J Assist Reprod Genet (2015) 32:999–1006
DOI 10.1007/s10815-015-0492-4

GENETICS

Assessment of aneuploidy formation in human blastocysts resulting from donated eggs and the necessity of the embryos for aneuploidy screening

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Received: 2 January 2015 / Accepted: 27 April 2015 / Published online: 9 May 2015

Abstract
Purpose To examine the prevalence of aneuploidy in human blastocysts resulting from donated eggs and embryo implantation after transfer of normal euploid embryos. Also, to assess the necessity of preimplantation genetic screening (PGS) for embryos produced with donor eggs.
Methods Blastocysts from donor-recipient cycles were biopsied for PGS (PGS group) and the samples were analyzed with DNA microarray. Euploid blastocysts were transferred to the recipients, and both clinical pregnancy and embryo implantation were examined and compared with embryos without PGS (control group).
Results After PGS, 39.1 % of blastocysts were abnormal, including aneuploidy and euploid with partial chromosome deletion and/or duplication. Transfer of normal euploid blastocysts brought about 72.4 % of clinical pregnancy, 65.5 % of ongoing/delivery and 54.9 % of embryo implantation rates; these rates were slightly higher than those in the control group (66.7, 54.0 and 47.8 %, respectively), but there was no statistical difference between the two groups. By contrast, the miscarriage rate was higher in the control group (19.2 %) than in the PGS group (9.5 %), but no statistical difference was observed. Transfer of two or more embryos did not significantly increase the ongoing/delivery rates in both groups, but significantly increased the twin pregnancy rates (50.0 % in the PGS group and 43.8 % in the control group).
Conclusion(s) High proportions of human blastocysts derived from donor eggs are aneuploid. Although pregnancy and embryo implantation rates were increased, and miscarriage rates were reduced by transfer of embryos selected by PGS, the efficiency was not significantly different as compared to the control, suggesting that PGS may be necessary only in some specific situations, such as single embryo transfer.

Keywords Aneuploidy · Single embryo transfer · Donor eggs · Implantation

Capsule Aneuploidy rate is high in human blastocysts resulting from donated eggs and, transfer of normal euploid embryos may facilitate the implementation of single embryo transfer without reducing live birth rate per embryo transfer.

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Introduction

Aneuploidy is one of the most detrimental factors affecting embryo implantation, and most birth defects are also caused by embryonic aneuploidy [1–3]. It has been reported that proportions of human preimplantation embryos with aneuploidy are increased in patients of advanced maternal age [3–5]. Recently, it has also been found that high proportions of human embryos were aneuploid in younger patients undergoing in vitro fertilization (IVF) [6, 7]. Donor eggs are usually collected from healthy, young and fertile women, and most eggs should have normal chromosomal integrity. However, these eggs are collected from women after controlled ovarian stimulation with high dosage of external gonadotropins, and then the eggs are exposed to in vitro environments for manipulations. Many factors may affect egg and/or embryo quality [8],

and these effects on the genetic and epigenetic alternations have not been examined and are still unknown.

Preimplantation genetic screening (PGS) by all chromosome DNA microarray has become an advanced technology to screen chromosomal integrity in human embryos, which has provided the best approach to examine structural and numeral abnormalities in all chromosomes in the preimplantation embryos [5, 7, 9, 10]. It has been found that PGS especially benefits patients of advanced maternal ages, recurrent miscarriage and previous spontaneous miscarriage, as aneuploidy is the main reason for unsuccessful embryo implantation in these populations of patients [5, 10–12].

Based on Society of Assisted Reproductive Technology (SART) data, live birth rates are only 5–10 % higher in patients who received donor eggs (www.sart.org) than those in patients who used their own eggs at the age of 34 or under. If aneuploidy is an important factor affecting embryo implantation in young patients undergoing IVF [7], it may also be a contributing factor affecting embryo implantation in the recipients who receive the donated eggs. According to a previous study, 17 % of human eggs collected from healthy women at ages of 22–25 during a natural cycle had spindle abnormalities [13]; it is possible that the embryos formed from this group of eggs would have chromosomal errors after fertilization, which in turn form aneuploidy [13].

