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Does ICSI for in vitro fertilization cause more aneuploid embryos?

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Abstract

Background: High proportion of human embryos produced by in vitro fertilization (IVF) is aneuploidy. Many factors are related to the prevalence of embryonic aneuploidies, such as maternal age, sperm quality, and in vitro manipulation of oocytes. Oocytes are usually inseminated by intracytoplasmic sperm injection (ICSI) procedures for preimplantation genetic testing. There is still no available information whether insemination procedures, regular IVF or ICSI, affect embryonic aneuploidies.

Methods: In this case report, a patient at her age of 47 years old received donated oocytes from a young donor for infertility treatment. Half of oocytes were inseminated by regular IVF and other half of oocytes were inseminated by ICSI. Fertilized oocytes were cultured to blastocyst stage and then biopsied for preimplantation genetic testing for aneuploidies (PGT-A). The proportions of aneuploidies were compared between two insemination procedures.

Results: Forty-seven oocytes were retrieved, 23 were inseminated by regular IVF and 24 were removed from enclosed cumulus cells for ICSI. Out of 24 oocytes, 21 oocytes at metaphase II were inseminated by ICSI. After fertilization assessment, it was found that 12 oocytes from regular IVF fertilized normally. Nine blastocysts (75%) were biopsied and 1 (11.1%) was aneuploidy. By contrast, 19 out of 21 oocytes inseminated by ICSI fertilized normally, 14 blastocysts (73.7%) were obtained and 7 (50.0%) were aneuploidy. Transfer of a euploid blastocyst from regular IVF resulted in a healthy baby delivery.

Conclusion: These results indicate that more embryos produced by ICSI are aneuploidy as compared with embryos produced by regular IVF. The results indicate that in vitro manipulation of oocytes for ICSI procedure may have adverse effect on human oocytes, and it may be one of the reasons causing aneuploid embryos in human IVF.

Keywords: Oocytes, Regular IVF, Blastocysts, ICSI, Aneuploidy

Background

Preimplantation genetic testing for aneuploidies (PGT-A) has been widely applied in human in vitro fertilization (IVF), and has been one of embryo selection approaches apart from embryo morphology and time-lapse culture with morphokinetic embryo selection [1–7]. However, high proportion of human embryos produced by IVF is aneuploidy that cannot be revealed by morphological assessment and morphokinetic embryo selection, thus PGT-A is considered as a valuable procedure to screen embryos' genetic status [1–5]. With PGT-A procedure, euploid embryos can be selected to transfer, which eventually can increase embryo implantation and reduce repeated implantation failures and birth defects [8–10].

Current PGT-A technology includes blastocyst biopsy and chromosomal screening by next generation sequencing (NGS) of whole chromosomes that can provide accurate chromosomal information including chromosomal number, chromosomal deletion and duplications [11–13]. Embryo biopsy at the blastocyst stage is a critical step to obtain samples for accurate chromosomal testing. For this

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purpose, oocyte insemination by intracytoplasmic sperm injection (ICSI) has been recommended as contamination by cumulus cells and/or sperm during regular IVF can be avoided [14].

Maternal age is one of the important factors affecting embryonic aneuploidy [15–27]. However, it has been found that aneuploidy rate in human IVF is quite high even when oocytes are collected from young patients or from young oocyte donors [15, 18–31]. The reasons for such high aneuploidy rates have not been addressed completely. Because most human aneuploid embryos are originated from oocytes [23, 32] and maternal age is an unquestionably factor to contribute to high aneuploidy rate in human embryos [15–19, 24]. Recently, a study reported that embryo aneuploidy rates from oocyte donor cycles are related to IVF laboratories [33]. Therefore, there must be other factors, except maternal ages, are related to embryo aneuploidy. One of the factors may be insemination procedures because oocyte second meiosis occurs during oocyte fertilization. Disruption of a normal meiosis can cause chromosomal errors during meiosis and eventually result in embryonic aneuploidy formation [23, 32, 34–36].

