Chromosomal Analysis of "Failed-fertilized" Human Oocytes Resulting from *In-vitro* Fertilization and Intracytoplasmic Sperm Injection

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Abstract

To evaluate the incidence of chromosomal abnormalities in "failed-fertilized" oocytes derived from *in-vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) procedure, a cytogenetic analysis was performed on 164 IVF and 64 ICSI oocytes. One hundred and eleven (67.7%) of the IVF and 56 (87.5%) of the ICSI oocytes were successfully karyotyped. Of 111 IVF oocytes, 73 (65.8%) exhibited normal haploid and 38 (34.2%) were abnormal. The abnormalities included 25 aneuploid (22.5%) (7 hyperhaploid and 18 hypohaploid), 11 diploid (9.9%) and 2 structural anomalies (1.8%). Of 56 ICSI oocytes, 52 (92.8%) were normal haploid and only 4 (7.2%) were aneuploid, with 2 hyperhaploid and 2 hypohaploid. The sperm nuclei were observed in 43 IVF oocytes (38.7%), composed of 38 (34.2%) premature chromosome condensation (PCC) and 5 (4.5%) decondensed sperm heads. Evidence of successful sperm delivery was found in all 56 ICSI oocytes ; 25.0 per cent (14/56) showed PCC, 17.9 per cent (10/56) showed decondensed sperm heads, and 57.1 per cent (32/56) showed intact sperm heads. This study suggested that about one-third of unfertilized oocytes exhibited chromosomal abnormalities. The difference of aneuploidy between IVF and ICSI oocytes needs further studies analysing a larger number of oocytes.

Key word : Chromosomal Analysis, "Failed – fertilized" Oocytes, *In-vitro* Fertilization, Intracytoplasmic Sperm Injection

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It has been reported that more than 70 per cent of human embryos die during the early stages of pregnancy[1,2]. In spite of improvements in *in-vitro* fertilization (IVF) programmes and technical advances in intracytoplasmic sperm injection (ICSI) procedure, nearly 80 per cent of the transferred embryos do not implant, and the abortion rate in this group of patients is twice that of spontaneous pregnancies. Chromosomal aberrations have been shown to be the major cause of the pregnancy wastage[3,4]. It is believed that these abnormalities are more frequent during the meiotic process, especially in females[5].

Chromosomal analysis, of "failed-fertilized" oocytes, could help to elucidate the phenomenon of early embryonic loss. For unfertilized oocytes, aneuploidy rates have been reported ranging from 10.9 per cent to 57.7 per cent[6], depending on technical characteristics and interpretation.

Cytogenetic studies of human oocytes became routine in the early 1980's[7,8] with the development of *in-vitro* fertilization techniques. The recent development of the intracytoplasmic sperm injection technique for the treatment of severe male factor of infertility has been argued that it may increase the incidence of chromosome abnormalities in the embryos derived from this technique[9]. Although many studies of chromosome abnormalities of unfertilized human oocytes after IVF have been reported[6,10-12], only a few cytogenetic studies were carried out using uncleaved zygotes after ICSI[13-15].

The present study evaluated the incidence of chromosomal abnormalities in failed-fertilized human oocytes after conventional IVF and ICSI procedures.

MATERIAL AND METHOD

Material for this study was obtained from a series of 62 patients (71 cycles) who underwent an IVF-embryo transfer and 32 patients (35 cycles) from the ICSI programme at Siriraj Hospital. A total of 228 oocytes were derived from April 1998 to March 2000, selection was based only on availability of both oocytes and staffs. The mean age of the female patients was 34.7 years (range 28-42) for IVF and 33.9 years (range 25-42) for ICSI cycles. The indications for IVF were tubal obstruction, endometriosis, ovulatory dysfunction, and unexplained infertility, and those for ICSI were previous failure of IVF and male subfertility.

Hormonal stimulation for multi-follicular growth was performed by a short protocol of gonadotropin-releasing hormone agonist (GnRHa) and human menopausal gonadotropin (hMG) regimen. Buserelin (600 μg) was administered intranasally from day 1 and hMG (150-300 IU) was injected from day 3 of the cycle followed by human chorionic gonadotropin (hCG) injection (10,000 IU) when at least two follicles reached 18 mm in diameter. About 34-36 hours after hCG injection, the follicles were aspirated under transvaginal ultrasound guidance. Semen was obtained by masturbation from the husbands and was prepared by the mini-Percoll gradient centrifugation method[16].

