Section: Obstetrics & Gynaecology

Original Research Article

COMPARISON OF CLINICAL OUTCOME OF IVF CYCLES WITH OR WITHOUT PREIMPLANTATION GENETIC SCREENING: A PROSPECTIVE OBSERVATIONAL CASE- CONTROL STUDY

Deepti Sharma[1], *Anuja Kamath*[2]

[1]*Assistant Professor, Department of Obstetrics and Gynaecology, VCSG Govt Medical College, Srinagar, Uttarakhand, India*
[2]*Senior Embryologist, Milann IVF center, Bengaluru, India*

Received *: 05/10/2025*
Received in revised form *: 23/10/2025*
Accepted *: 26/10/2025*

Corresponding Author:
Dr. Deepti Sharma,
Assistant Professor, Department of Obstetrics and Gynaecology, VCSG Govt Medical College, Srinagar, Uttarakhand, India.
Email: drdeeptisharma04@gmail.com

DOI: 10.70034/ijmedph.2025.4.123

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (4); 682-689

ABSTRACT

Background: The objective is to compare clinical outcome of women undergoing frozen transfer of euploid blastocysts identified by PGS with women undergoing frozen transfer of non-PGS blastocysts. This is a prospective observational case-control study. Study was carried out at tertiary care center.

Materials and Methods: A total of 58 patients underwent IVF followed by PGS by Next Generation Sequencing (NGS) technique between May 2019 to April 2020 with either of the following indications were included in the case group:(a) Advanced maternal age (AMA) ≥ 35 yrs (b) Previous >2 implantation failure after transfer of at least 4 good quality embryos including fresh and frozen embryo transfer cycles (RIF) (c) History of recurrent pregnancy loss (RPL). During the same time period 72 patients undergoing self-cycles of frozen embryo transfer with good quality blastocyst transfer without PGS having either of the above indications were included in control group. Statistical Analysis Continuous variables were presented as mean± standard devaiation. Statistical significance was evaluated using students t test for continuous variables and chi-square test for categorical variables. A p value of <0.05 is considered significant. In addition, Kaplan–Meier estimator is used to calculate time to pregnancy. Outcome measures: Primary outcome was ongoing pregnancy rate, Secondary outcome measures were Implantation rate, clinical pregnancy rate, miscarriage rate, multiple pregnancy rate & time to pregnancy in RIF patients.

Results: Ongoing pregnancy rate(p=0.008) was significantly higher in PGS group; Miscarriage rate(p=0.009), multiple pregnancy rate(p=0.009) were significantly lower in PGS group. Implantation rate and clinical pregnancy rate although higher in PGS group but did not reach clinical significance. Additionally, there were significantly lesser no. of embryos transferred in PGS group(p=<0.0001); and there was significantly reduced time to achieve pregnancy(p=<0.0001) found in RIF patients. In subgroups Ongoing pregnancy rates were significantly higher with PGS in AMA(p=0.02); and increased clinical pregnancy(p=0.003) and ongoing pregnancy rates(p=0.03) in AMA+RIF group.

Conclusion: By this study it has been concluded that use of PGS by NGS technique in indicated cases leads to improved clinical outcome in terms of improved ongoing pregnancy rates; reduced miscarriage and multiple pregnancy rate and lesser time to achieve pregnancy in RIF patients. Patients either with AMA or AMA+RIF are significantly benefited.

Keywords: Preimplantation genetic screening (PGS), Next Generation Sequencing (NGS), In vitro fertilization (IVF).

INTRODUCTION

Despite the numerous advances in the field of IVF; likelihood to achieve a live birth is about 54-54.9% in young patients and 26-42.2% in patients with advanced maternal age.[1] Its success depends upon various factors such as genetic composition of embryos, endometrial receptivity and a proper

embryo transfer.[2-4] In relationship to embryos; about 50% of cleavage stage embryos produced in vitro have been found to be chromosomally abnormal which increases to about 80% in women over 40yrs of age.[5-9] Although some abnormal embryos undergo arrest at D3 or 5; most of them continue to grow and more than 40% of blastocyst are abnormal in women with advanced maternal age.[10] Most of the chromosomal abnormalities are incompatible with implantation or birth, thereby negatively affecting the success rates of IVF.[11]

In the past years in order to combat this obstacle, in IVF multiple embryos have been transferred so as to increase the probability of achieving at least single live birth; which has its own drawback of multiple pregnancies leading to increased obstetric and perinatal complications.[12-14]

In order to avoid this complication Embryo Selection (ES) methods have been developed to select best one or two embryos for transfer.[15-17] Ideally single euploid embryo with maximum implantation potential must be chosen for transfer, thus decreasing multiple pregnancy rates and increasing the likelihood to achieve pregnancy and live birth.[18-20] ES methods include non-invasive methods e.g., morphological selection, and morpho kinetic selection and invasive method i.e., Preimplantation Genetic Screening (PGS).[16,17,21,22]

