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Pre-implantation genetic diagnosis and pre-implantation genetic screening: two years experience at a single center

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Objective
Indications for preimplantation genetic diagnosis (PGD)/preimplantation genetic screening (PGS) cycles and clinical outcomes were evaluated at CHA Gangnam Medical Center.

Methods
This is retrospective cohort study. All patients (n=336) who went through *in vitro* fertilization (IVF)-PGD/PGS cycles (n=486) between January 2014 and December 2015 were included in Fertility Center of CHA Gangnam Medical Center. Patients underwent IVF-PGD/PGS with 24-chromosome screening. Patients with euploid embryos had transfer of one or 2 embryos in a fresh cycle with any subsequent frozen embryo transfer (ET) cycle. Compared implantation, clinical pregnancy, ongoing pregnancy, and early abortion rates were the main outcome measures.

Results
The most common indication for PGD/PGS was recurrent spontaneous abortion (n=160). The chromosome rearrangement cases (n=116) included 24 Robertsonian translocations, 60 reciprocal translocations, 3 inversions, 2 deletions, 4 additions, and 23 mosaicisms. PGS cases rather than the PGD cases showed higher implantation rates (26.4% vs. 20.3%), ongoing pregnancy rates (19.5% vs. 16.4%), and clinical pregnancy rates (28.6% vs. 23.3%). Implantation rates (30.3% vs. 23.7%), clinical pregnancy rates (39.2% vs. 25.2%), and ongoing pregnancy rates (25.7% vs. 17.5%) were significant higher in the blastocyst evaluation group than cleavage stage evaluation group.

Conclusion
This was the largest study of PGD/PGS for 2 years at a single center in Korea. The pregnancy outcomes of PGD cases are slightly lower than PGS cases. It was confirmed again that success rate of PGD/PGS is higher if biopsy was done at blastocyst than cleavage stage.

Keywords: *In vitro* fertilization; Preimplantation genetic diagnosis

Introduction

Embryo aneuploidy is one of the most important factor for maintaining pregnancy. Over 25% of all miscarriages are due to aneuploidy embryo [1]. Also, embryo aneuploidy is known as most common cause of implantation failures in *in vitro* fertilization (IVF) [2]. It is well known that aneuploidy rates in the preimplantation embryos dramatically increase with maternal age [3,4]. For the last 2 decades, screening strategies to select euploid embryos have emerged as a major interest. A preimplantation genetic screening (PGS) to select euploid embryo to transfer in couples who do not have specific disease is widely used as a good screening tool before going through IVF-embryo transfer (ET) [5]. Indication for PGS varies depending on center. In general, advanced maternal age, recurrent

pregnancy loss, recurrent IVF failure, severe male factor infertility, confirmed chromosomal rearrangement carrier, and bad obstetric history could be indications.

Even though PGS is good for detecting aneuploidy embryo, it is controversial to adopt as routine use for all who is in IVF cycles. One retrospective study concluded that routine use of PGS in fresh IVF cycles should be postponed until the effectiveness and safety of the procedure are more established [6]. Other retrospective study demonstrated a clear benefit more of PGS in patients over 37 years old who have euploid embryos available for transfer, but it did not demonstrate a benefit when all patients were included in the study [2].

A preimplantation genetic diagnosis (PGD) is the method to avoid the transmission of genetic disease such as recessive monogenic disorders, dominant monogenic disorder, sex-linked disorders, chromosomal disorders or human leukocyte antigen matching [5,7]. Also, diagnosis of embryos for chromosome abnormalities including aneuploidy screening has been invigorated by introduction of microarray-based testing methods, which allowed analysis of 24 chromosomes [8]. Fluorescence in situ hybridization (FISH) was the most commonly applied method to determine the chromosomal constitution of an embryo. However, the FISH method has a restricted application analyzing all chromosomes. Recently, comprehensive chromosomal screening strategies such as array comparative genomic hybridization (aCGH) started to be applied to PGD/PGS. Therefore, it became possible to check by PGS whether ploidy contains all 24 chromosomes before IVE-ET [9].

In Korea, rising age of marriage and pregnancy are huge social issues. As a result, the number of infertile couples has rapidly increased. At the same time, interest and demand for PGD/PGS has increased. In this study, data including 486 PGD and PGS cycles for 2 years at CHA Gangnam Medical Center was reviewed and outcomes were analyzed.