In the present case report, a patient received IVF treatment with donor oocytes with half of oocytes being inseminated by regular IVF and other half of oocytes being inseminated by ICSI. We found that different aneuploidy rates were present between two insemination procedures, thus we report this case and the information may be meaningful for physicians and clinical embryologists.

Methods

Donor stimulation for oocyte retrieval

The oocyte donor (34 years old) underwent controlled ovarian stimulation for 11 days with a combination of daily injection of 150 IU recombinant follicle-stimulating hormone (Gonal-F, EMD Serono, MA, USA) and 150 IU of a combination of follicle stimulating hormone and luteinizing hormone (Menopur, Ferring Pharmaceuticals, NJ, USA). On day 7, 0.25 mg gonadotropin releasing hormone antagonist (Cetrotide, EMD Serono) was given daily until triggering for oocyte maturation by 4 mg gonadotropin-releasing hormone agonist (Lupron) on Day 12 and 13. Oocytes were retrieved at 36 h after the first Lupron and then cultured in Global™Total medium at 37 °C in an atmosphere of 5.5% CO_2, 6% O_2, and balanced N_2 under humidified conditions.

Oocyte insemination by ICSI and regular IVF

For ICSI, cumulus cells were removed by using hyaluronidase (Fujifilm-Irvine Scientific, CA, USA) at 4 h after oocyte retrieval and metaphase II oocytes were injected 5 h after retrieval. For regular IVF, oocyte-cumulus complexes were inseminated directly in the organ culture dishes with 135,000 motile sperm/ml at 5 h after retrieval.

Assessment of fertilization, embryo quality and blastocyst biopsy

Fertilization was assessed 18 h after insemination, and normal fertilization was characterized by two distinct pronuclei and two polar bodies. Embryo quality was evaluated by an inverted microscope on days 3, 5 and 6. Blastocysts at days 5 and 6 were biopsied using a modified inner zona biopsy method. Briefly, after holding the blastocyst to a proper position, a small hole in the zona was opened by the ZILOS-tk™ laser system (Hamilton Thorn Bioscience Inc., MA, USA) and a 20 μm polished biopsy pipette (Sunlight Medical, Jacksonville, FL, USA) was inserted inside zona through this hole. A few trophectoderm cells (5–10 cells) were aspirated into biopsy pipette inside the zona and then pull the pipette out of the zona. After assisted cutting (one or two pulses) with laser at the edge of the front opening of biopsy pipette, a fast mechanical friction between holding pipette and biopsy pipette was used to separate the cells from blastocyst. The biopsied cells were collected in PCR tubes and stored at − 20 °C freezer until processing for NGS. All blastocysts were cryopreserved for later frozen embryo transfer (FET).

Chromosome analysis in the blastocysts

Biopsied samples were analyzed by a commercial genetic testing company (Invitea, San Francisco, CA, USA) using Illumina platform with a FAST-SeqS next generation of sequencing method and associated bioinformatics pipeline validated for accurate detection of whole chromosome number, segmental (≥10 Mb) aneuploidy, polyploidy and UPiD (chromosomes 1–16. 18, and X).

Blastocyst vitrification, warming and transfer to recipient

The biopsied blastocysts were vitrified using a vitrification device and kit (Fujifilm-Irvine Scientific). Both equilibration solution and vitrification solution were warmed in original vials at 37 °C for at least 30 min before use. Briefly, collapsed blastocysts by a laser pulse were equilibrated in 100 μl drop of equilibration solution for 2 min, and then 45 s in 100 μl drop of vitrification solution (both steps were performed on a 37 °C warming stage) before loading to vitrification device. All blastocysts were vitrified individually and then stored in liquid nitrogen until warming for FET.