However, morphological selection of embryos that is routinely used in IVF gives very little information about chromosomal composition of embryos.[23] For this reason, Preimplantation genetic screening (PGS) has been developed as a method for selection of chromosomally normal embryos following embryo biopsy and genetic assessment.[24,25] These normal embryos have higher potential for implantation and survival to term, thus decreasing miscarriage rates and improving IVF success rates.[26] For PGS, in order to obtain genetic material; biopsy can be done at different stages of embryo development ranging from polar body biopsy to trophectoderm biopsy. Of these methods; trophectoderm biopsy done on D5 or D6 has been documented to have better clinical outcomes,[27] which further are improved with utilization of Comprehensive chromosomal screening (CCS) method. CCS has the advantage of performing complete 24 chromosomal analyses thus, providing better tool for selection of genetically normal embryo.[28] In recent years Next generation sequencing (NGS) has been developed and found to be highly accurate and efficient technique of PGS-CCS.[29]

Patients with recurrent implantation failure have also been found to have higher proportion of chromosomally abnormal embryos (67.4%) as compared to controls (36.3%) and application of PGS in this group of patients improves IVF outcomes,[30] and may decrease time to achieve pregnancy. Further around 50-70% of spontaneous miscarriages are a result of some form of chromosomal abnormality in the embryo,[31] and some of these can result in recurrent pregnancy loss, thus indicating the utilization of PGS in this group; particularly in those who had previous aneuploid miscarriage.[32]

The aim of this study is to evaluate the clinical outcome with and without the application of PGS in patients undergoing IVF with any of these indications: a) advanced maternal age(AMA) b) recurrent implantation failure(RIF) c) recurrent pregnancy loss (RPL).

MATERIALS AND METHODS

Study design and participants: This is a prospective observational case control study conducted at a tertiary a care center from May2019to April 2020. The study protocol was approved by the Institutional Ethics Committee.

All patients gave written informed consent for IVF-PGS. A total of 56 patients underwent 59 cycles of ICSI followed by PGS through NGS technique with either of the following indications were included in the case group:

(a) Advanced maternal age≥ 35 yrs (b) Previous >2 implantation failure after transfer of at least 4 good quality embryos including fresh and frozen embryo transfer cycles (c) History of recurrent pregnancy loss.

During the same time period 72 patients undergoing self-cycles of frozen embryo transfer with good quality blastocyst transfer without PGS having either of the above indications were included in control group.

Ovarian stimulation, embryo biopsy, PGS, vitrification and frozen embryo transfer:

All patients in both the groups underwent controlled ovarian stimulation with either of standard long agonist or flexible antagonist protocol. In long agonist protocol after confirming pituitary suppression on day 2 or day 3 of menstrual cycle stimulation was initiated with either recombinant FSH (Gonal-f, Merck-Serono) alone or in combination with human menopausal gonadotropin (HMG;Humog HP, Bharat Serum Vaccine). Starting dose was selected on the basis of age, day 2/3 FSH levels, BMI, AMH levels and antral follicle count(AFC), with adjustments made according to patient's response.

In antagonist cycles stimulation was started on day 2 or day 3 of cycle and antagonist- Ganirelix (Orgalutron, Organon) 0.25 mg s/c was started when either the lead follicle is of 14mm or S. E2 levels ≥400 pg/ml. Serial monitoring of ovarian response was done by transvaginal ultrasound (TVS) and serum E2, LH and P4 assays. When at least 3 follicles reached 17 mm size, recombinant HCG (Ovitrelle, Merck-Serono) 250 or 500 mcg s/c was administered. Transvaginal ultrasound guided oocyte retrieval was performed 34-35 hrs following HCG injection under intravenous sedation with either single lumen (Gynetics, Vitrolife) or double

lumen (Swemed) oocyte retrieval needle depending upon no. of follicles present.

In all the patients mature oocytes (MII) were injected with sperm by Intracytoplasmic sperm injection (ICSI) as per the standard operating procedure of the center. Fertilization check was done 18hrs post ICSI. 2PN embryos were further cultured in cleavage stage (G1, vitrolife) media. Embryos were graded according to Istanbul Consensus workshop on embryo assessment (33): cleavage-stage embryos, Grade 1 (G1) (good): <10% fragmentation, stage-specific cell size, and no multinucleation. Grade 2 (G2) (fair): 10%–25% fragmentation, stage-specific cell size for majority of cells, and no evidence of multinucleation. Grade 3 (G3) (poor): severe fragmentation (>25%), cell size not stage specific, and with evidence of multinucleation.