Materials and methods

This is a retrospective cohort study from January 2014 to December 2015 at the Fertility Center of CHA Gangnam Medical Center. The samples were obtained during 116 PGD cycles undertaken for 76 couples, and 370 PGS cycles for 260 couples. Indications for PGS and PGD are such as recurrent spontaneous abortion, recurrent implantation failure, chromosomal rearrangement carrier which are PGD cases, couples had bad

obstetric history, old age, male factor infertility, and others. This study was approved by the Institutional Review Board of CHA Gangnam Medical Center (#GCI-16-43).

Patients were either down-regulated using a gonadotropin-releasing hormone (GnRH) agonist or a GnRH antagonist. GnRH agonists (Lorelin®; Dongkook Pharmaceutical Co., Ltd., Seoul, Korea) were used in "long" protocols, and for GnRH antagonist cycles, patients were started on 0.25 mg of Cetrotide (Merck-Serono, Darmstadt, Germany) or Ganirelix (Orgalutran®; Organon, Oss, The Netherlands) according to a flexible dosing scheme. Ovarian stimulation started on the 2nd or 3rd day of the menstrual cycle, and the initial dose of gonadotropin was individualized for each patient according to the woman's age, anti-Müllerian hormone (AMH), basal follicle-stimulating hormone (FSH) levels, antral follicle count, and previous ovarian response to ovarian stimulation. The daily dose of gonadotropin was adjusted for each individual according to the serum estradiol (E2) concentration, follicular growth and numbers were assessed by ultrasound. A 5,000–10,000 IU dose human chorionic gonadotropin (hCG) (Ovidrel®; Merck-Serono) was administered when at least 2 follicles reached 18 mm in diameter. Oocyte retrieval was scheduled 34–36 hours after triggering of final mature oocyte by transvaginal ultrasound-guided puncture of follicles. Retrieved oocytes were fertilized 3–6 hours later, by intracytoplasmic sperm injection. The luteal support was provided for all patients with progesterone vaginal suppositories or progesterone intramuscular injection from the day of oocyte retrieval.

Biopsy was performed as below. A single blastomere of cleavage stage embryo or approximately 5 cells from the trophectoderm layer of blastocyst were analyzed using bacterial artificial chromosome (BAC)-aCGH. The biopsied cells were transferred to polymerase chain reaction (PCR) tubes. The whole genome amplification was performed using the GenomePlexÒ single Cell Whole Genome Amplification kit (Sigma-Aldrich, St. Louis, MO, USA) and the manufacturer's protocol. The amplified DNAs were labeled with Cy-3 and Cy-5 dCTP using a random priming method for 3 hours. The labeled DNAs were hybridized with the 1440 human BAC array (Macrogen, Seoul, Korea) for overnight. The slides were washed and the hybridization images were acquired with a GenePix4000B dual-laser scanner (Axon Instruments, Union City, CA, USA) and analyzed with MacViewer software.

Once biopsy results confirmed at least one euploid embryo, patients were scheduled for a fresh or frozen ET cycle. One

or 2 euploid embryos were transferred after evaluation of the embryo quality. The transfer procedure was performed under transabdominal or transvaginal ultrasound guidance.

The pregnancy outcome was measured using the fresh cycle and any subsequent frozen transfer, assuming all frozen transfer as one PGD/PGS cycle. Primary outcome measure was implantation rates. Ongoing pregnancy rates, clinical pregnancy rates, and early abortion rates were assessed as secondary outcomes. The implantation rates were defined as the number of gestational-sac(s) divided by the number of embryo transferred. In case of monozygotic twin, it is considered as simple implantation. Ongoing pregnancy rates were defined as the number of patients continuing pregnancy more than 12 weeks divided by total PGD/PGS cycles. Early abortion rates were defined as the number of miscarriage divided by the number of cycles including thawing ET cycles which had serum β-hCG level more than 20 at 11–13 days after ET. Clinical pregnancy rates were defined as number of cycles including

thawing ET cycles which had serum β-hCG level more than 20 at 11–13 days after ET divided by total PGD/PGS cycles.

