Preimplantation Genetic Screening (PGS) in Infertile Female Age ≥ 35 Years by Fluorescence in Situ Hybridization of Chromosome 13, 18, 21, X and Y

Charoenchai Chiamchanya MD*, Pachara Visutakul MD, PhD**,
Nuntana Gumnarai BSc***, Wanasaya Su-angkawatin MSc****

** Department of Obstetrics and Gynecology, Faculty of Medicine, Thammasat University, Pathumthani*
*** Office of Graduate Studies, Faculty of Medicine, Thammasat University, Pathumthani*
**** Infertility Clinic, Thammasat University Hospital, Pathumthani*
***** Faculty of Medicine, Thammasat University, Pathumthani*

Introduction: *It is common in infertile couples that the female partner age ≥ 35 years, that some of them require assisted reproductive technology (ART) for their treatment, it is also well known that in this female age group increases the chance of chromosome aneuploidy in offsprings. It is known that the antenatal diagnosis may have the ethical dilemma and psychological impact. Therefore, the preimplantation genetic screening (PGS) may have a role in this ART group.*

Objective: *The present study had the objective to compare the incidence of normal, abnormal embryos and also aneuploidy of each chromosome, i.e. 13, 18, 21, X and Y, between 2 subgroups of age i.e. the age 35-39 years and 32-39 years vs. the age ≥ 40 years in both female and male partners respectively.*

Materials and Method: *This prospective study was performed in 20 infertile couples attending the Fertility Clinic at Thammasat University Hospital during the years 2006-2007 of which the female partner aged ≥ 35 years had to use the ART. The PGS was performed by FISH technique with 5 probes to detect the 13, 18, 21, X and Y chromosomes. The comparative analysis was made between 2 subgroups of both female and male partner aged, as mentioned above in the incidence of normal, abnormal embryos and aneuploidy of each chromosome by Chi-square test and Fisher's exact test with statistical significance if $p < 0.05$.*

Results: *The abnormal embryos in the female partner age ≥ 40 years were higher than those of the age 35-39 years (72.4% vs. 52.5%, $p = 0.07$) but with no statistical significance. No different results were obtained in the comparable male partner age groups (56.8% vs. 61.4%, $p = 0.66$).*

The normal female and male embryos in the female partner age ≥ 40 years were lower than those of the age 35-39 years (10.4% vs. 25.4%, $p = 0.08$ and 17.2% vs. 22.1%, $p = 0.60$ respectively) but with no statistical significance.

The normal female and male embryos in the male partner age ≥ 40 years and the age 32-39 years were also compared with no significant differences (20.5% vs. 20.5%, $p = 1.00$ and 22.7% vs. 18.2%, $p = 0.60$, respectively).

The percentage of embryos with aneuploidy of chromosome 18 in the female partner age ≥ 40 years was significantly higher than that of the age 35-39 years (72.0% vs. 45.0%, $p = 0.003$).

The pregnancy rate in the presented PGS study was 12.5% but unfortunately was associated with a high abortion rate of 100%.

Conclusion: *It was found in the present study that the incidence of abnormal embryos trend to increase in the female partner aged ≥ 40 years compared to the aged 35-39 years although with no statistical significance. However, the incidence of embryos with aneuploidy of chromosome 18 was higher in females aged ≥ 40 years with statistical significance, whereas the male partner age had no impact on the abnormality or normality of the embryo. The abortion rate was very high (100%) probably may be due to inadequate choice of probes, inappropriate fixation technology and small sample size. However, the results obtained in this study indicate that the PGS should be considerably performed with strong indication only.*

Keywords: *Preimplantation Genetic Screening (PGS), Assisted Reproductive Technology (ART), Chromosomal aneuploidy, Maternal age, Male partner age*