Blastocysts were graded as follows: 1 – early; 2 – blastocyst; 3 – expanded; 4 – hatched/hatching; inner cell mass: 1 (good) – prominent, easily discernible, with many cells that are compacted and tightly adhered together; 2 (fair) – easily discernible, with many cells that are loosely grouped together; 3 (poor) – difficult to discern, with few cells; trophectoderm: 1 (good) – many cells forming a cohesive epithelium; 2 (fair) – few cells forming a loose epithelium; 3 (poor) – very few cell

When at least one good quality embryo was present on day 3, decision was taken to go ahead with biopsy and Laser assisted zona drilling was done with the help of laser (Octax,MTG) to assist hatching and embryos were then cultured in blastocyst culture media(G2, vitrolife) media. On day 5 embryos that have reached blastocyst stage and started hatching were transferred from culture dish to biopsy dish containing zwitter ion based media (GMOPS, vitrolife) which was then moved to micromanipulator (RI Nikon); where under 400x magnification hatched out trophectoderm cells (4-8cells) were pulled with biopsy pipette (Flat micropipette, Origio) and laser (Octax,MTG) was used to lyse cell junctions. Biopsied cells were washed 4-5 times successively in buffer media (PVP media, Igenomix) and loaded in 2 µl of phosphate buffered saline; both present in biopsy kit and kept at -120°C and transferred to genetic lab for PGS by NGS technique by maintaining cold chain.

The biopsied blastocysts were then vitrified; as the results of PGS were made available after 14-20 days. In control group good quality blastocysts (3-1-1) according to istanbul consensus were vitrified. An open system using cryolock with 15% ethylene glycol, 15% dimethylsulfoxide(DMSO),and 0.5 mol/L sucrose as cryoprotectants (SAGE vitrification kit, Origio) was used to vitrify the embryos. Once the results of PGS are available; same were communicated to the patient and when minimum of one euploid embryo was present; patient was planned for FET cycle. In cases where no euploid embryos were found; no transfer was done after counselling of patient.

All frozen embryo transfer cycles (FET) were HRT cycles where endometrial preparation was done with daily dose of orally administered 6mg of estradiol (Progynova; Zydus Cadila). Endometrial evaluation was done by TVS; and when endometrial thickness was >8mm with multilayered morphology, it was considered adequate for implantation. After this endometrial priming was done by injectable progesterone (Gestone, 50 mg; Ferring) for 5 days. During endometrial preparation on day 9 if endometrial thickness was <7mm, transdermal preparation of estradiol (Oetragel, Besins) was added and dose of oral estrogen was increased to 12 mg. If after 7 days of increased estrogen dose; endometrial thickness was <7 mm the cycle was cancelled.

One or two euploid embryos in case group and one or two embryos in control group were selected and were thawed on day of transfer using 1.0M sucrose(Sage-thawing kit, Origio) and incubated for at least 2 hrs in bench top incubator(K-system,G185) for blastocele to re-expand. Transfer of only good quality blastocysts was done under ultrasound guidance using Sure-Pro Ultra catheter (Wallace,Origio).

All patients were given Luteal phase support with estradiol (Progynova; Zydus Cadila) and vaginal progesterone (Susten;Sun Pharmaceutical Ind. Ltd) for 14 days. S. BHCG was done after 14 days of embryo transfer and luteal phase support was continued till 10 weeks when pregnancy was achieved. A clinical pregnancy was defined by presence of one or more gestational sacs seen on Transvaginal ultrasonography after 3-4 weeks of positive BHCG.

Time to pregnancy (TTP) was calculated in patients with RIF; i)from the day of decision making for first embryo transfer which was noted from patient's medical records till day of positive BHCG.

ii)and/or from day of embryo biopsy till day of positive βHCG.

OUTCOMES MEASURED:

Primary outcome: Ongoing pregnancy rate was defined as number of gestational sacs with fetal heartbeat present at 12 weeks of gestation per embryo transfer cycle

Secondary outcome: Implantation rate which was defined as the number of gestational sacs visible on TVS divided by the number of embryos transferred per patient. Clinical pregnancy was defined as a positive serum beta-hCG (b-hCG) with transvaginal sonographic evidence of a gestational sac with fetal heart beat at 6 weeks of gestation and Clinical pregnancy rate was defined as clinical pregnancy divided by number no. of embryo transfer cycles, miscarriage rate was defined as the number of implanted pregnancies or clinical pregnancies lost before 20 weeks of gestation divided by the total number of clinical pregnancies, multiple pregnancy rate was defined as number of pregnancies with >1 gestational sacs seen on scan per clinical pregnancy

& time to pregnancy was estimated in in RIF patients.

Statistical method: Data was collected in excel sheet Microsoft world 2010 version and analyzed.

Continuous variables were presented as mean± standard devaiation.

Statistical Significance was evaluated using students t test for continuous variables and c2 test for categorical variables.

A p value of <0.05 is considered significant.

Kaplan–Meier estimator is used to calculate time to pregnancy.

RESULTS

Participant flow: Participant flow is depicted in fig.1. Out of the total of 130 patients; 58 were in PGS group and 72 in non-PGS group; and had undergone IVF cycles.

Baseline and stimulation cycles characterstics: Two groups were similar in baseline characteristics of age, FSH, LH, AMH levels, AFC, total dose of gonadotropins, stimulation days, peak E2, and p4 levels on day of HCG trigger. [Table 1]. Majority of patients had secondary infertility (66% in PGS and 62.5% in non-PGS group). Most of the patients had antagonist protocol for COS (85.7% in PGS and 87.5% in non-PGS group)

Embryological characterstics: Two groups did not differ in no. of oocytes retrieved, no. of MII oocytes, no. of fertilized oocytes, no. of cleaved embryos, no. of embryos cultured to blast and no. of embryos on D5. [Table 2].