Results

Baseline characteristics of the subjects are presented in Table 1. The chromosome rearrangements which were PGD cases (n=116) included 24 Robertsonian translocations, 60 reciprocal translocations, 3 inversions, 2 deletions, 4 additions, and 23 mosaicism. The chromosomally normal patients who went through PGS requested embryo testing as part of an IVF cycle because of recurrent spontaneous abortion (n=160), recurrent implantation failure (n=145), advanced maternal age (n=81), bad obstetric history (n=66) (Ex: stillbirth or birth history of Down or Edward syndrome), severe male factor infertility (n=8), and others (n=3). Mean maternal age was 37.5±4.3 years old, mean number of embryo biopsy per cycle was 5.6±3.1, and

Table 1. Characteristics of participants

Characteristics	PGD cases (n=116)	PGS cases (n=370)	Total (n=486)	*P*-value
Mean female age (yr)	35.4±4.1	38.2±4.2	37.5±4.3	<0.010
Infertility duration (yr)	4.3±2.9	4.4±2.6	4.3±2.7	0.751
BMI (kg/m^2)	21.7±3.5	21.3±2.7	21.4±21.4	0.154
AMH (ng/mL)	3.8±3.3	3.5±3.1	3.6±3.1	0.346
FSH (mIU/mL)	8.2±1.9	8.4±3.2	8.4±3.0	0.268
No. of embryo biopsied	6.2±3.4	5.4±3.1	5.6±3.1	0.017
Euploidy rates (%)	27.9±22.1	28.3±24.0	28.2±23.5	0.885
Clinical outcomes (%)				
Implantation rates	20.3 (32/158)	26.4 (121/459)	24.8 (153/617)	N/S
Ongoing pregnancy rates	16.4 (19/116)	19.5 (72/370)	18.7 (91/486)	N/S
Early abortion rates	22.2 (6/27)	34.0 (36/106)	31.6 (42/133)	N/S
Clinical pregnancy rates	23.3 (27/116)	28.6 (106/370)	27.4 (133/486)	N/S

PGD, preimplantation genetic diagnosis; PGS, preimplantation genetic screening; BMI, body mass index; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; N/S, not significant.

Table 2. Clinical outcome according to biopsy timing

Variables	PGD cases		PGS cases	
	Cleavage stage	Blastocyst	Cleavage stage	Blastocyst
No. of cycles	90	26	322	48
Implantation rates (%)	17.8 (21/118)	27.5 (11/40)	25.5 (102/400)	32.2 (19/59)
Ongoing pregnancy rates (%)	14.4 (13/90)	23.1 (6/26)	18.3 (59/322)	27.1 (13/48)
Early abortion rates (%)	23.5 (4/17)	20.0 (2/10)	35.6 (31/87)	26.3 (5/19)
Clinical pregnancy rates (%)	18.9 (17/90)	38.5 (10/26)	27.0 (87/322)	39.6 (19/48)

PGD, preimplantation genetic diagnosis; PGS, preimplantation genetic screening.

overall euploidy rates were 28.2%±23.5%. Euploid embryo did not exist in 113 cycles. There were no other infertility factors (uterine, peritoneal, etc.). There was no case of embryo demise after biopsy. There were 4 embryos without result due to analysis failure (1 in PGD group, 3 in PGS group). Analysis failure can happen due to whole genome amplification failure or unclear result. Total implantation rates were 24.8%, ongoing pregnancy rates were 18.7% and clinical pregnancy rates were 27.4%. Implantation rates were 20.3% in the PGD cases and 26.4% in the PGS cases. Clinical pregnancy rates were 23.3% in the PGD cases and 28.6% in the PGS cases. Ongoing pregnancy rates were 16.4% in the PGD cases and 19.5% in the PGS cases.

The dataset was divided by biopsy timing and this is presented at Table 2. Clinical outcome including implantation rates, ongoing pregnancy rates, and clinical pregnancy rates were higher when biopsy was done at blastocyst stage. Table 3 presents clinical outcomes according to biopsy timing.

Cleavage stage analysis was undertaken in 412 cases, and blastocyst evaluation was in 74 cases. Clinical pregnancy rates (38.2%), ongoing pregnancy rates (25.0%), and implantation rates (30.3%) were significant higher in blastocyst evaluation group. Early abortion rates were higher in cleavage stage analysis group.

Table 4 presents clinical outcome of PGD group according to age. Euploidy rates and clinical pregnancy rates decrease as age gets older. Reversely, early abortion rate increases as age gets older. Table 5 presents clinical outcome of PGS group according to age. Clinical outcome tendency was similar to PGD group.