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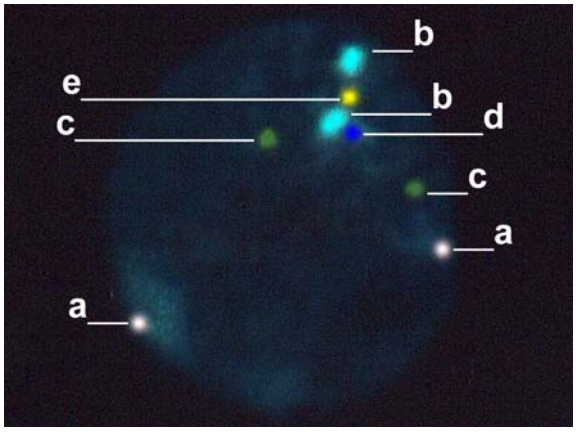
Correspondence to: Chiamchanya C, Department of Obstetrics and Gynecology, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand. Phone: 0-2411-3256, Fax: 0-2411-3256, E-mail: sitci@mahidol.ac.th

It is well known that the advanced female age ≥ 35 years increases the chance of chromosome aneuploidy in offsprings[1]. Infertility is also the problem in couples who were married late, and it is common in infertile couples that some of the female-partner age ≥ 35 years require assisted reproductive technology (ART) for their treatments. As it is also well known that the conventional prenatal diagnosis (PND) such as chorionic villous sampling (CVS), amniocentesis and cordocentesis may have the ethical dilemma and psychological impact when the abnormal fetus is diagnosed, most pregnant females had to termination pregnancy and some couples carried abnormal genetic disease risking the chance of repeated abortion, either spontaneous or induced, after PND. Pre-implantation genetic screening (PGS) is a method to prevent transferring the abnormal genetic embryo to females who had ART treatment by biopsy one or two blastomeres from cleavage stage embryo, usually at 8-cell stage, and analyzed by fluorescence in situ hybridization (FISH) for numerical chromosomal abnormality such as aneuploidy or structural chromosomal abnormality such as reciprocal translocation and Robertsonian translocation and/or polymerase chain reaction (PCR) for single gene disorder, without affecting the viability of the embryo[2-4]. To avoid this problem in infertile females at risk and to select unaffected embryos to be transferred in this ART group is the advantage with PGS over conventional ART plus PND. Due to the limitation of the number of cells available for genetic testing, timeframe of embryo before transfer, this cannot wait for full karyotyping by culture cells to produce metaphase chromosomes for G banding technique and the common chromosome aneuploidy in newborn such as trisomy 21,18 found in females age ≥ 35 years were more than single gene defect, therefore, the present study aimed to examine the PGS in the embryos of the infertile female in this age group before embryo transfer in ART treatment by selection to detect the abnormal of common chromosomes such as 13, 18, 21, X and Y by FISH technique.

Material and Method

The prospective study was approved by the ethical committee of Thammasat Faculty of Medicine and performed in voluntary infertile couples of which the female partners were aged ≥ 35 years who had to undergo the ART treatment in Thammasat University Hospital during the years 2006-2007. The control ovarian hyperstimulation was done by short and long protocol with recombinant FSH (Gonal F®, Serono, Italy) and GnRH agonist (Suprefact® nasal spray, Hoechst AG, Germany). The ovulation induction was made with hCG 10,000 units (Pregnyl®, Organon, Netherland) after the 2 leading follicles with 18 millimeters diameter was found by transvaginal ultrasound monitoring. The oocyte retrieval was performed transvaginally 36 hours after the hCG injection with double lumen needle No.16 (Cook®,Cook, Australia) by transvaginal ultrasound guidance (Aloka SSD-500, Aloka, Japan). The sperm preparation was processed by gradient centrifugation technique with sperm preparation medium (Sil-Select Plus™, Fertipro, Belgium). The insemination was carried out by standard methods of either in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) according to the indication. The fertilization of embryo was checked 16-18 hours after insemination and cultured in culture medium (G-3series®, Vitrolife, Sweden) until 8 cells stage was obtained, then the embryo biopsy was carried out by laser technique (Zilos-tk®, Hamilton Thorne Research, USA). The PGS was performed by FISH technique with 5 probes (MultiVysion PGT®, Vysis, USA) to detect the 13, 18, 21, X and Y chromosomes. After obtaining the blastomere cell and placed into a microdrop of culture medium in a 35 millimeters Petri dish. Gently aspirated the blastomere cell into the pipette and transferred it to the Petri dish containing the hypotonic solution, placing it within the engraved circle under the stereomicroscope. Allow the blastomere cell to remain in the hypotonic solution for 5 minutes. Move the blastomere from the hypotonic solution and transfer to a microscope slide with the small amount of hypotonic solution. Allow the blastomere cell to dry in the drop of hypotonic solution on the slide while constantly viewing under the inverted microscope. Carefully observe the blastomere cell until drying is almost complete. Just prior to complete drying of the hypotonic solution, aspirate a small amount of fixative and drop the fixative onto the blastomere cell. Constantly observe the cell under the inverted microscope while the fixative begins to dry. Just before the drying process is completed place another drop of fixative onto the cell. Repeat several times until the cytoplasm surrounding the cell has dissolved leaving only the nucleus. Draw two circles around the cell on the underside of the glass slide to allow for easier cell location after the hybridization procedure. Place the slide in the coplin jar containing the methanol until ready to begin hybridization. Immediately before hybridization remove the slide from the methanol solution and allow to air dry. Place the specimen slide on the HYBrite surface. Apply 3