On the other hand, in order to reduce multiple embryo implantation, single embryo transfer has been recommended [14, 15]. Blastocyst transfer has been considered to be one of the successful approaches to achieve the implementation of single embryo transfer [16]. However, aneuploid blastocyst formation is still a major issue in embryos produced in vitro [5, 17]. According to SART annual report, average number of embryos for transfer in recipients decreased from 2.0 in 2010 to 1.6 in 2013, indicating that at least half of the recipients still had two embryos for transfer, which may cause high multiple birth rates. However, multiple birth rates were not reported by SART (www.sart.org). In order to increase the implantation opportunity of embryos after single embryo transfer, it is necessary to select the best quality of embryos for transfer. Both blastocyst culture and blastocyst PGS would be useful methods for embryologists to select the embryos with high implantation potential, which could further enhance the implementation of single embryo transfer. If the implantation rate can be improved by transfer of a single blastocyst after PGS, multiple pregnancy can be avoided. Recently, it has also been reported that a high aneuploidy rate (53.2 %) was observed in embryos resulting from donor eggs and 88.1 % of the embryo aneuploidy were of a maternal source, suggesting the necessity of PGS for embryos resulting from donor eggs [18]. Although this was the first report to show that high proportions of human embryos from donor eggs were aneuploidy, the authors did not examine the embryo implantation after the transfer of euploid blastocysts [18]. Therefore, in the present study, we retrospectively collected data on PGS of blastocysts derived from donor eggs in our clinic during the past 2 years and aimed to analyze whether a high prevalence of aneuploidy is also present in human embryos derived from donor eggs in our clinic and also to evaluate the efficiency and necessity of PGS in patients who received donor eggs.

Materials and methods

Ethical statement

Patients undergoing IVF, egg donation and PGS signed written consents for all kinds of laboratory and clinical procedures. All egg donors were anonymous in the present study. The data was retrospectively collected from the medical records at the clinic from September 2012 to August 2014 and the study was approved by New England Institutional Review Board (NEIRB 14–504).

Donor stimulation and egg retrieval

Egg donors were stimulated with a combination of Follistim (Organo Inc, Roseland NJ, USA), Gonal-F (EMD Serono, Rockland MA, USA), Menopur (Ferring Pharmaceuticals, Parsippany NJ, USA) and/or Bravella (Ferring Pharmaceuticals) beginning 2–3 days after the onset of menses. The initial starting total dose was 150–375 IU and was adjusted subsequently as the stimulation progressed. To prevent an LH surge, a GnRH antagonist, Ganirelix or Cetrorelix (Organo Inc.), was given when the leading follicle was 13–14 mm or when the estradiol level was 400 pg/ml. Human chorionic gonadotropin (hCG), Ovidrel (Serono USA), or a GnRH agonist, leuprolide acetate (Teva North America, North Wales PA, USA), was injected to induce final oocyte maturation when at least two dominant follicles reached a diameter of >18 mm. Eggs were retrieved under IV sedation via transvaginal ultrasound between 35 and 37 h after hCG or leuprolide acetate administration.

Recipient populations

Recipients' embryos for PGS were considered as study group and recipients' embryos without PGS were considered as control group. Because biopsied samples need to be sent to reference laboratories for analysis, all embryos in the PGS group were cryopreserved for later frozen embryo transfer (FET). In order to reduce the factors that may affect embryo implantation, only recipients who had all embryos cryopreserved without PGS were selected in the control group and recipients who received fresh embryo transfer were not included in the control group. Data were collected and analyzed from both groups

of recipients who had the first attempt of FET during the study period.