For warming, blastocyst was exposed to a thawing solution (Fujifilm-Irvine warming kit) at 37 °C for 1 min, transferred to a dilution solution for 3 min and finally to a washing solution for 10 min with a solution change after 5 min at room temperature. After completion of the

warming process, blastocyst was cultured in Global™Total medium for 2 h before transfer.

For preparation of embryo transfer, the patient received 2 mg estradiol (Estrace, Warner Chilcott, NJ, USA) vaginally, 0.1 mg estradiol patch (Estradiol Transdermal System, Noven Pharmaceuticals, NJ, USA) every 3 days, and 400 mg progesterone (Cyclogest), twice a day, was administered on 15th day of estradiol treatment. The blastocyst was transferred on the sixth day of progesterone administered and progesterone was continued daily until the first serum β-hCG test 2 weeks after transfer. Ongoing pregnancy was supported by continued estradiol and progesterone until 11 weeks of pregnancy. Pregnancy was initially confirmed 14 days after embryo transfer by a serum β-hCG assay. Four weeks after embryo transfer, when a gestational sac and a heartbeat appeared, the patient was diagnosed as having a clinical pregnancy. The patient was then monitored by an obstetrician until childbirth.

Case presentation

A 45 years old patient and her 44 years old male partner had 5 years infertility treatment with 10 previous failed IVF cycles. The male partner had normal semen analysis results (4 ml semen with 109 $x10^6$sperm/ml and 41% motility). The patients decided to use donor oocytes for coming IVF treatment. Forty-seven oocytes were retrieved from an oocyte donor. Twenty three oocytes were inseminated by regular IVF and 21 oocytes at metaphase II (out of 24 oocytes) were inseminated by ICSI. As shown in Table 1, 16 oocytes inseminated by regular IVF and 19 oocytes inseminated by ICSI fertilized normally to form 2 pronuclei. All fertilized oocytes cleaved at Day 3 examination, and 9 and 14 blastocysts were obtained from regular IVF and ICSI, respectively. After PGT-A, it was found that 1 out of 9 blastocysts from regular IVF was aneuploid. However, 7 out of 14 blastocysts from ICSI were aneuploid.

The details of chromosomal status and embryo quality of each blastocyst were shown in Table 2. It would appear that there is no relationship between embryo quality and chromosomal status of embryos. For example, 9 good blastocysts (both inner cell mass and trophectoderm) and 6 fare blastocysts (either inner cell mass or trophectoderm) were euploid while 6 good blastocysts and 2 fare blastocysts were aneuploid.

After transfer of one euploid blastocyst (#1 embryo) resulting from regular IVF, the blastocyst implanted and a healthy girl (weight 3266 g) was delivered at gestation of 40 weeks and 5 days by cesarean section.

Discussion

Application of PGT-A in human IVF has been increased significantly in recent few years and the benefits of PGT-A is to transfer euploid embryos that can increase embryo implantation and reduce birth defects. The reason for use of PGT-A is that high proportions of human embryos produced by IVF are aneuploidy that was found not only in patients with advanced maternal age [16, 20–22, 25, 37], but also in young patients [15, 28–30] or patients with donated oocytes for IVF [19, 31].

The origin of embryonic aneuploidies mainly is from oocytes by chromosomal errors during meiosis I and/or meiosis II [32, 34, 35]. Usually errors in meiosis I occur in in vivo during oocyte maturation, but errors during meiosis II occur during insemination in vitro [23, 32, 35]. Therefore, in vitro manipulation of oocyte and/or in vitro conditions may affect oocyte meiosis II. For PGT-A, oocytes are usually inseminated by ICSI, not by regular IVF, thus ICSI procedures (cumulus removal and ICSI procedure itself) increase more opportunities for oocytes to be exposed to sup-optimal conditions. The purpose for use of ICSI for PGT-A is to assure that cumulus cells around oocytes have been removed and only one sperm is used for insemination, so that there are limited cumulus cells attached to zona pellucida and no sperm around the oocyte, which can reduce the contamination of cumulus cells and/or sperm for more accurate genetic testing.