microlitters of MultiVysion probe solution to the area on slide where the cell is contained. Apply a 12 millimeters round coverslip over the target area immediately after applying the probe solution. Cut a piece of parafilm and put it over the slide and coverslip to keep the probe solution from drying out during hybridization. Close the lid of the HYBrite and started the hyb/melt program, then carry the process through the 73°C for 5 minutes (melt) and the 37°C for 4 hours (hybridization). When the HYBrite program is completed, remove the slide from the HYBrite. Remove the parafilm and coverslip from the slide. Placed slide in the 0.4X SSC/0.3% NP-40 at 73°C in the coplin jar immediately after removing the coverslip in the coplin jar, incubate for 5 minutes. Remove the slide from the wash solution and place the slide in the coplin jar containing 2X SSC/0.1% NP-40 at room temperature and incubate for 1 minute. Remove the slide from the wash solution and place vertically in a dark area on a paper towel to dry completely. Apply 3 microlitters of AntifadeII onto the target area and place a 12 millimeters round coverslip over the AntifadeII solution. View the slide under the fluorescence microscopy and appropriate filter sets, such as chromosome 13, 18, 21, X and Y by red, aqua, green, blue and yellow colors, respectively. The normal blastomere would be presented with diploid set of all of these 5 chromosomes (Fig. 1). If another aneuploidys such as haploid, triploid, tetraploid set or absence of any of these 5 chromosomes were presented, the blastomere must be labeled as abnormal. The normal embryos were then cultured until reaching blastocyst stage and transferred into intrauterine cavity with embryo transfer catheter (K-JET®, Cook, Australia) under transabdominal ultrasound guidance (Aloka SSD-500, Aloka, Japan). The comparative analysis was made between 2 subgroups of both female and male partner ages, *i.e.* the females aged 35-39 years vs. ≥ 40 years and the males aged 32-39 years vs. ≥ 40 years. The incidence of normal, abnormal embryos and also aneuploidy of each chromosome were analyzed by Chi-square test and Fisher's exact test with statistical significance if $p < 0.05$.



Note: chromosome 13 = a, chromosome 18 = b, chromosome 21 = c, chromosome X = d, chromosome Y = e

Fig. 1 Demontration of FISH of chromosome 13, 18, 21, X and Y in a normal blastomere

Results

A total of 20 infertile couples were included in the present study, the range of age of the female partners and male partners were 35-43 and 32-63 years respectively. The mean duration of infertility was 4.4 ± 3.1 years. The incidence of the primary and secondary infertility were 60% and 40% respectively and all of them had no history of a previous child with congenital anomaly. The causes of infertility in both female and males were mainly undetectable (55% and 40% respectively). The results of semen analysis were the following; 100.1 ± 70.5 million/mililitre of concentration, 43.1 ± 22.6% of motility and 22.9 ± 16.2% of normal morphology. The mean numbers of MII oocyte retrieval per case were 10.8 ± 7.3 (Table 1).

The fertilization rate, cleavage rate and blastocyst rate were 70.7%, 92.9% and 52.0% respectively, of which 35.5% of fertilized embryos were kept frozen (Table 2).