A total of 162 blastocysts were biopsied out of which 57 were found to be euploid and 84 were aneuploid and 21 had no diagnosis as in 15 no DNA was detected, 5 had insufficient DNA, and in 1Whole Genome Amplification(WGA) failed.

FET cycle charaterstics and clinical outcome: Out of 58 patients in PGS group 36 had frozen embryo transfer of 54 euploid blastocysts in 37 FET cycles. A total of 36 patients underwent first FET cycle and 1 patient underwent second FET cycle also. 3 excess euploid blastocysts are frozen. 21 patients had no euploid blastocyst to transfer.

In non-PGS group 72 patients have undergone IVF cycles followed by FET cycles with transfer of morphological good quality blastocysts (according to Istanbul Consensus Workshop 2011).

In PGS group significantly lesser no. of embryos were transferred as compared to control group. [Table 3] to achieve a higher Implantation rate and clinical pregnancy rate but that does not reach statistically significant level.

With significantly lower miscarriage rates; ongoing pregnancy rate is significantly higher in PGS group thus, implying that PGS helps in selection of viable euploid embryos [Table 3]

Also, multiple pregnancy rate is significantly lower in PGS group [Table 3]; thus, enabling transfer of lesser no. embryos with decrease risk of multiple pregnancy.

Further, AMA group had maximum no. of aneuploid blastocyst followed by RM and AMA+RIF group; and least in RIF group.as maternal. [Table 4]

Chromosome 16 and 15 were found to be most commonly aneuploid.

Mean time to pregnancy in recurrent implantation failure (RIF) patients was 949 days (CI: 534.46-1364.13) before PGS was done and it reduced to 134 days (CI: 88.0-180.3) after PGS was done (p value:<0.0001) which is even less than the control group (Mean 217 days; CI 110.4-325.3) (p value:<0.0001) [Figure 2]

Table 1: Baseline and Stimulation characteristics

	PGS group(n=58)	Non PGS group (n=72)	P value
Maternal age	34.7(4.0)	33.7(3.3)	0.1
Duration of infertility	5.3(3.3)	5.5(3.0)	0.72
Primary Infertility n (%)	22(39.2%)	27(37.5%)	
Secondary Infertilty n (%)	34(66.0%)	45(62.5%)	
BMI (kg/m2)	25.7(3.7)	25.2(5.0)	0.53
FSH	7.4(3.5)	6.6(1.8)	0.09
LH	6.2(3.9)	6.4(3.7)	0.76
AMH(ng/ml)	3.5(2.7)	4.6(3.7)	0.06
AFC	11.2(4.0)	11.9(3.9)	0.32
Antagonist Protocol n(%)	47(85.7%)	63(87.5%)	
Long Agonist Protocol n(%)	11(19.6%)	9(12.5%)	
Total dosage of gonadotropins	2961(1922)	2555(1627)	0.19
Days of stimulation	10.18(1.45)	10.11(1.37)	0.78
PEAK E2(pg/ml)	1584.7(797.1)	1646.82(720.0)	0.64
PEAK P4(ng/ml)	0.76(0.45)	0.92(0.67)	0.12

Table 2: Embryological Characterstics

	PGS Group	NON PGS GROUP	P value
No. Of Oocytes Retrieved	10.38(5.49)	12.1(6.35)	0.10
No. Of Mii Oocytes	6.72(3.59)	7.38(3.25)	0.27
Fertilized Oocytes	6.6(3.4)	7.1(3.4)	0.4
Cleaved Oocytes	6.6(3.4)	7.0(3.4)	0.5
No. Of Embryos On D3	5.16(2.97)	5.69(2.65)	0.74
No. Of Embryos Cutured To Blast	4.35(2.35)	4.08(1.40)	0.41

No. Of Embryos On D5	3.40(1.75)	3.40(1.18)	1.0

Table 3: FET Cycle Characterstics and Clinical Outcome

	PGS GROUP N=58	NON PGS GROUP(n=72)	P value
Endometrial thickness	9.8(1.7)	9.4(1.0)	0.09
No. Of embryos transferred	1.43(0.50)	1.79(0.40)	<0.0001
Implantation rate	22/54=40.7%	43/129=33.3%	0.38
Clinical pregnancy rate/et cycle	19/37=51.3%	28/72=38.8%	0.15
Miscarriage rate/et cycle	4/37=10.8%	24/72=33.3%	0.009
Ongoing pregnancy/et cycle	18/37=48.6%	19/72=26.3%	0.008
Multiple pregnancy	1/19=5.2%	6/28=21.4%	0.009