As a standard procedure for ICSI, cumulus cells need to be removed before ICSI and oocytes are inseminated after the oocytes are exposed to air under a controlled temperature conditions. Although ICSI has been used in human IVF for more than 30 years and live birth after ICSI and regular IVF did not show any differences [38, 39]. Very little information is available whether ICSI procedure can cause more oocytes to form aneuploid embryos because regular IVF is not used to inseminate oocytes if PGT-A is applied to the resulting embryos. In the present study, our case report, for the first time, indicates that ICSI procedures can cause more embryos to form aneuploidy as compared with regular IVF. The

Table 1 Fertilization and embryo development after regular IVF and ICSI

Insemination method	No. of oocytes	Oocytes at M-II[a]	Fertilization			No. of blastocysts	No. of aneuploidy
			0PN	3PN	2PN		
Regular IVF	23	NA	5	2	16 (69.6%)	9 (56.2%)	1 (11.1%)
ICSI	24	21	2	0	19 (90.5%)	14 (73.7%)	7 (50.0%)

[a]*M-II* metaphase II, *PN* Pronuclei

Table 2 Quality and chromosomal status of blastocysts from regular IVF and ICSI

Embryo #	Insemination method	Chromosomal Status	Blastocyst quality[a]	Embryo status
1	Regular IVF	46, XX	Good/Good	Transferred
2	Regular IVF	46, XX	Good/Good	Frozen
3	Regular IVF	46, XX	Good/Good	Frozen
4	Regular IVF	46, XX	Fare/Good	Frozen
5	Regular IVF	46, XY	Good/ Fare	Frozen
6	Regular IVF	46, XY	Good/Good	Frozen
7	Regular IVF	46, XY	Fare /Good	Frozen
8	Regular IVF	46, XY	Good/Good	Frozen
9	Regular IVF	47, XY, +16	Good/ Fare	Frozen
10	ICSI	46, XX	Good/Good	Frozen
11	ICSI	46, XX	Good/Good	Frozen
12	ICSI	46, XX	Fare /Good	Frozen
13	ICSI	46, XY	Fare /Good	Frozen
14	ICSI	46, XY	Good/Good	Frozen
15	ICSI	46, XY	Good/Good	Frozen
16	ICSI	46, XY	Fare /Good	Frozen
17	ICSI	45, XY,-16	Good/Good	Frozen
18	ICSI	48, XY, +3, +4	Good/Good	Frozen
19	ICSI	47, XY, +22	Good/Good	Frozen
20	ICSI	47, XX, +3	Good/Good	Frozen
21	ICSI	46, XY, del(7) (q32q34)	Good/Good	Frozen
22	ICSI	46, XY, del(3) (q21)	Fare /Good	Frozen
23	ICSI	46, XY, dup(7)(p11.2p22)	Good/Good	Frozen

[a]Inner cell mass/trophectoderm

reason for this may be due to oocytes' meiosis II process being exposed to a suboptimal in vitro conditions.

Temperature fluctuation is one of most critical factors affecting meiotic spindle organization which has been found in many mammals [40–45], and human oocytes are especially sensitive to temperature fluctuations [45]. It has been found that meiotic spindle in human oocyte depolymerized after the temperature dropped to 35 °C and spindles can recover after oocytes were returned to 37 °C, but limited recovery was also found in some oocytes [46–51].

Recently, it has also been reported that embryonic aneuploidy rates varied between physicians in same IVF laboratory and also varied among different clinics when oocytes were collected from young, healthy donors [33]. The reasons for these differences are unknown. Because many factors exist during IVF treatment, such as patient stimulation protocols, donor differences, different laboratory set up, laboratory environmental differences, and embryologist's skill to perform ICSI. Our data from the present case may suggest that manipulation of oocytes in vitro including ICSI procedure itself may affect meiosis II, which eventually affect embryonic aneuploidy formation.