Egg insemination, embryo culture and blastocyst biopsy

Matured eggs in both study and control groups were inseminated by intracytoplasmic sperm injection (ICSI) 5–6 h after retrieval and inseminated eggs were cultured in Global medium supplemented with 10 % SPS after ICSI. Fertilization was examined 16–18 h after ICSI and normally fertilized eggs (zygotes) were cultured in Global medium supplemented with 10 % SPS at 37 °C in a humidified atmosphere of 5.5 % CO_2, 5 % O_2 and balanced nitrogen until day 6 after inseminations.

At day 3, a hole about 20 μm was opened in the zona pellucida using the ZILOS-tk™ laser system (Hamilton Thorn Bioscience Inc., MA USA) for embryos in the PGS group, but not in the control group (without PGS). On day 5, embryos for biopsy were examined with an inverted microscope, and if trophectoderm (TE) cells started to hatch from the opening in the zona pellucid, some hatched TE cells (~10) were biopsied using a 20 μm polished biopsy pipette with assisted cutting by the laser. Blastocyst biopsy was performed on TE cells at days 5 and 6 depending on blastocyst development. After biopsy, the embryo proper was cultured in Global medium supplemented with 10 % SPS for 1–2 h before vitrification. The biopsied cells were washed with a washing buffer provided by PGS laboratories, placed in tubes with cell lysis buffer and were then frozen at −20 °C before being processed for microarray. DNA microarray assay was performed by commercial companies including Pacgenomics, Reprogenetics and Natera.

Blastocyst vitrification, warming and embryo transfer

All blastocysts in both study and control groups were vitrified after the blastocoele was completely collapsed by using Irvine vitrification kit (Irvine Scientific, Irvine, CA USA). Briefly, blastocysts were equilibrated in the equilibration solution for 2 min and then transferred into the vitrification solution (both steps were performed on a warming stage). The blastocysts were finally loaded onto a vitrification straw within 45 s. All embryos were vitrified individually and then stored in liquid nitrogen until warming for FET.

For warming, blastocysts were exposed to warming solution (Irvine warming kit) at 37 °C for 1 min. Blastocysts were then transferred to a dilution solution for 3 min and then to a washing solution for 10 min with a solution change after 5 min at room temperature. Assisted hatching was performed in the blastocysts after warming in the control group using the ZILOS-tk™ laser system. No further assisted hatching was performed in the blastocysts that had been biopsied for PGS. After completion of the warming process, blastocysts were washed with Global medium supplemented with 10 % SPS and then cultured in the same medium for 2–4 h before transfer. Blastocyst quality was assessed using standard assessments developed by the SART [19].

Patient preparation for embryo transfer

All patients for embryo transfer received estradiol orally and transvaginally. Intramuscular administration of progesterone oil was initiated after about 14 days of estradiol treatment. Endometrium thickness was measured on the day of progesterone administration. Embryo transfer occurred on the sixth or seventh day of progesterone administration and progesterone was continued until the first serum β-hCG test 2 weeks after transfer. Ongoing pregnancies were supported by continued estradiol and progesterone.

Pregnancy and implantation assessment

Fourteen days after embryo transfer, pregnancy was checked by a serum β-hCG assay. When the β-hCG was>5 mIU/mL the patients were regarded as having a biochemical pregnancy. Four weeks after embryo transfer, when a gestational sac and a heart beat appeared ultrasonographically, the patients were diagnosed as having a clinical pregnancy. Miscarriage rates and ongoing/delivery rates were calculated based on the current updated data.

Statistical analysis

Interval data was analyzed by one-way analysis of variance, and categorical differences between groups were analyzed by Chi-square. Donor ages, recipient ages, number of embryos transferred and E_2 level at trigger day were reported as mean± standard deviation (SD), and data were compared between groups with Student's *T*-test. If the P value was less 0.05, it was considered to be statistically different.