Table 4: Ploidy of blastocysts in patients with different indications for PGS

Primary indication	No. of patients	No. of embryos biopsied	Euploid embryos	Aneuploid embryos
AMA	19	57	17/57(29.8%)	32/57(56.1%)
RIF	20	58	21/58(36.2%)	28/58(48.2%)
AMA+RIF	13	34	12/34(35.2%)	18/34(52.1%)
RM	5	13	7/13(53.8%)	6/13(46.1%)
TOTAL	58	162	57/162(33.7%)	84/162(49.7%)

Table 5: No. and percentage of embryos with different chromosomal abnormalities

Monosomy	30(35.7%)
Trisomy	16(19.0%)
2 Abnormal chromosomes	7(8.3%)
3 Abnormal chromosomes	4(4.7%)
4 Abnormal chromosomes	5(5.9%)
Complex abnormal chromosomes	19(22.6%)
Segmental chromosomes abnormality	2(2.3%)
Mosaic aneuploidy	1(1.1%)
Total	84

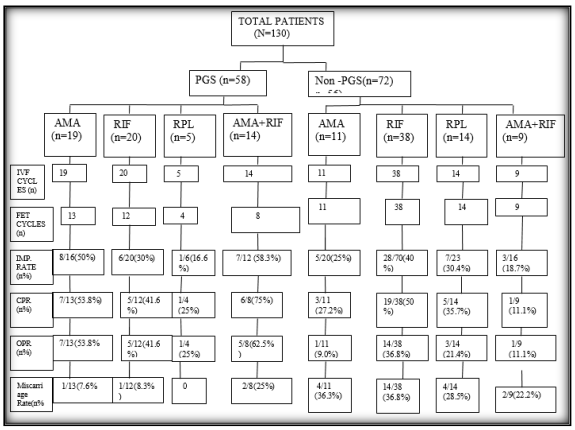

Figure 1: Pateints Flow Chart

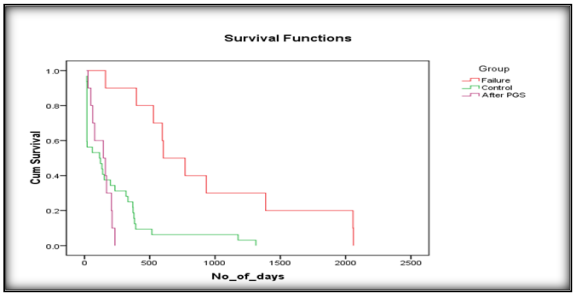

Figure: 2 Kaplan Meir Curve Showing Time To Pregnancy In Rif Pateints Befor And After Pgs And Comapred To That Of Control Group

DISCUSSION

PGS is being used for more than a decade to select euploid embryos for transfer in IVF cycles, so as to improve pregnancy rates; but still there is an ongoing controversy for its use. In 1990s PGS with florescent in situ hybridization technique (FISH) on polar body or cleavage stage biopsy was done and first pregnancy was reported in 1995.[5] One of the first meta-analysis by Mastenbroek et al,[34] in 2011 concluded that PGS lowers live birth rate in women with advanced age. Drawbacks of the study was that all the studies that were included; had used FISH method on D3 embryo biopsy for PGS which has technical inefficiency of limited number and regions of chromosomes analyzed; and increased chromosomal mosaicism and thus increased false positive rates.

With the evolution; newer technologies utilizing whole genome amplification (WGA) and CCS from cells derived from trophectoderm on D5 or D6 embryo have been developed.[35] These techniques include comparative genomic hybridization arrays (aCGH), single nucleotide polymorphism microarrays (SNP), quantitative polymerase chain reaction and recently, next generation sequencing (NGS). These technologies aim to improve not only clinical results but also time to pregnancy and, most importantly, take home baby rates. Among these NGS is rapidly emerging technique with specificity of 99.8% and sensitivity of 100% for aneuploidy detection.[36] The advantages of NGS is that in addition to detection of aneuploidies it can also allow simultaneous detection of single gene disorders, translocations, and abnormalities of mitochondrial genes from the same biopsy sample without the need for unique different platforms for each.[36]

In the present study NGS was used for genetic analysis of trophectoderm cells of D5 or D6 embryos. Benefits of biopsy on D5 or D6 embryo are removal of trophectoderm cells for biopsy has minimal or no impact on embryo development potential as compared to D3 embryo biopsy,[37] it also provides more DNA templates than D3 embryo thus improving sensitivity and specificity of PGS; cells derived from trophectoderm are more representative of inner cell mass chromosomal composition; and are less likely to be mosaic.[38]

A RCT by Coates et al has concluded better ongoing pregnancy rate and live birth rate in NGS based PGS on trophectoderm cells followed by transfer of euploid embryos in frozen cycles.[39] Frozen embryo transfer cycles have the advantage of more physiological estradiol(E2) levels as compared to fresh cycles in which Supraphysiological E2 levels reduces the endometrial receptivity leading to poorer outcomes.[40]

Further the indications for PGS in IVF are the conditions where there is increased risk of embryo aneuploidy such as advanced maternal age,[41-49] recurrent implantation failures,[50,51] recurrent miscarriages.[52,53] In our study we hypothesize the utility of NGS based PGS after trophectoderm biopsy on D5 embryos with transfer of euploid embryos in frozen transfer cycles in improving clinical outcome in these group of patients.