For IVF, oocytes were transferred to four-well dishes containing universal IVF medium (Medi-Cult, Copenhagen, Denmark) and incubated for 4-6 hours at 37°C, 5 per cent CO_2 in air before insemination. For ICSI, oocytes were preincubated for 3-4 hours, then treated with 80 IU/ml hyaluronidase for 30-60 seconds. The microinjection procedure was performed as described by Palermo et al[9]. Briefly, injection pipettes with an inner diameter of 5-6 micron were used to aspirate spermatozoa from the sperm droplet and to place them into the 10 per cent polyvinylpyrrolidone. Sperm was immobilized and loaded into the injection pipette and injected into the center of the oocyte after aspirating some ooplasm. The injected oocytes were washed four times and incubated in the culture medium at 37°C, 5 per cent CO_2 in air. Two days after insemination, all normally fertilized embryos were transferred or cryopreserved.

The failed-fertilized oocytes were prepared for assessing the chromosomal content using the method described by Tarkowski[17]. Briefly, the oocyte was treated with hypotonic solution (1% sodium citrate) for 10-15 minutes at room temperature. Each treated oocyte was then transferred in a microdrop of sodium citrate to a grease-free slide. Under close monitoring with a dissecting microscope, a freshly prepared fixative (methanol : acetic acid, 3:1) was added drop by drop. The slide was left to air dry and then stained with Giemsa solution for 20 minutes.

Chi-square test was used to analyze the difference in number of chromosomally abnormal

Table 1. Chromosomal analysis of "failed-fertilized" oocytes obtained from IVF and ICSI.

	No. of oocytes (%)*		p-value
	IVF	ICSI	
Oocytes used for study	164	64	
Analyzable oocytes	111	56	
Normal	73 (65.8)	52 (92.8)	
Abnormal	38 (34.2)	4 (7.2)	< 0.001
Aneuploidy	25 (22.5)	4 (7.2)	0.013
Diploidy	11 (9.9)	-	0.017
Structural anomaly	2 (1.8)	-	0.55

* Values in parentheses are per cent of analyzable oocytes

Table 2. Details of aneuploidy found in IVF and ICSI "failed-fertilized" oocytes.

	No. of aneuploidy (%)		p-value
	IVF (n=25)	ICSI (n=4)	
Hyperhaploidy	7 (28.0)	2 (50.0)	0.57
Hypohaploidy	18 (72.0)	2 (50.0)	

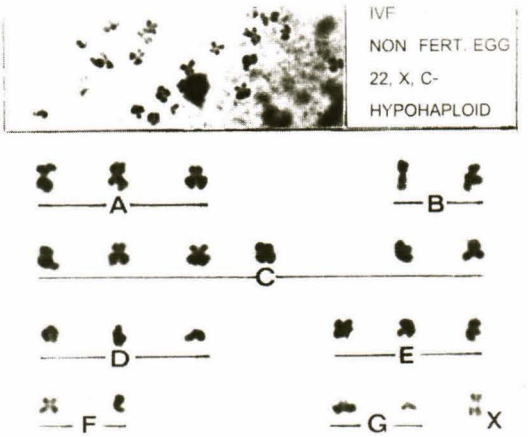

Fig. 1. Chromosome spread and karyotype of a hypohaploid oocyte (22, X, -C) prepared after IVF.

oocytes between IVF and ICSI failed-fertilized oocytes. This test was also used to determine the different types of aneuploid chromosomes and abnormal sperm heads found in IVF and ICSI oocytes.

All oocytes used in this study were donated by infertile couples treated in our clinic. The study was approved by the ethical committee of the Faculty of Medicine Siriraj Hospital, Mahidol University.

RESULTS

Of 164 oocytes collected from IVF, 111 oocytes (67.7%) were analyzable, and of 64 oocytes from ICSI, 56 oocytes (87.5%) could be analyzed. In the remaining 61 oocytes, the analysis was impossible because of scattered chromosomes, or poorly spread chromosomes.

The results of chromosomal analysis in 111 failed-fertilized oocytes from IVF and 64 oocytes from ICSI are shown in Table 1. The karyotypes of 73 oocytes (65.8%) from IVF were normal and 38 (34.2%) were abnormal. For 56 analyzable oocytes from ICSI, 52 (92.8%) exhibited a normal haploid, only 4 (7.2%) were abnormal. The incidence of abnormal chromosomes was significantly higher in IVF than in ICSI oocytes ($p<0.001$).

Of the abnormal IVF oocytes, 25 (22.5%) were aneuploid, 11 (9.9%) were diploid, and 2 (1.8%) had structural chromosomal anomalies. For ICSI oocytes, only 4 (7.2%) of aneuploid were found. The incidence of aneuploidy was significantly higher in IVF oocytes ($p<0.05$).

