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SHORT COMMUNICATION

Embryonic Chromosomal Abnormalities Obtained After Rescue Intracytoplasmic Sperm Injection of 1-Day-Old Unfertilized Oocytes

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***Purpose*:** To study if second day intracytoplasmic sperm injection (ICSI) results in chromosomal abnormalities in the embryos.
***Methods*:** Rescue ICSI was performed on 14 metaphase II (MII) oocytes after unsuccessful conventional IVF, four were fertilized. Fluorescent in situ hybridization (FISH) was performed on these four embryos and was informative for three.
***Results*:** There were two tetraploid embryos, one mosaic embryo with trisomy 21, tetrasomy 18, and tetrasomy for sex chromosomes in one cell and trisomy 22 in another cell.
***Conclusions*:** We discourage the use of second day ICSI due to the observed increase in chromosomal abnormalities in these embryos.

KEY WORDS: FISH; IVF; ICSI; preimplantation development.

INTRODUCTION

Unexpected fertilization failure after conventional in vitro fertilization (IVF) is an important and distressing problem to the couple and the physician. The causes of fertilization failure can be multiple, occurring at any stage of the fertilization process. Since the use of ICSI overcomes some of the causes of fertilization failure, the use of second day ICSI has been suggested and employed as a possible approach in this unexpected situation (1–3), or even has been suggested as a prognostic test for future procedures (4).

Until now, there are several reports on fertilization rates, pregnancy rates, and embryo quality in embryos achieved by this method. Reported fertilization rates using this procedure vary from as low as 28% to as high as 60% (1–3,5), however; pregnancy rates in general are very low (1–3), and higher 3PN formation has been observed (2,3,6). The timing of rescue ICSI is crucial since as the oocyte ages, the success rate decreases, with decrease in normal fertilization rates and increase in 3PN formation (6). In a recent report, the ideal timing for ICSI on aged oocytes has been reported to be between 19 and 22 h after initial oocyte insemination (5).

The chromosomal constitution of embryos obtained after this procedure has been tested in only two reports. One report using Giemsa staining showed that 5 out of 10 embryos were abnormal (6), and the other report using FISH in two embryos showed that one of them was aneuploid and the other was normal (3).

Here we report the case of a couple with an unexpected fertilization failure in whom rescue ICSI was performed 21 h after the initial insemination and FISH analysis of the embryos for chromosomes 13, 16, 18, 21, 22, X, and Y, revealed that all of them were chromosomally abnormal.

Case Report

A healthy couple, female 35 years old and male 39 years old, was evaluated after a history of primary infertility with operated severe endometriosis. The couple previously had undergone three artificial inseminations, and three IVF cycles. The woman had normal ovulatory cycles and a normal hysterosalpingogram. Her hormonal profile on day 3 of the cycle was normal. The seminogram was normal, with a concentration of 32 millions/mL, a progressive motility of 80%, and 30% normal morphology.

Ovarian stimulation was carried out by standard procedures and oocyte retrieval performed as described elsewhere (7). Twenty two oocytes were obtained and were inseminated 5 h after oocyte pick-up, with 100,000 progressive motile spermatozoa/mL. Fertilization was checked 18 h later; two were normally fertilized (two polar bodies and two pronuclei), one had two polar bodies, and no pronuclei, 11 were unfertilized with one polar body and no pronuclei and 8 were metaphase I. On the second day, 21 h after the initial insemination, ICSI was performed on the 11 previously unfertilized MII, 1-day old oocytes and on the 3 initially MI oocytes, which had reached MII stage by this time. Normal fertilization was achieved in 4 of the 11 previously unfertilized metaphase II, 1-day old oocytes and in none of the initially metaphase I oocytes.

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On day 3 after ICSI, the four embryos were fixed for FISH analysis using methanol: acetic acid [3:1]. At the day of the fixation, two embryos were arrested at the two-cell stage, one was arrested at the three-cell stage, and one had developed to 7-cell stage.

FISH procedure was carried out using probes for chromosomes 13, 16, 18, 21, 22, X, and Y (Vysis Inc., Downers Grove, IL) in three consecutive rounds as previously described (8). Briefly, one round for chromosomes 13 and 21, followed by a second round for chromosomes 16 and 22, and a third round for chromosomes X, Y, and 18 was performed. FISH results were as follows; two-cell stage embryos were tetraploids with one having a sex chromosome constitution of XXYY, and the other XXXX, the embryo at three-cell stage was noninformative by FISH analysis and the embryo at seven-cell stage was a mosaic with chromosomal abnormalities observed in 2 out of 7 cells analyzed, there was trisomy for chromosome 21, tetrasomy for chromosome 18 and the sex chromosomes in one cell, and trisomy 22 in another cell (Fig. 1).