Discussion

In this study, result of PGD/PGS cycle at single center was analyzed and outcomes depending on patient's age, reason of PGD/PGS, and timing of biopsy were compared. It is well known that aneuploidy highly contributes to decreased implantation rates and early pregnancy loss [10]. After PGS was introduced, there has been many technological studies to improve pregnancy outcome of PGS. Previously, FISH was base technology of PGS that was widely used on one or 2 blastomere cells from day 3 of embryo biopsy. Especially, multicolor FISH typically with 5–9 probes in 2 sequential hybridization became the standard method [11]. However, FISH has tech-

Table 3. Clinical outcome according to biopsy timing

Variables	Cleavage stage	Blastocyst stage
No. of cycles	412	74
Implantation rates (%)	23.7 (123/518)	30.3 (30/99)
Ongoing pregnancy rates (%)	17.5 (72/412)	25.7 (19/74)
Early abortion rates (%)	33.7 (35/104)	24.1 (7/29)
Clinical pregnancy rates (%)	25.2 (104/412)	39.2 (29/74)

Table 4. Clinical outcome of preimplantation genetic diagnosis group according to age

Variables	≤30 yr	31–35 yr	≥36 yr
No. of cycles	9	57	50
Euploidy rates (%)	40.6±24.5	30.0±19.9	24.0±23.2
Implantation rates (%)	20.0 (3/15)	20.7 (19/92)	19.6 (10/51)
Ongoing pregnancy rates (%)	33.3 (3/9)	17.5 (10/57)	12.0 (6/50)
Early abortion rates (%)	0 (0/4)	20.0 (3/15)	37.5 (3/8)
Clinical pregnancy rates (%)	44.4 (4/9)	26.3 (15/57)	16.0 (8/50)

Table 5. Clinical outcome of preimplantation genetic screening group according to age

Variables	≤30 yr	31–35 yr	≥36 yr
No. of cycles	13	99	258
Euploidy rates (%)	32.8±19.0	36.2±20.4	23.9±24.7
Implantation rates (%)	45.5 (10/22)	28.2 (51/181)	23.4 (60/256)
Ongoing pregnancy rates (%)	15.4 (2/13)	30.3 (30/99)	15.5 (40/258)
Early abortion rates (%)	50.0 (3/6)	26.8 (11/41)	37.3 (22/59)
Clinical pregnancy rates (%)	46.2 (6/13)	41.4 (41/99)	22.9 (59/258)

nical limitations such as hybridization failure, signal overlap and splitting. Also, only restricted number of chromosomes can be interpreted by FISH. RCTs showed a decrease or no improvement in live birth rates per cycle done by FISH [11]. To overcome drawbacks of FISH, new genetic testing technologies that allows all 24 chromosomes to be analyzed was introduced [11]. For instance, PCR, aCGH, single nucleotide polymorphism microarray, and next-generation sequencing were reported as complete chromosome analyzing tools. Among this, array CGH is widely used around the world as it is known to be reliable, accurate, and relatively rapid for whole chromosome analysis [11]. aCGH is genomic hybridization method which requires labelled DNA from both test and control sample which are hybridized to DNA microarray [5]. And then quantitative deviation is analyzed by scanning fluorescent signal to detect both aneuploidy (chromosome copy number) and unbalanced chromosome translocation. aCGH is known to have many strengths. Whole chromosome can be simultaneously analyzed and copy number change of tiny level like 5–10 kbp of DNA sequences of can be detected. Also, identification of microdeletion and duplication is possible. Especially, whole results come out in 24 hours.

In order to improve effectiveness of PGS, there has been many studies to determine when to biopsy the embryo. Recently, 1 RCT concluded that blastocyst biopsy had no measurable impact and may be used safely when embryo biopsy is indicated. In contrast, cleavage stage biopsy markedly reduced potential of embryonic reproduction [12]. Also, Blastocyst biopsy has possibility to analyze more cells and have higher chance to detect the presence of mosaicism in embryo than 3-day biopsy [5]. Several RCTs showed transfer at blastocyst stage is more recommended due to higher implantation rate [5,13]. Also, comprehensive chromosome screening-using PGS as blastocyst biopsy improved IVF outcomes [10].