Results

As shown in Table 1, data from 31 cycles in the PGS group and 39 cycles in the control group were collected and compared. There were no statistical differences between the two groups in terms of donor ages (25.04±3.11 vs 24.80±2.71), fertilization rates (88.3 vs 87.4 %), cleavage rates at day 3 (98.4 vs 99.5 %) and blastocysts rates (66.3 vs 68.7 %). Due to the cost of PGS, some patients with more than eight blastocysts decided to have PGS on a portion of their blastocysts, which resulted in some blastocysts being cryopreserved without PGS. Average blastocysts analyzed for each recipient were 9.94±4.64 (range from 3 to 21). In the present study, two patients did not have normal blastocyst for transfer although all blastocysts (one had seven blastocysts and another had

Table 1 Comparison of laboratory observations in patients undergoing donated egg in vitro fertilization with or without PGS

Observations	No. of cycles with PGS	No. of cycles without PGS	*P* value
No. of cycles	31	39	NA
Donor age (Mean±SD)	25.04±3.11	24.80±2.71	NS
Total No. of eggs inseminated	563	459	NA
Total No. (%) of eggs fertilized	497 (88.3)	401 (87.4)	NS
Total No. (%) of eggs cleaved	489 (98.4)	399 (99.5)	NS
Total No. (%) of Blastocysts	324 (66.3)	274 (68.7)	NS
Total No. (%) of blastocysts analyzed	308[a] (95.1)	NA	NA
Average No. of blastocysts analyzed (range)	9.94±4.64 (3–21)	NA	NA
Total No. (%) of samples with data after PGS	294 (95.5)	NA	NA
Total No. (%) of samples without data after PGS	14 (4.5)	NA	NA
Total No. (%) of normal euploid blastocysts	179 (60.9)	NA	NA
Average No. (%) of euploid blastocysts (range)	5.77±3.58 (0–15)	NA	NA
Total No. (%) of abnormal blastocysts[b]	115 (39.1)	NA	NA

NS Not statistically different, *NA* Not applicable, *PGS* Preimplantation genetic screening

[a] Some blastocysts were not biopsied and analyzed

[b] Abnormal blastocysts include aneuploidy and euploid with deletion and/or duplication

three blastocysts) were biopsied and analyzed, but others had at least one (ranged from 2 to 15) normal euploid blastocyst for transfer.

After blastocyst biopsy and DNA microarray analysis, 95.5 % (294/308) of samples had chromosome data and 4.5 % [14] of samples did not have DNA signal due to low DNA amount. Out of the analyzed samples, 179 (60.9 %) were normal blastocysts and 115 (39.1 %) were abnormal blastocysts. Each recipient had average of 5.77±3.58 normal embryos. As shown in Fig. 1a, out of the abnormal samples, 86 (74.7 %) samples were aneuploidy including 44 with one chromosome anomalies and 42 with two or more chromosome