DISCUSSION

The causes of fertilization failure can be multiple. Fertilization failure after IVF can be caused by failure of sperm penetration, failure of oocyte activation, and defects in pronuclei apposition (9). Also, there could be a problem of timing in the visualization of the pronuclei that they have already disappeared or the formation is retarded. This situation may account for the increase in 3PN obtained after rescue ICSI in an already fertilized oocyte. It is possible that the fertilization failure may be due to an abnormality in the sperm but then rescue ICSI is going to aggravate this situation using the same sperm in an aged oocyte. Whatever explanation that we may seek for this unwanted situation, second day ICSI seems to be a simplistic approach to solve it.

We report here the presence of chromosomal abnormalities in all embryos resulting from rescue ICSI analyzed by FISH. The presence of chromosomal abnormalities in a cohort of embryos with low quality (arrested, irregular embryos) can be attributed to many factors. Nevertheless, since the

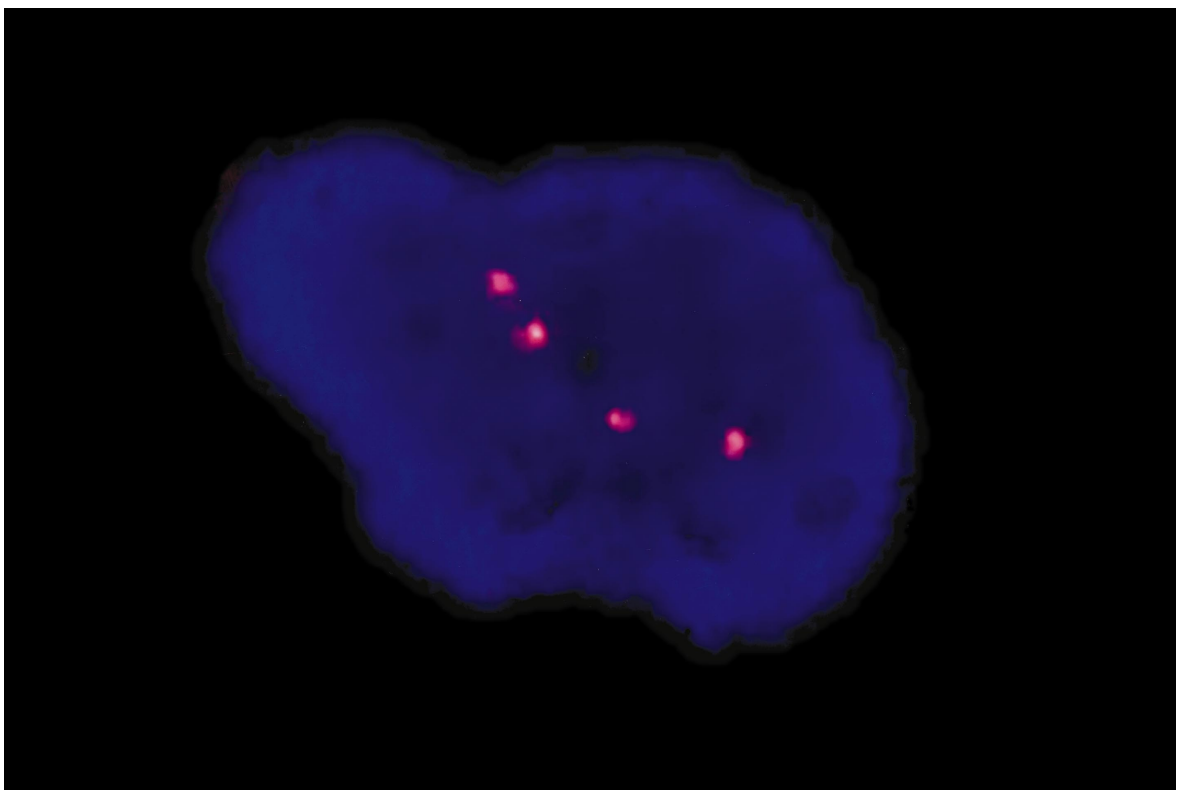

Fig. 1. Tetraploid embryo showing four signals for chromosome X (spectrum orange). Magnification 1000×.

origin of these embryos is second day ICSI, it is important to bear in mind the high probability of chromosomal abnormalities as well as the low embryo quality that may occur due to the intervention. Our findings agree with the previous reports that considered this approach as an unsafe procedure because of the high risk of chromosomal abnormalities.

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