The details of aneuploidy found in IVF and ICSI failed-fertilized oocytes are shown in Table 2. Aneuploidy of 25 IVF oocytes consisted of 7 hyperhaploids (28.0%) and 18 hypohaploids (72.0%) (Fig. 1). Of 4 aneuploid ICSI oocytes, both 2 hyperhaploids (Fig. 2) and 2 hypohaploids (50% each) were found. The rates of hyper - and hypohaploidy between IVF and ICSI oocytes were not significantly different ($p = 0.57$). This may be due to the small numbers of ICSI oocytes available for analysis.

Table 3 shows sperm nuclei in the analyzable oocytes obtained from both procedures. The sperm nuclei could be observed in 43 (38.7%) of the failed- fertilized IVF oocytes. These unfertilized oocytes were in fact penetrated by a sperm but failed to develop further. In these oocytes, 38 (34.2%) showed premature chromosome condensation (PCC) of sperm nuclei and 5 (4.5%) decondensed sperm head. Unlike IVF, the sperm nuclei could be demonstrated in all of 56 failed–fertilized

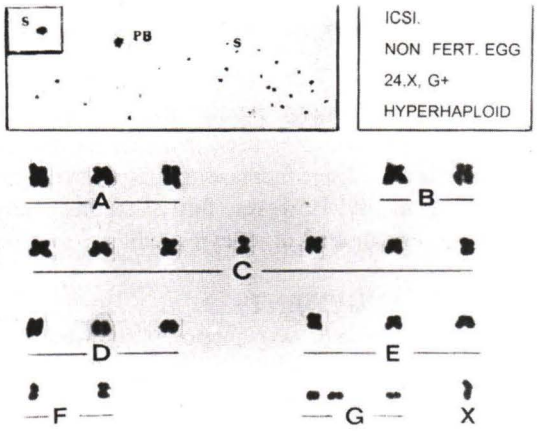

Fig. 2. Chromosome spread and karyotype of a hyperhaploid oocyte (24, X, +G) prepared after ICSI (PB = polar body, S = sperm).

ICSI oocytes ; PCC was observed in 14 (25.0%), decondensed sperm head in 10 (17.9%), and intact sperm head in 32 (57.1%) of the oocytes.

DISCUSSION

Failure of fertilization in IVF may be caused by suboptimal culture system, the abnormal sperm, or abnormal oocytes. Besides the use of the ICSI procedure in severe male subfertility has generated more concern about causing chromosomally abnormal embryos. Chromosomal analysis of failed-fertilized oocytes obtained from both procedures is important in determining the causal factors and mechanisms of chromosomal aberrations. The unfertilized oocytes after conventional IVF in this study showed 34.2 per cent of chromosomal abnormalities. This agreed with many other studies[18-22], although previous data reported the incidence of chromosomal abnormalities varied considerably from 18 per cent to 66.7 per cent[6,12,23-25]. The incidence of hypohaploidy and hyperhaploidy in this study was 16.2 and 6.3 per cent, respectively. The significantly more hypohaploid than hyperhaploid oocytes may due to some artificial loss of chromosomes during slide preparation. However, the findings confirm the previously suggested hypothesis of a high incidence of nondisjunction at meiosis I. Kamiguchi[6] suggested that hypohaploid oocytes are caused not only by nondisjunction but also by anaphase lagging, giving the higher incidence of hypohaploidy than that of hyperhaploidy. In the present study, the incidence of diploidy was high (9.9%). The diploid oocytes were produced because of no extrusion of the first polar body in the meiosis I. The incidence of structural anomaly (1.8%) was slightly lower than that previously reported. Aneuploidy rate found in this study for failed-fertilized oocytes after ICSI was very low (7.2%), when compared to 22.5 per cent in IVF oocytes. This may be partly due to the well selected oocytes in the ICSI procedure. Other studies showed the incidence of aneuploidy rate after ICSI ranged from 5.9 per cent [15] to 32 per cent[13,14]. A larger number of uncleaved zygotes after ICSI is needed to give more reliable information regarding the frequency of chromosomal abnormality.

Table 3. Sperm nuclei contained in "failed-fertilized" oocytes obtained from IVF and ICSI.

	No. of oocytes (%)*		p-value
	IVF	ICSI	
Premature chromosome condensation (PCC)	38 (34.2)	14 (25.0)	0.298
Decondensed sperm head	5 (4.5)	10 (17.9)	0.010
Intact sperm head	-	32 (57.1)	<0.001

* Values in parentheses are per cent of analyzable oocytes

Failure of postfertilization development was observed in 43 of the 111 cases studied (38.7%). Other investigators reported the incidence ranged from 22.8 per cent to 36 per cent[20,23,26]. These oocytes are arrested in metaphase II in spite of the presence of sperm in the individual ooplasm. The sperm head is found in various stages, i.e. intact sperm head without transformation, decondensed sperm head, or PCC. Premature chromosome condensation is a phenomenon in which the chromosomes of the oocyte are arrested in metaphase II, allowing the persistence of cytoplasmic chromosome condensing factors, leading to the induction of PCC in the sperm nucleus[25,27]. The frequency of this phenomenon varies from 3 per cent to 16.9 per cent[21,23], but could be as high as 58.2 per cent, depending on the manner of calculation[23]. In this study, the incidence of PCC was 34.2 per cent for unfertilized oocytes from IVF, and 25 per cent from ICSI oocytes.