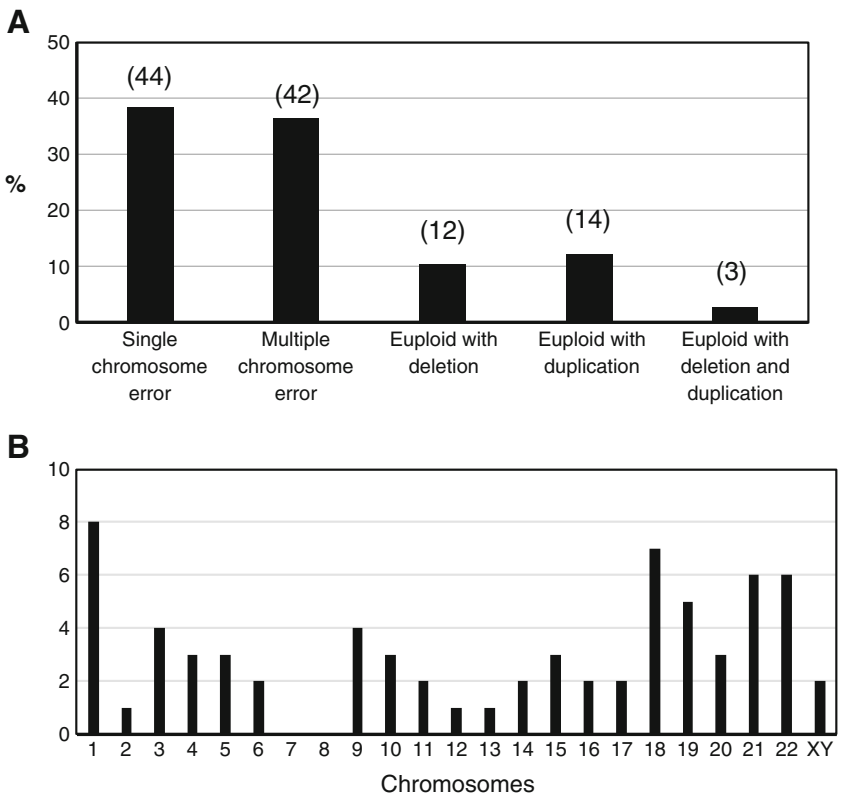

Fig. 1 Microarray results of abnormal chromosomes in human blastocysts derived from donated eggs. **a** Abnormal chromosome distribution in the samples examined in the study. Data was based on 115 abnormal samples. Numbers of samples are included in the parenthesis. **b** Distribution of a single chromosome error in the abnormal embryos. Data was based on 44 samples

anomalies. The remaining 29 samples were euploid but had microdeletion [12], duplication [14] or both [3].

After analyzing the single chromosome errors, we found that chromosome errors could occur in most chromosomes except chromosomes 7 and 8 (Fig. 1b). However, if all chromosomes in all abnormal embryos were analyzed, we found that chromosome errors could occur in any chromosomes (data is not shown).

When we analyzed the degrees of aneuploidy in the individual cases, as shown in Fig. 2, we found that there were only three cases (# 6, 15 and 25) in which all embryos were normal, and there were two cases (# 14 and 16) in which all embryos were abnormal. Others had abnormal embryos between 10 and 70 % (3 cases had 10–19 %, 10 cases had 20–40 %, 12 cases had 41–60 % and one case had 70 % abnormal embryos, respectively). Twenty nine recipients had at least one euploid embryo after PGS and FETs were performed in these patients.

As shown in Table 2, 29 recipients had FET in the PGS group and 39 had FET in the control group. There was no statistical difference in the recipients' ages (43.84±5.56 vs 40.31±5.98) between the two groups and most recipients (79.3 % in the PGS group and 71.8 % in the control group) were of advanced maternal age between 40 and 54 years old. For embryo transfers, we did not find statistical differences in terms of good quality embryos (88.2 vs 88.4 %), average number of embryos transferred (1.76 vs 1.77), clinical pregnancy rate (72.4 vs 66.7 %), miscarriage rates (9.5 vs 19.2 %), ongoing/delivery rates (65.5 vs 54.0 %) and embryo implantation rates (54.9 vs 47.8 %) although there was an increased tendency of clinical pregnancy, ongoing/delivery and implantation rates as well as a decreased tendency of miscarriage rate in the PGS group.

When we further analyzed the outcomes after different number of embryos were transferred, as shown in Table 3, we found that increasing number of embryos for transfer did not significantly increase the ongoing/delivery rates in both the PGS group and control group. In the present study, all patients who received EFT had at least one good embryo for transfer while four patients (three in the PGS and one in the control groups) had one good and two fair embryos transferred as they had a few previous embryo implantation failures. However, transferring more embryos did not increase embryo implantation rates. By contrast, a decreased tendency of embryo implantation was observed as the number of transferred embryos was increased. It would appear that the implantation rate was higher if a single embryo was transferred in the PGS group as compared to the control, but the difference was not statistically significant. Twin pregnancy was observed in the recipients with transfer of two (50 %) or three embryos (50 %) in the PGS group and of two embryos (43.8 %) in the control group. Triplet pregnancy was not observed in the present study.