The total of 98 embryos were included in PGS study with 7 embryos not "cleavaged" and 3 embryos considered technical failures due to technical error, therefore, 88 embryos were analyzed. The abnormal embryos in the female partners aged 35-39 years vs. ≥ 40 years were 52.5% vs. 72.4% ($p = 0.07$), and the abnormal embryos in the males aged 32-39 years vs. ≥ 40 years were 61.4% vs. 56.8% ($p = 0.66$). The normal female and male embryos in female partners aged 35-39 years vs. ≥ 40 years were 25.4% vs. 10.4% ($p = 0.08$), and 22.1% vs. 17.2% respectively ($p = 0.60$). Whereas the normal female and male embryos in male partners aged 32-39 years vs. ≥ 40 years were 20.5% vs. 20.5% ($p = 1.00$) and 18.2% vs. 22.7% ($p = 0.60$) respectively as shown in Table 3.

The X chromosome aneuploidy in female partners aged 35-39 years vs. ≥ 40 years were 20.0% vs. 12.0% ($p = 0.32$), whereas, the Y chromosome aneuploidy were 7.5% vs. 0.0% ($p = 0.19$) the chromosome 13 aneuploidy were 57.5% vs. 56.0% ($p = 0.17$),

Table 1. Demographic data of 20 infertile couples

	Range	$\bar{x} \pm SD$	n (%)
Age (years)			
Female	35-43	38.3 ± 3.5	-
Male	32-63	40.8 ± 6.7	-
Duration of infertility (years)	1-10	4.4 ± 3.1	-
Type of infertility			
Primary	-	-	12/20 (60.0)
Secondary	-	-	8/20 (40.0)
History of previous child with anomaly	-	-	0/20 (0.0)
Cause of infertility			
Female factors			
No abnormal detection	-	-	11/20 (55.0)
Tubal factor	-	-	8/20 (40.0)
Endometriosis	-	-	1/20 (5.0)
Male factors			
No abnormal detection	-	-	8/20 (40.0)
Asthenoteratozoospermia	-	-	6/20 (30.0)
Asthenozoospermia	-	-	3/20 (15.0)
Azoospermia	-	-	2/20 (10.0)
Teratozoospermia	-	-	1/20 (5.0)
Semen analysis	21.1-235.0	100.1 ± 70.5	-
Count (mil/ml)	9.0-79.0	43.1 ± 22.6	-
Motility (%)	6.0-57.0	26.1 ± 16.3	-
Progressive motile concentration (PMC %)			
Normal morphology (%)	1.0-47.0	22.9 ± 16.2	-
Oocyte retrieval per case			
M II	3-32	10.8 ± 7.3	-
M I	0-6	0.9 ± 1.5	-

Table 2. Fertilization rate, cleavage rate and blastocyst rate

Insemination methods	Oocyte insemination (n)	Fertilization rate, n (%)	Cryopreservation rate, n (%)	Cleavage rate, n (%)	Blastocyst rate, n (%)
IVF	27	9/27 (33.3)	5/9 (55.6)	3/4 (75.0)	3/4 (75.0)
ICSI	188	143/188 (76.1)	49/143 (34.3)	88/94 (93.6)	48/94 (51.1)
Total	215	152/215 (70.7)	54/152 (35.3)	91/98 (92.9)	51/98 (52.0)

Table 3. The normal and abnormal embryos among the female and male age groups

	Total, n (%)	Female partner aged, n (%)			Male partner aged, n (%)		
		35-39 years	≥ 40 years	p-value	32-39 years	≥ 40 years	p-value
Normal embryo							
Female	18/88 (20.5)	15/59 (25.4)	3/29 (10.4)	0.08	9/44 (20.5)	9/44 (20.5)	1.00
Male	18/88 (20.5)	13/59 (22.1)	5/29 (17.2)	0.60	8/44 (18.2)	10/44 (22.7)	0.60
Total	36/88 (40.9)	28/59 (47.5)	8/29 (27.6)	0.07	17/44 (38.7)	19/44 (43.4)	0.66
Abnormal embryo	52/88 (59.1)	31/59 (52.5)	21/29 (72.4)	0.07	27/44 (61.4)	25/44 (56.8)	0.66

Note : Total of 91 cleavaged embryos were included in the PGS study with 3 embryos having technical failure, therefore only 88 embryos were analysed

