมในการตรวจสอบการปฏิสนธิภายหลังการฉีดอสุจิเข้าภายในไข่ คือ ที่ 2, 6 และ 18 ชั่วโมง

คำสำคัญ : การปรากฏของ second polar body, การปรากฏของ pronuclear, การฉีดอสุจิเข้าภายในไข่

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* ภาควิชาสูติศาสตร์นรีเวชวิทยา, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ 10700