In the present case report, we used a new blastocyst biopsy method in which blastocysts were biopsied inside the zona pellucida. This method can be used to avoid any contamination of cumulus cells and sperm during regular IVF. Therefore, for insemination of oocytes in patients for PGT-A, it is not necessary to use ICSI procedures. We have used this new method to perform blastocyst biopsy during the past few years and found it is an easy to use biopsy procedure. The times for embryos to be exposed to air and the time for biopsy can be reduced by this method, and we also found that limited laser cutting is required during biopsy and excessive laser cutting application may damage embryos and biopsied cells.

Embryo morphology does not exactly represent chromosomal status in the embryos, which has been reported by use of different morphological assessments, including simple embryo morphological assessment or recently developed time-lapse morphokinetic embryo selection [51–53]. That is the reason that PGT-A is still the most valuable method to screen chromosomal status in human embryos [54–59], while embryo biopsy for PGT is one of the most challenged procedures in an IVF laboratory.

Although we manipulate human oocytes for ICSI under a well-controlled temperature and other conditions, it would appear that these conditions are not the optimal conditions for human oocytes. Further improvement is still necessary to avoid adverse effects on oocytes during in vitro manipulation of oocytes for ICSI.

Previous studies have examined cytogenetic results of spontaneous abortions following IVF and ICSI but the results were contradictory [60–62]. Lathi and Milki found that significantly higher aneuploidy rate in the abortuses of patients who conceived with ICSI than that with IVF [60], while other studies did not find the difference between regular IVF and ICSI [61, 62]. The different results from these studies may be resulted from sample collection. Many factors in these studies, such as the proportions of miscarriage out of total clinical pregnancy, were not known. Furthermore, only one sample was collected from each patient, so the chromosomal status in other embryos that have not been transferred in the same patients were not known either. While in the present case report, we analyzed all embryos produced by either regular IVF or ICSI from single oocyte retrieval cycle, thus the differences between patients, or between cycles were avoided, which makes the results to be more reliable.

Conclusion

The present case report indicates that more human embryos produced by ICSI are aneuploidy as compared with the embryos produced by regular IVF. The higher aneuploidy rate may be related to in vitro manipulation of oocytes for ICSI including cumulus cell removal, ICSI procedure itself and/or temperature fluctuations during the processing. These processes may be suboptimal conditions for oocytes to undergo meiosis, therefore further improvement of in vitro conditions for ICSI procedure may be necessary. In addition, blastocyst biopsy can be performed inside zona pellucida to avoid contamination of cumulus cells and/or sperm for chromosomal analyses of biopsied cells, thus oocytes for PGT can be inseminated by regular IVF if semen analysis shows a normal sperm number, motility and morphology. Further comparison of aneuploidy rate in human embryos produced by regular IVF and ICSI remains necessary.

Abbreviations

FET: Frozen embryo transfer; ICSI: Intracytoplasmic sperm injection; IVF: In vitro fertilization; PGT-A: Preimplantation genetic testing for aneuploidies; M-II: Metaphase II; PN: Pronuclei

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Authors' contributions

XN, JL, and FG collected and analyzed the data. XN, JL, FG and WHW interpreted the results, and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The primary data for this study is available from corresponding author on reasonable request.

Ethics approval and consent to participate

The patients signed the consents for all laboratory and clinical procedures including controlled ovarian stimulation, fertilization of oocytes with ICSI and regular IVF, embryo cryopreservation, embryo biopsy for PGT-A. The data was collected from medical records at the clinic and laboratory, and the study with IVF and PGT-A was approved by New England Institutional Review Board (NEIRB 14–504).

Consent for publication

Written informed consent was obtained from the patient for the publication of any data included in this article and other reports without their personal identification.

Competing interests

The authors declare that they have no competing interests.

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