In our study it has been found that PGS leads to significantly increased ongoing pregnancy rate (48.6% vs 26.3%); thereby decreasing miscarriage rates significantly (13.5% vs 33.3%) thus reducing emotional and mental trauma that patient has to undergo with miscarriages. In present study it has been shown that with PGS significantly lesser number of embryos were transferred (1.43± 0.5 vs 1.79± 0.4) with reduction in multiple pregnancy rates (5.2% vs 21.4%); there by reducing its obstetric and perinatal adverse effects.

Aneuploidy rate of embryos increases with increased maternal age; leading to implantation failure and miscarriages also, it has been concluded that there is increased proportion of more complex aneuploidies with advanced maternal age.[54] In this study more than half of embryos were found to be aneuploid in AMA; and AMA +RIF groups; hence PGS is indicated in these group of patients in order to select euploid embryos. It has been found in our study that PGS in AMA and AMA+RIF groups had significantly increased ongoing pregnancy rates as compared to control group (53.8% vs 33.3%; p value 0.02); (62.5%vs 11.1%) respectively. In AMA+ RIF group CPR (75%vs11.1%; p value 0.003) was also increased significantly. In these groups implantation rate has been found to be increased but not significantly; with no significant difference in miscarriage rates. Our findings are in conjunction with the previous studies which have found increased CPR and OPR in AMA population with no difference in miscarriage rate.[55]

Less than half of embryos were aneuploid in RIF group and in this group no statistically significant difference has been found in clinical outcomes; which was also been found in a study by Rubio et al.[43] In this group of patients PGS has led to significantly reduced time to achieve pregnancy; there by reducing the number of transfers needed to achieve pregnancy. This can lead to benefit in RIF population that transfer of euploid embryo at an earlier stage leads to reduced time to pregnancy; which in turn will reduce social and emotional burden. This is in corroboration with a recent study in which in AMA population PGS led to lesser number of embryo transfer cycles per live birth as compared to control group and also time to pregnancy was found to be less thereby also indicating that PGS leads to decreased miscarriage rate.[56]

In present study in RM group there is more proportion of euploid embryos, with no significant difference in clinical outcomes noted; although the ploidy status of previous miscarriages is not known. However, a retrospective study by Al-asmar et al. has recommended the role of PGS in couple with previous aneuploid miscarriage; as they were found to have increased aneuploidy rate.[57]

Strengths of this study is use of NGS technique of PGS done on trophectoderm cells of D5 embryos. All cycles were frozen embryo transfer cycles of euploid embryos both in cases and control groups.

Limitations of the study is its small sample size. High cost associated with PGS and vitrification of embryos is one of the reasons for this. PGS is an invasive procedure; with removal of cells from embryos; and its epigenetic effects are not known. Few studies that have been conducted so far have shown no difference in prematurity, low birth weight and major congenital malformation in children born after embryo biopsy as compared to IVF only,[58] also no evidence of neurodevelopmental adverse effect has been found in 9yrs children born after D3 embryo biopsy.[59]

Lastly the rate limiting step in PGS is extended culture of embryos to blastocyst stage; and patients whose embryo fail to develop till D5 especially in AMA group of patients; and they were left with no embryos to transfer.

CONCLUSION

Our study has showed that PGS done by NGS technique leads to increase in ongoing pregnancy rate with decrease in miscarriage rate in indicated groups; with most benefit in patients with AMA and AMA+RIF. Also, it leads to lesser no. of embryos transferred and a valid tool for embryo selection and thereby helps in transfer of viable embryo with highest potential to give pregnancy; and also leads to decrease in multiple pregnancy and its related complications.

Further selection of embryos with PGS has shown a decreased time to achieve pregnancy in RIF patients; who are burdened with repeated embryo transfer as repeated negative pregnancy results. With the application of PGS in this group will lessen their burden and this is one of the emerging benefits of PGS application.