Next, we investigated if final oocyte maturation triggers can affect aneuploidy formation in the resulting embryos. In this study, two different triggers were used for final oocyte maturation: hCG (Ovidrel) and GnRH agonist (Luprolide Acetate). Usually Ovidrel was used if E_2 level was low while Luprolide acetate was used if E_2 was high at trigger day to avoid hyper stimulation, so we divided the cases into two groups. We found that the E_2 level was significantly (P=0.04) higher in the Luprolide acetate group (5064.69±2428.67 pg/ml) than in the Ovidrel group (3368.13±1558.97 pg/ml). However, the aneuploidy rates did not show statistical difference between the two groups although it was higher in the Ovidrel group (42.3 %) than in Luprolide acetate group (35.7 %).

Discussion

Aneuploid embryo is one of the major factors affecting human IVF success, and such an effect could be reduced by transfer of euploid embryos selected by all chromosome PGS [5, 7]. An age-related reduced embryo implantation can also be

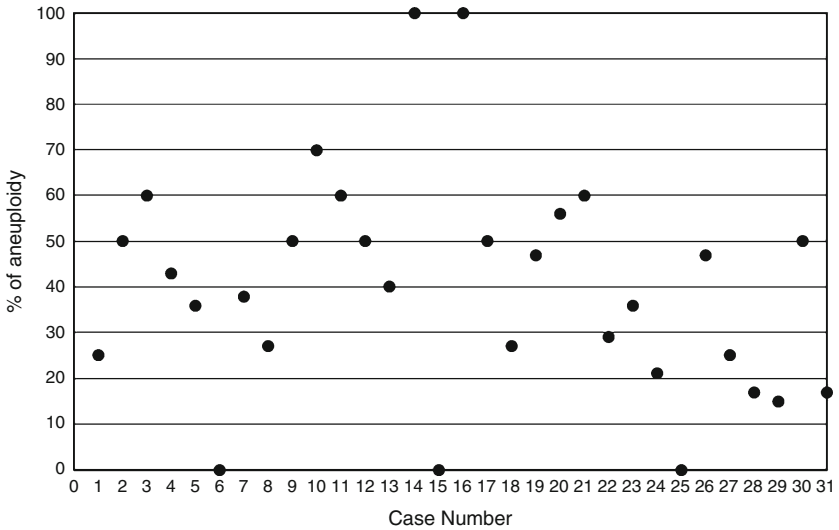

Fig. 2 Degree of abnormal chromosome distribution in 31 donor-recipient cycles in the study. Percentages of aneuploidy were calculated based on each individual cycles. X-axis shows the assigned cycle number and y-axis shows the percentage of aneuploidy blastocysts

Table 2 Comparison of clinical observations in patients undergoing donated egg in vitro fertilization with or without PGS

Observations	No. of cycles with PGS	No. of cycles without PGS	*P* Value
No. of Recipients received embryo transfer	29	39	NA
Age of recipients (Mean±SD)	43.84±5.56	40.31±5.98	NS
No. (%) of recipients of≥40 years old	23 (79.3)	28 (71.8)	NS
Total No. of embryos transferred	51	69	NA
No. (%) of good quality embryos	45 (88.2)	61 (88.4)	NA
Mean No. of embryos transferred	1.76±0.64	1.77±0.48	NS
No. (%) of clinical pregnancy	21 (72.4)	26 (66.7)	NS
No. (%) of miscarriage	2 (9.5)	5 (19.2)	NS
No. (%) of ongoing/delivery	19 (65.5)	21 (54.0)	NS
No. (%) of embryos implanted	28 (54.9)	33 (47.8)	NS