Table 4. The chromosome aneuploidy among the female and male age groups

	Female partner age, n (%)			Male partner age, n (%)		
	35-39 years	≥ 40 years	p-value	32-39 years	≥ 40 years	p-value
Chromosome						
X	8/40 (20.0)	3/25 (12.0)	0.32	6/37 (16.2)	5/31 (16.1)	0.63
Y	3/40 (7.5)	0/25 (0.0)	0.19	3/37 (8.1)	0/31 (0.0)	0.26
13	23/40 (57.5)	14/25 (56.0)	0.17	21/37 (56.8)	16/31 (51.6)	0.67
18	18/40 (45.0)	18/25 (72.0)	0.003*	22/37 (59.5)	14/31 (45.2)	0.24
21	22/40 (55.0)	15/25 (60.0)	0.11	22/37 (59.5)	15/31 (48.4)	0.36

* $p < 0.05$

Table 5. The pregnancy rate and abortion rate

Insemination methods	Pregnancy rate n (%)	Abortion rate n (%)
IVF	0/1 (0.0%)	-
ICSI	2/15 (13.3%)	2/2 (100.0%)
Total	2/16 (12.5%)	2/2 (100.0%)

Note : Of the total 20 couples, all embryos of 4 couples were abnormal by the PGS , therefore only 16 couples had some normal embryos to transfer

the chromosome 18 aneuploidy were 45.0% vs. 72.0% (p = 0.003) and the chromosome 21 aneuploidy were 55.0% vs. 60.0% (p = 0.11) respectively as seen in Table 4.

The X chromosome aneuploidy in male partners aged 32-39 years vs. ≥ 40 years were 16.2% vs. 16.1% (p = 0.63), whereas the Y chromosome aneuploidy were 8.1% vs. 0.0% (p = 0.26), the chromosome 13 aneuploidy were 56.8% vs. 51.6% (p = 0.67), the chromosome 18 aneuploidy were 59.5% vs. 45.2% (p = 0.24) and the chromosome 21 aneuploidy were 59.5% vs. 48.4% (p = 0.36) respectively as shown in Table 4.

The pregnancy rate, abortion rate and live birth rate were 12.5%, 100% and 0.0% respectively (Table 5).

Discussion

In the present study, the authors found the incidence of normal embryos of females aged ≥ 35 years was 40.9% which is similar to those of an other study (40.0%)[5]. The incidence of abnormal embryos in the females aged 35-39 years and ≥ 40 years (52.5% and 72.4%) were less than those of an other study (69.0% and 82.0%)[6]. The incidence of chromosome 13, 18, 21 aneuploidy in the females aged 35-39 years and ≥ 40 years (45.0-57.5% and 56.0-72.0%) were higher than those of the other study (37.0% and 53.0% respectively)[7]. However, there was a trend of increase of abnormal embryos in the females aged ≥ 40 years compared to those of 35-39 years (72.4% vs. 52.5%, p = 0.07) with a trend of decrease of normal female embryos in the females aged ≥ 40 years compared with those of aged 35-39 years (10.4% vs. 25.4%, p = 0.08) although with no statistical significance. These may be due to the small sample size in the present study and further study may be required. The present results were similar to those of a large study of Munne et al, 2007[8] in that the advanced maternal age was related to chromosome abnormalities. The authors also found that the male partner age had no impact on the embryo normality which is similar to those of other data[12]. The total range of incidence of chromosome 13, 18, 21 aneuploidy in the females age ≥ 35 years obtained in the present study were 45.0-72.0% and when compared between the females aged 35-39 years vs. ≥ 40 years the incidence was 57.5% vs. 56.0% (p = 0.17), 45.0% vs. 72.0% (p = 0.003) and 55.0% vs. 60.0% (p = 0.11) respectively. It could be noted that only chromosome 18 aneuploidy was significantly increased in the females aged ≥ 40 years when compared to the females aged 35-39 years. The present result was more or less comparable to those of Parikh FR et al, 1999[9] who found the chromosome 13, 18, 21 aneuploidy increased in the females aged ≥ 35 years. However, although the total incidence of chromosome 13, 18 and 21 aneuploidy in the present study and of other studies were quite high in the females aged ≥ 35 years (45.0-72.0%, in the present study) but it was generally found in the newborn less frequently[10-12]. This finding may

be due to a high abortion rate which often associates with abnormal embryos[13]. The technical error in the present study was 3.3% (3 in 91 embryos) is also comparable to those of other studies (4.7%)[6]. However, the very high abortion rate obtained in the present study (100%) is different from those of other studies. This discrepancy may be due to inadequate choice of probes, inappropriate fixation technology and small sample size[6]. Therefore, in the authors opinion the PGS should be considerably performed with strong indication and strict precautions.