All failed-fertilized oocytes derived after ICSI contained a sperm head. The percentages of PCC between IVF and ICSI (p=0.298) were not different. However, 57.1 per cent of sperm in ICSI oocytes did not transform, whereas, none of sperm in the IVF oocytes remained intact. This implied that some of failed- fertilized oocytes were not ready for fertilization. They may have lacked some oolemma and ooplasmic factors, causing the failure of sperm to enter the oocyte in IVF and even after the enforcement of sperm by ICSI, causing the failure of sperm to transform to male pronuclei.

In conclusion, cytogenetic analysis of "failed-fertilized" human oocytes showed approximately one-third of fertilization failure, the causes were genetic or physiological and lay with the oocyte. The procedure of ICSI that pierces into the egg cytoplasm did not increase the rate of chromosomal abnormality. However, further studies analysing a larger number of oocytes will be needed.

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การวิเคราะห์โครโมโซมของไข่ที่ไม่ปฏิสนธิและตัวอ่อนที่ไม่แบ่งตัวจากการปฏิสนธินอกร่างกายและการฉีดอสุจิเข้าไปในไข่

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เพื่อศึกษาอุบัติการณ์ของโครโมโซมผิดปกติในไข่ที่ไม่ปฏิสนธิจากวิธีการปฏิสนธินอกร่างกาย (IVF) และในตัวอ่อนที่ไม่แบ่งตัวจากวิธีการฉีดอสุจิเข้าไปในไข่ (ICSI) จึงได้นำไข่ที่ไม่ปฏิสนธิจากวิธีแรกจำนวน 164 ใบ และตัวอ่อนที่ไม่แบ่งตัวจากวิธีหลังจำนวน 64 ใบ มาตรวจวิเคราะห์โครโมโซมดู ไข่จากวิธีแรกสามารถตรวจโครโมโซมได้ 111 ใบ (67.7%) และจากวิธีหลังตรวจได้ 56 ใบ (87.5%) สำหรับไข่จากวิธีการปฏิสนธินอกร่างกาย พบว่าโครโมโซมปกติ 73 ใบ (65.8%) และผิดปกติ 38 ใบ (34.2%) ในจำนวนที่ผิดปกตินั้น 25 ใบ (22.5%) เป็น aneuploid (hyperhaploid 7 ใบ และ hypohaploid 18 ใบ) 11 ใบ (9.9%) เป็น diploid และ 2 ใบ (1.8%) เป็น structural anomaly ส่วนตัวอ่อน 56 ใบ จากวิธีการฉีดอสุจิเข้าไปในไข่ พบว่าโครโมโซมปกติ 52 ใบ (92.8%) และผิดปกติเพียง 4 ใบ (7.2%) โดยเป็น hyperhaploid 2 ใบ และ hypohaploid 2 ใบ เมื่อตรวจดูนิวเคลียสของอสุจิ พบในไข่จากวิธีการปฏิสนธินอกร่างกาย 43 ใบ (38.7%) โดย 38 ใบ (34.2%) เป็น premature chromosome condensation (PCC) และ 5 ใบ (4.5%) เป็น decondensed sperm heads ส่วนในตัวอ่อนที่ไม่แบ่งตัว 56 ใบ จากวิธีการฉีดอสุจิเข้าไปในไข่ พบว่ามีอสุจิเข้าไปในไข่ทุกใบ โดยเป็น PCC ร้อยละ 25 decondensed sperm heads ร้อยละ 17.9 และ intact sperm heads ร้อยละ 57.1 จากการศึกษานี้สรุปได้ว่าประมาณ 1 ใน 3 ของไข่ที่ไม่ปฏิสนธิมีความผิดปกติของโครโมโซม ส่วนความแตกต่างของ aneuploidy ที่พบในไข่จากวิธีทั้งสองจำเป็นจะต้องมีการศึกษาต่อโดยต้องวิเคราะห์ในไข่ที่มีจำนวนมากกว่านี้

คำสำคัญ : การวิเคราะห์โครโมโซม, ไข่ที่ไม่ปฏิสนธิ, ตัวอ่อนที่ไม่แบ่งตัว, การปฏิสนธินอกร่างกาย, การฉีดอสุจิเข้าไปในไข่

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