NS Not statistically different, *NA* Not applicable, *PGS* Preimplantation genetic screening

improved by the transfer of selected euploid embryos with PGS [5]. Aneuploid embryos are present in infertile patients at various maternal ages with an increased tendency as maternal ages are increased [5, 7, 10, 20, 21]. Embryos from healthy egg donors should have low embryonic aneuploidy rates, but a previous study by Sills et al. [18] and our present study indicate that high aneuploidy rates are present in human embryos from donor eggs. PGS for embryos derived from donor eggs are not routinely performed due to the high cost and lack of necessity, thus the case numbers in both studies are limited. Although we did not examine the origination (maternal or paternal source) of the aneuploidy in the present study, the study by Sills et al. showed that 88.1 % of these aneuploidy were of a maternal source, suggesting that the majority of aneuploidy was from eggs, not from sperm in these embryos [18].

Recently, Yang et al. investigated the aneuploidy formation in human embryos derived from young patients (less than 35 years old) and also found that approximately 40 % of blastocysts were aneuploid [7], a rate which was similar to our previous studies with the same age group [5, 10]. In Yang's study, they found that transfer of PGS-selected embryos resulted in a higher implantation rate as compared with transfer of non-PGS embryos, suggesting that PGS is necessary not only for patients of advanced maternal ages, but also for young women [7].

According to a previous study, 17 % of human eggs collected from healthy young women (22–25 years old) at natural cycle had meiotic spindle abnormalities [13]. It is estimated that the aneuploidy rate would be the same or greater than this rate if embryos are formed from this group of eggs [13]. However, there is no direct evidence to verify the relationship between spindle morphology and embryonic aneuploidy with human eggs. If all eggs with an abnormal meiotic spindle would form aneuploid embryos, then embryos from natural cycles may also have high aneuploidy rates after insemination. Unfortunately, this kind of study has not been performed due to the difficulties with human oocytes. Previous data [18] and our present data indicate that the aneuploidy rates in the embryos produced from eggs collected from ovarian stimulation are between 39.1 and 53.2 %, which were higher than the proportion (17 %) of eggs with abnormal meiotic spindles in young women at natural cycle [13]. It is unknown whether aneuploid embryos could be increased by ovarian stimulation, egg manipulation in vitro under suboptimal conditions [22–24] or other factors. In a previous study, it was reported that extra ovarian stimulation can increase aneuploidy

Table 3 Summary of clinical outcome (ongoing/delivery rate and implantation rate) in recipients after PGS and embryo transfer with donated eggs

Clinical outcomes	No. of embryos transferred	Cycles with PGS	Cycles without PGS	*P* value
Ongoing/delivery rate (%)	×1	7/10 (70.0)	5/10 (50)	NS
	×2	10/16 (62.5)	16/28 (57.1)	NS
	×3	2/3 (66.7)	0/1 (0)	NS
Implantation rate (%)	×1	7/10 (70.0)	5/10 (50.0)	NS
	×2	15/32 (46.8)	23/56 (41.4)	NS
	×3	3/9 (33.3)	0/3 (0)	NS
Twin pregnancy rate (%)	×1	0/6 (0)	0/5 (0)	NS
	×2	5/10 (50.0)	7/16 (43.8)	NS
	×3	1/2 (50.0)	0	NS

NS Not statistically different, *PGS* Preimplantation genetic screening

formation in human IVF [25], but no further evidence to indicate that stimulation can increase the risk of aneuploidy. High aneuploidy rates observed in human IVF may be due to multiple factors, such as maternal age [5, 7, 10] and in vitro manipulation of eggs [22–24]. However, a comprehensible conclusion may need more basic and clinical investigations.

Based on SART data during the past 10 years, we found that live birth rates were about 5–10 % higher in patients who received donor eggs (50–57 %) (www.sart.org) than those in patients who were less than 35 years old with their own eggs (43–47 %). If aneuploidy is a significant problem in young patients undergoing IVF [7], it may also be a contributing factor in the recipients who receive donated eggs. However, it was found that PGD/PGS was performed only in less than 4 % of donor egg IVF cycles and this percentage for PGS has not changed during the past decade. By contrast, PGS for patients receiving IVF with their own eggs has significantly increased during the past few years, especially in patients of advanced maternal age, which may be due to improvement of PGS technology by all chromosome microarray and dramatically reduced PGS cost. If PGS could also be used in embryos from young patients or embryos from donated eggs, embryo implantation should be increased significantly as those observed in patients of advanced maternal age [5, 7, 10, 17, 20, 21].