Conclusion

The incidence of a normal embryo in the females aged ≥ 35 years (40.9%) in the present study was similar to those of other studies. The incidence of an abnormal embryos trended to increase while the normal embryo trended to decrease in the female aged ≥ 40 years when compared to the females aged 35-39 years although with no statistical significance. The total range of incidence of 13, 18, 21 chromosome aneuploidy in female partners aged ≥ 35 years found in the present study were also high (45.0-72.0%). However, only the incidence of embryos with aneuploidy of chromosome 18 was significantly increased in the females aged ≥ 40 years, whereas the male partner age had no impact on the abnormality or normality of embryo. The abortion rate of the present study was also very high, possibly due to inadequate choice of probes, inappropriate fixation technology and small sample size. Therefore, it is concluded that the PGS should be considerably performed with strong indication and precautions.

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การวินิจฉัยความผิดปกติทางพันธุกรรมก่อนการฝังตัวของตัวอ่อนของโครโมโซมคู่ที่13,18, 21, X และY ในหญิงที่มีบุตรยากอายุตั้งแต่ 35 ปี ขึ้นไป โดยขบวนการ fluorescence in situ hybridization (FISH)

เจริญไชย เจียมจรรยา, พัชรา วิสุตกุล, นันทนา ก้ำนารายณ์, วนัสยา สุอังคะวาทิน

บทนำ: *ในคู่สมรสที่มีบุตรยากจะพบบ่อยว่าฝ่ายหญิงมีอายุตั้งแต่ 35 ปีขึ้นไป ซึ่งกลุ่มนี้จะมีโอกาสมีบุตรที่มีความผิดปกติของโครโมโซมมากขึ้น บางรายต้องรักษาด้วยขบวนการช่วยปฏิสนธิด้วยเทคโนโลยีช่วยการเจริญพันธุ์ เป็นที่ทราบกันว่าการตรวจวินิจฉัยความผิดปกติของทารกในครรภ์ อาจมีข้อถกเถียงด้านจริยธรรม และผลกระทบต่อจิตใจ ดังนั้นการวินิจฉัยความผิดปกติทางพันธุกรรมก่อนการฝังตัวของตัวอ่อน จึงอาจจะมีบทบาทในคู่สมรสดังกล่าว*

วัตถุประสงค์: *การศึกษานี้มีวัตถุประสงค์ที่จะศึกษาเปรียบเทียบอุบัติการณ์ของตัวอ่อนที่ปกติและที่ผิดปกติ รวมทั้งความผิดปกติของแต่ละโครโมโซม ในโครโมโซมคู่ที่ 13, 18, 21, X และ Y ในหญิงและชาย 2 กลุ่มอายุ คือ 35-39 ปี (ในหญิง), 32-39 ปี (ในชาย) เปรียบเทียบกับกลุ่ม 40 ปีขึ้นไป*

วัสดุและวิธีการ: *เป็นการศึกษาไปข้างหน้าในคู่สมรสที่มีบุตรยากจำนวน 20 คู่ ในโรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ ระหว่างปี พ.ศ. 2549-2550 ที่ฝ่ายหญิงมีอายุตั้งแต่ 35 ปีขึ้นไป และมีข้อบ่งชี้ที่ต้องรักษาด้วยขบวนการช่วยปฏิสนธิด้วยเทคโนโลยีช่วยการเจริญพันธุ์ โดยทำการวินิจฉัยความผิดปกติทางพันธุกรรมในโครโมโซมคู่ที่ 13, 18, 21, X และY โดยขบวนการ fluorescence in situ hybridization (FISH) ก่อนทำการฝังตัวของตัวอ่อนแล้วทำการศึกษาเปรียบเทียบอุบัติการณ์ของตัวอ่อนที่ปกติ และผิดปกติในทั้งหญิงและชาย 2 กลุ่มดังกล่าวข้างต้น โดยใช้สถิติ Chi-square test และ Fisher's exact test โดยตั้งระดับความสำคัญทางสถิติไว้ที่ $p < 0.05$*