REFERENCES

1. National Summary Report for 2014 by Society of Assisted Reproductive Technology(SART)
2. Dahdouh EM, Balayla J, Garcia-Velasco JA. Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials. Reprod Biomed Online 2015;30:281–9.
3. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril 2011; 96:344–8.
4. Mains L, van Voorhis BJ. Optimizing the technique of embryo transfer. Fertil Steril 2010;94:785–90.
5. Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. Fertil Steril 1995;64:382–91.
6. Hardarson T, Hanson C, Lundin K, Hillensjo T, Nilsson L, Stevic J, et al. Pre- implantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. Hum Reprod 2008;23:2806–12.
7. Munne S. Preimplantation genetic diagnosis for aneuploidy and transloca- tions using array comparative genomic hybridization. Current Genomics 2012; 13:463–70.
8. Kroon B, Harrison K, Martin N, Wong B, Yazdani A. Miscarriage karyotype and its relationship with maternal body mass index, age, and mode of conception. Fertil Steril 2011;95:1827–9.
9. Harton GL, Munne S, Surrey M, Grifo J, Kaplan B, McCulloh DH, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril 2013;100:1695–703.
10. Schoolcraft WB, Fragouli E, Stevens J, Munné S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. Fertil Steril. 2010;94:1700–1706.
11. Munné S, Bahçe M, Sandalinas M, Escudero T, Márquez C, Velilla E, Colls P, Oter M, Alikani M, Cohen J. Differences in chromosome susceptibility to aneuploidy and survival to first trimester. Reprod Biomed Online. 2004;8:81–90.
12. Chambers GM, Ledger W. The economic implications of multiple preg- nancy following ART. Semin Fetal Neonatal Med 2014;19:254–61. Murray SR, Norman JE. Multiple pregnancies following assisted reproduc- tive technologies—a happy consequence or double trouble? Semin Fetal Neonatal Med 2014;19:222–7.
13. Gocmen A, Guven S, Bagci S, Cekmez Y, Sanlikan F. Comparison of maternal and fetal outcomes of IVF and spontaneously conceived twin pregnancies: three year experience of a tertiary hospital. Int J Clin Exp Med 2015;8:6272–6.
14. Bromer JG, Ata B, Seli M, Lockwood CJ, Seli E. Preterm deliveries that result from multiple pregnancies associated with assisted reproductive technolo-gies in the USA: a cost analysis. Curr Opin Obstet Gynecol 2011;23:168–73
15. Montag M, Toth B, Strowitzki T. New approaches to embryo selection. Reprod Biomed Online 2013;27:539–46.
16. Basile N, Caiazzo M, Meseguer M. What does morphokinetics add to embryo selection and in-vitro fertilization outcomes? Curr Opin Obstet Gynecol 2015;27:193–200.
17. Sakkas D. Embryo selection using metabolomics. Methods Mol Biol 2014; 1154:533–40.
18. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertil- ization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril 2013;100:100–7.e1.
19. Forman EJ, Tao X, Ferry KM, Taylor D, Treff NR, Scott RT Jr. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum Reprod 2012;27:1217–22.
20. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a ran-domized pilot study. Mol Cytogenet 2012;5:24.
21. Basile N, Vime P, Florensa M, Aparicio Ruiz B, Garcia Velasco JA, Remohi J, et al. The use of morphokinetics as a predictor of implantation: a multicentric study to define and validate an algorithm for embryo selection. Hum Reprod 2015;30:276–83.
22. Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod 2011;26:2658–71.
23. Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. Mol Hum Reprod 2014;20:117–26.
24. Munne S, Cohen J, Sable D. Preimplantation genetic diagnosis for advanced maternal age and other indications. Fertil Steril 2002;78:234–6.
25. Rubio C, Rodrigo L, Mir P, Mateu E, Peinado V, Milan M, et al. Use of array comparative genomic hybridization (array-CGH) for embryo assessment: clinical results. Fertil Steril 2013;99:1044–8.
26. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. diagnosis of major chromosome aneuploidies in human preimplantation embryos. Human Reprod. 1993;8:2185–2191.
27. Elias M Dahdouh, Jacques Balayla , Juan Antonio García-Velasco , Impact of blastocyst biopsy and comprehensive chromosome screening technology on preimplantation genetic screening: a systematic review of randomized controlled trials, Reproductive Bio Medicine Online (2015) 30, 281–289.
28. Gutierrez-Mateo C, Colls P, Sanchez-Garcia J, Escudero T, Prates R, Ketterson K, et al. Validation of microarray comparative genomic hybridi- zation for comprehensive chromosome analysis of embryos. Fertil Steril 2011;95:953–8.
29. Yang et al, Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study; BMC Medical Genomics (2015) 8:30
30. T Pehlivan, C Rubio, L Rodrigo, J Romero, Impact of preimplantation genetic diagnosis on IVF outcome in implantation failure patients Vol 6. No 2. 232–237 Reproductive BioMedicine Online;
31. Silver RM, Branch D. 2007. Sporadic and Recurrent Pregnancy Loss. In Clinical obstetrics: The fetus & mother, 3rd ed. (ed. Reece EA, Hobbins JC), pp. 143–160. Blackwell Publishing, Boston
32. Nasser Al-Asmar, Vanessa Peinado, María Vera, Chromosomal abnormalities in embryos from couples with a previous aneuploid miscarriage (Fertil Steril! 2012;98:145–50.
33. ALPHA Scientists In Reproductive Medicine, ESHRE Special Interest Group Embryology. Istanbul consensus workshop on embryo assessment: Proceedings of an expert meeting. Reprod Biomed Online 2011;22:632-46.
34. Masternbroek S, Twisk M, van der Veen F, Repping S. Preimplantation ge- netic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update 2011;17:454–66.
35. Sermon K, Capalbo A, Cohen J, Coonen E, De Rycke M, De Vos A, et al. The why, the how and the when of PGS 2.0: current practices and expert opin- ions of fertility specialists,