Blastocyst transfer has been widely adopted in many IVF clinics, and it has also been suggested that blastocyst transfer is the best option for single embryo transfer [26], which is the better way to select higher quality embryos for transfer by morphological characteristics. Accumulated data indicated that the pregnancy rate with single embryo transfer in patients with IVF was lower than that of multiple embryo transfer [27], but the accumulated pregnancy rates were not different between single embryo transfer and multiple embryo transfer in IVF patients [27]. Although Sills et al. examined the details of aneuploidy formation in human embryos derived from donor eggs, they did not examine the pregnancy and embryo implantation of the embryos after PGS [18]. In the present study, after comparison of the PGS and non-PGS embryo transfer in recipients, we did not find that PGS could significantly increase the clinical pregnancy and embryo implantation rates. Although the difference was not statistically significant between the PGS and the control groups, the miscarriage rate was reduced after transfer of embryos selected by PGS, suggesting that PGS should benefit IVF patients who receive donor eggs. The lack of statistical differences between the two groups may be due to the limited case number in the present study; it may mask the difference, thus more data from a large number of transfer cycles are necessary to draw a more reliable conclusion whether PGS can significantly improve IVF outcomes in patients who receive donor eggs, as those observed in patients with their own eggs [5, 7, 9, 10]. Currently, it may be difficult to obtain such data, as donor cycles account for only ~20 % of total IVF cycles, and most donor cycles did not process PGS on the resulting embryos.

A high ongoing/delivery rate and embryo implantation rate were observed after single embryo transfer in the PGS group, and the rates did not change if two or more embryos were transferred. Similar results were observed in patients without PGS. Although transfer of two or more embryos may slightly change the pregnancy rate, but such a change was limited in donor egg cycles, which may be different from those reported with patients' own eggs [27]. This may be due to the overall better egg quality from donors than from patients themselves. For donor eggs, if patients have one good embryo, it is not necessary to transfer more embryos to establish pregnancy, and transfer of the extra embryos will definitely increase twin pregnancy. PGS may facilitate the implementation of single embryo transfer without reducing pregnancy rate per embryo transfer.

The degrees of aneuploidy varied significantly among donors. From the present study, we found that only in three cycles, all embryos were normal, while all others had various levels/degrees (10–100 %) of chromosomal abnormalities. Most had 20–60 % aneuploid embryos. These data were similar to those in IVF patients less than 35 years old observed in previous studies with patients' own eggs [5, 7, 10, 28] and donor eggs [18]. These results indicate that aneuploidy is widely present in human embryos produced by IVF.

In the present study, although all donors were stimulated with external gonadotropins, our data indicate that aneuploidy formation may not be related to E_2 level, an indicator of stimulation degree, and final oocyte maturation induced by either hCG or Lupron did not affect aneuploidy formation.

In conclusion, our study indicates that the proportions of aneuploidy are high in human embryos produced by IVF from donated eggs. PGS of blastocysts should be considered to be one of the approaches to improve embryo implantation if single embryo transfer is implemented. PGS may not be necessary if multiple embryos are transferred. However, considering the medical complexity of multiple pregnancy in humans, single embryo transfer should be considered to be the most important approach to reduce multiple pregnancy. Similar aneuploidy rates were observed in patients less than 35 years old with their own eggs or in recipients with donor eggs, indicating that aneuploidy is not only a factor affecting embryo implantation in patients of advanced maternal age, but also in young patients and egg donors. As PGS has become a routine laboratory procedure, consideration of PGS for more patients may increase overall pregnancy and reduce miscarriage, especially for single embryo transfer.

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