ผลการศึกษา: *ความผิดปกติของตัวอ่อนทั้งหมดในหญิงอายุตั้งแต่ 40 ปี ขึ้นไป มีมากกว่าหญิงอายุ 35-39 ปี คิดเป็นร้อยละ 72.4 และ 52.5 ตามลำดับ แต่ไม่มีนัยสำคัญทางสถิติ ($p = 0.07$) สำหรับในชายกลุ่มอายุตั้งแต่ 40 ปีขึ้นไป เปรียบเทียบกับอายุ 32-39 ปี ไม่มีความแตกต่างกันทางสถิติ คิดเป็นร้อยละ 56.8 และ 61.4 ตามลำดับ ($p = 0.66$)*

ตัวอ่อนปกติทั้งเพศหญิงและเพศชายในหญิงอายุตั้งแต่ 40 ปี ขึ้นไป มีน้อยกว่าอายุ 35-39 ปี คิดเป็นร้อยละ 10.4 และ 25.4 ($p = 0.08$) กับ 17.2 และ 22.1 ($p = 0.60$) ตามลำดับ แต่ไม่มีความแตกต่างกันทางสถิติ สำหรับตัวอ่อนเพศหญิงและเพศชายปกติในชายอายุตั้งแต่ 40 ปี ขึ้นไป เปรียบเทียบกับชายอายุ 32-39 ปี คิดเป็นร้อยละ 20.5 และ 20.5 ($p = 1.00$) กับ 22.7 และ 18.2 ($p = 0.60$) ตามลำดับ ซึ่งก็ไม่มีความแตกต่างกันทางสถิติเช่นกัน

ความผิดปกติในโครโมโซมคู่ที่ 18 ของตัวอ่อนในหญิงอายุตั้งแต่ 40 ปีขึ้นไป พบมากกว่าหญิงอายุ 35-39 ปี คิดเป็นร้อยละ 72.6 และ 45.0 ตามลำดับ อย่างมีนัยสำคัญทางสถิติ ($p = 0.003$) สำหรับอัตราการตั้งครรภ์ในการศึกษานี้เท่ากับร้อยละ 12.5 แต่มีอัตราการแท้งที่สูงมาก คือ ร้อยละ 100

สรุป: *ในการศึกษานี้พบว่า อุบัติการณ์ของตัวอ่อนที่ผิดปกติมีแนวโน้มเพิ่มขึ้น ในหญิงอายุตั้งแต่ 40 ปีขึ้นไป เปรียบเทียบกับหญิงอายุ 35-39 ปี ถึงแม้ว่าจะไม่มีนัยสำคัญทางสถิติ ($p = 0.07$) อย่างไรก็ตาม พบว่าอุบัติการณ์ของตัวอ่อนที่มีความผิดปกติของโครโมโซมคู่ที่ 18 เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในหญิงที่มีอายุตั้งแต่ 40 ปีขึ้นไป ในขณะที่อายุของฝ่ายชายไม่มีผลต่อความผิดปกติหรือปกติของตัวอ่อน นอกจากนี้ยังพบว่าตัวอ่อนปกติที่ผ่านขบวนการวินิจฉัยความผิดปกติทางพันธุกรรมก่อนการฝังตัว (preimplantation genetic screening; PGS) แล้วมีอัตราการแท้งสูงมาก ทั้งนี้อาจเนื่องจากการตรวจเพียง 5 โครโมโซมอาจไม่เพียงพอเพราะอาจมีความผิดปกติของโครโมโซมอื่นที่อาจเป็นสาเหตุของการแท้ง และยังอาจมีปัญหาทางเทคนิคของ PGS กับขนาดตัวอย่างที่น้อยเกินไป ดังนั้น PGSจึงควรทำด้วยความระมัดระวังเมื่อมีข้อบ่งชี้ทางการแพทย์เท่านั้น*