In this study, 486 PGD and PGS cycles were conducted by array CGH and biopsy at both cleavage stage and blastocyst to compare the outcome by biopsy timing. Tables 2 and 3 showed implantation rates, ongoing pregnancy rates, and clinical pregnancy rates were higher when embryo was biopsied at blastocyst. Conversely, early abortion rates were lower when embryo was biopsied at blastocyst. So this led to conclusion that biopsy at blastocyst is more favorable. Generally, blastocyst biopsy can be performed at 5 days. After biopsy, it takes 24 hours more to analyze all chromosomes so miss of embryo transferring timing might happen. Because of that,

freezing the embryos is commonly done and skip the cycle when 5 days biopsy is conducted. The only downside of blastocyst biopsy is that patient may skip the cycle for embryo. But higher pregnancy rates may anticipate. Also, blastocyst biopsy has an advantage in cases of embryo cryopreservation and verification [5].

From this study, it is known that pregnancy rates and implantation rates were lower in PGD group even mean female age was significantly younger than PGS group. As Table 1 shows that implantation rates, clinical pregnancy rates and ongoing pregnancy rates were lower in PGD group than PGS group. This implies that chromosome rearrangement of parents could be more important factor than age for pregnancy. So, we could make possibilities mentioned below. First, chromosome rearrangement carrier, included at PGD case, might have lower outcome due to unknown effect at embryo which can decrease implantation & pregnancy rate, although it was euploidy embryo. The embryo might be perfect in number, but there could be quality problem. Second, there's possibility of unknown effect produced at PGD case might have adverse effect on mother's susceptibility. This hypothesis might mean some factor which is concerned with implantation of pregnancy maintaining could be lower or not present in chromosome rearrangement carrier. But above 2 possibilities are not proved, and study with more specific design should be done in order to find out. And in PGS cases, variable indications are included, so comparing with PGD cases is not much meaningful. So, analysis of each outcomes was more focused, instead of comparing 2 groups.

It could be expected that embryo of PGD group has higher probability of aneuploidy embryo, as it was already confirmed that parents' chromosome was abnormal before conducting PGS. Nevertheless, the number of cycles which all embryos were abnormal was higher in control group (24.86%) than in the chromosome rearrangement carriers group (18.10%). This opposite result comes out from age issue. The mean age of PGS group is older than PGD group which means age is very important factor to make aneuploidy embryo. Therefore, we analyzed the dataset in the women under 35 years old, and the PGS group displayed more euploid embryos than PGD group (35.8% vs. 31.3%). As a result, younger group has higher chance of getting euploid embryos, and in group under 35 years old, PGS group has higher possibility of getting euploid embryos compared to PGD group.

As seen at Table 4, most patients in PGD group had no

chance of getting pregnant if patient was old age. Out of the group of 40 years of age or older, only one case resulted in pregnancy which eventually ended up as miscarriage. It is uncertain due to small sample size but it is thought to be harder to get pregnant in couples having abnormal chromosome compared to couples having normal karyotype when euploid embryo was transplanted in both group.

As Table 3 shows, early abortion rates were higher in the group biopsied at cleavage stage than in the group biopsied at blastocyst. When embryo development or quality is below average or number of embryos is small, culture to blastocyst is unable and biopsy is done at cleavage stage. This might lead to higher abortion rate as quality might be lower although it was euploidy embryo.

Limitations exist in this study. First, even though findings are broadly similar to those from other study, they have not validated on a large population or large-scale basis. The fact that these data only come from a single institution can be both strength and limitation. Second, although subdivision of patients by various indications and analysis of clinical outcomes were done in this study, there was no comparison to non-PGD/PGS ET done group. Therefore, it could not be proved statistically how much effective PGD/PGS was to increase pregnancy rate in this center.

In summary, our study shows clinical outcomes of PGD/PGS for 2 years at a single center. This is known to be the largest study in Korea. This study showed that the pregnancy outcomes of chromosome rearrangement carriers are slightly lower than others. Also, we could reconfirm that PGD/PGS success rates get higher if biopsy was done at blastocyst. Aneuploidy screening in IVF cycle has clear benefits. But this does not imply universal application of PGD/PGS is preferred. It's not clear yet to whom PGD/PGS may benefit. Further studies are desirable to define better on more specific patients' conditions to improve PGD/PGS results and develop more effective methods.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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