molecular biologists, and embryologists. Mol Hum Reprod 2016;22:845–57.
36. Francesco Fiorentino, Anil Biricik, Sara Bono, Letizia Spizzichino et al .Development and validation of a next-generation sequencing–based protocol for 24-chromosome aneuploidy screening of embryos; Fertil Steril 2014;101:1375–82
37. Scott, R.T., Jr., Upham, K.M., Forman, E.J., Zhao, T., Treff, N.R., 2013b. Cleavage-stage biopsy significantly impairs human embry- onic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil. Steril. 100, 624– 630.
38. Capalbo, A., Wright, G., Elliott, T., Ubaldi, F.M., Rienzi, L., Nagy, Z.P., 2013b. Fish reanalysis of inner cell mass and trophecto- derm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mo- saicism at the blastocyst stage. Hum. Reprod. 28, 2298–2307.
39. Coates et al, Optimal euploid embryo transfer strategy, fresh versus frozen, after preimplantation genetic screening with next generation sequencing: a randomized controlled trial, (Fertil Steril 2017;107:723-30)
40. Arslan M, Bocca S, Arslan EO, Duran HE, Stadtmauer L, Oehninger S. Cu- mulative exposure to high estradiol levels during the follicular phase of IVF cycles negatively affects implantation. J Assist Reprod Genet 2007; 24:111–7.
41. Hanson C, Hardarson T, Lundin K, Bergh C, Hillensjo T, Stevic J, et al. Re- analysis of 166 embryos not transferred after PGS with advanced reproduc- tive maternal age as indication. Hum Reprod 2009;24:2960–4.
42. Orris JJ, Taylor TH, Gilchrist JW, Hallowell SV, Glassner MJ, Wininger JD. The utility of embryo banking in order to increase the number of embryos available for preimplantation genetic screening in advanced maternal age patients. J Assist Reprod Genet 2010;27:729–33.
43. Rubio C, Bellver J, Rodrigo L, Bosch E, Mercader A, Vidal C, et al. Preimplan- tation genetic screening using fluorescence in situ hybridization in patients with repetitive implantation failure and advanced maternal age: two ran-domized trials. Fertil Steril 2013;99:1400–7.
44. Milan M, Cobo AC, Rodrigo L, Mateu E, Mercader A, Buendia P, et al. Re- defining advanced maternal age as an indication for preimplantation ge- netic screening. Reprod Biomed Online 2010;21:649–57.
45. Platteau P, Staessen C, Michiels A, van Steirteghem A, Liebaers I, Devroey P. Preimplantation genetic diagnosis for aneuploidy screening in women older than 37 years. Fertil Steril 2005;84:319–24.
46. Gianaroli L, Magli MC, Ferraretti AP, Munne S. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. Fertil Steril 1999;72:837–44.
47. Kuliev A, Verlinsky Y. The role of preimplantation genetic diagnosis in women of advanced reproductive age. Curr Opin Obstet Gynecol 2003; 15:233–8.
48. Schoolcraft WB, Katz-Jaffe MG, Stevens J, Rawlins M, Munne S. Preimplan- tation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. Fertil Steril 2009;92:157–62.
49. Munne S, Magli C, Bahce M, Fung J, Legator M, Morrison L, et al. Preim- plantation diagnosis of the aneuploidies most commonly found in sponta- neous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. Prenat Diagn 1998;18:1459–66.
50. Blockeel C, Schutyser V, de Vos A, Verpoest W, de Vos M, Staessen C, et al. Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. Reprod Biomed Online 2008;17:848–54.
51. Greco E, Bono S, Ruberti A, Lobascio AM, Greco P, Biricik A, et al. Compar- ative genomic hybridization selection of blastocysts for repeated implanta- tion failure treatment: a pilot study. Biomed Res Int 2014;2014:457913.
52. Munne S, Chen S, Fischer J, Colls P, Zheng X, Stevens J, et al. Preimplanta- tion genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. Fertil Steril 2005;84:331–5.
53. Shahine LK, Lathi RB. Embryo selection with preimplantation chromosomal screening in patients with recurrent pregnancy loss. Semin Reprod Med 2014;32:93–9.
54. Franasiak et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening (Fertil Steril 2014;101:656–63)
55. Hey-Joo Kang, Alexis P. Melnick, Joshua D. Stewart et al, Preimplantation genetic screening: who benefits? Fertil Steril! 2016;106:597–602.
56. Rubio et al, In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study, Fertil Steril 2017;107:1122–9
57. Nasser Al-Asmar et al; Chromosomal abnormalities in embryos from couples with a previous aneuploid miscarriage (Fertil Steril 2012;98:145–50).
58. S. Desmyttere,M. De Rycke, C. Staessen, I. Liebaers et al, Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD, Human Reproduction, Vol.27, No.1 pp. 288–293, 2012
59. Derk Kuiper, Anne Bennema, Sacha, Bastide-van Gemert, Developmental outcome of 9-year-old children born after PGS: follow-up of a randomized trial Human Reproduction, Vol.33, No.1 pp. 147–155, 2018.