Aneuploidy screening by array comparative genomic hybridization improves success rates of *in vitro* fertilization: A multicenter Indian study

Aditi Kotdawala, Deven Patel, Javier Herrero[1], Rajni Khajuria[2], Nalini Mahajan[3], Manish Banker

Nova IVI Fertility, Ahmedabad, Gujarat, [1]Nova IVI Fertility, Bengaluru, Karnataka, [2]Iviomics Research Pvt. Ltd., [3]Nova IVI Fertility, New Delhi, India

Address for correspondence:
Ms. Aditi Kotdawala, Nova IVI Fertility, 108, Swastik Society, Navrangpura, Ahmedabad - 380 006, Gujarat, India.
E-mail: aditi.kotdawala@novaivifertility.net

Received: 17.05.2016
Review completed: 04.09.2016
Accepted: 24.11.2016

Access this article online

Quick Response Code:

Website: www.jhrsonline.org

DOI: 10.4103/0974-1208.197630

ABSTRACT

OBJECTIVE: To evaluate the usefulness of preimplantation genetic screening (PGS) using array comparative genomic hybridization (aCGH) in the Indian population. **MATERIALS AND METHODS:** This is a retrospective, multicenter study including 235 PGS cycles following intracytoplasmic sperm injection performed at six different infertility centers from September 2013 to June 2015. Patients were divided as per maternal age in several groups (<35, 35–36, 37–38, 39–40, and >40 years) and as per indication for undergoing PGS. Indications for performing PGS were recurrent miscarriage, repetitive implantation failure, severe male factor, previous trisomic pregnancy, and advanced maternal age (≥35). Day 3 embryo biopsy was performed and analyzed by aCGH followed by day 5 embryo transfer in the same cycle or the following cycle. Outcomes such as pregnancy rates (PRs)/transfer, implantation rates, miscarriage rates, percentage of abnormal embryos, and number of embryos with more than one aneuploidy and chaotic patterns were recorded for all the treated subjects based on different age and indication groups. **RESULTS:** aCGH helped in identifying aneuploid embryos, thus leading to consistent implantation (range: 33.3%–42.9%) and PRs per transfer (range: 31.8%–54.9%) that were obtained for all the indications in all the age groups, after performing PGS. **CONCLUSION:** Aneuploidy is one of the major factors which affect embryo implantation. aCGH can be successfully employed for screening of aneuploid embryos. When euploid embryos are transferred, an increase in PRs can be achieved irrespective of the age or the indication.

KEY WORDS: Aneuploidy, array comparative genomic hybridization, chromosome aberrations, preimplantation genetic screening

INTRODUCTION

Human embryo development occurs through a process that encompasses reprogramming, sequential cleavage divisions, mitotic chromosome segregation, and embryonic genome activation. Chromosomal abnormalities in the germ cells and/or preimplantation embryos may arise during such developmental processes and are a major cause of spontaneous miscarriage or birth defects. Aneuploidy, an alteration in number of chromosomes, is one such chromosomal aberration which is commonly observed in early stage human embryos.[1] Trisomic and monosomic embryos account for at least 10% of human pregnancies and for the women nearing the end of their reproductive lifespan, the incidence of aneuploidy may exceed to 50%.[2]

It is usually perceived that aneuploidy rates are higher in oocytes and embryos in women with advanced maternal age (AMA) which results in increase in spontaneous abortions,

How to cite this article: Kotdawala A, Patel D, Herrero J, Khajuria R, Mahajan N, Banker M. Aneuploidy screening by array comparative genomic hybridization improves success rates of in vitro fertilization: A multicenter Indian study. J Hum Reprod Sci 2016;9:223-9.

thereby reducing ongoing implantation rates (IRs).[3] It might be associated with both maternal meiosis I and meiosis II nondisjunction events.[4] Recent studies in humans and model organisms have shed light on the complexity of meiotic defects, providing evidence that the errors associated with advancing age in human females is not attributable to a single factor but, to an interplay between oogenetic defects and other endogenous and exogenous factors.[2]

Aneuploidy might also be a contributing factor in other infertile populations; for example, despite other potential causes, an abnormal embryonic karyotype was found to be the most frequent cause of recurrent miscarriage (RM).[5] In the same study, the percentage of cases with RM of truly unexplained origin was limited to 24.5%. While the diagnosis of repetitive implantation failure (RIF) remains a clinical challenge, embryonic aneuploidy has been proposed as one of the leading causes behind it.[6,7]

Even, male factor (MF) infertility, resulting from sperm chromosomal abnormalities, is considered to be due to an impairment of the meiotic process.[8,9] This could also be supported by a higher incidence of abnormal karyotypes described in the miscarriages of couples undergoing intracytoplasmic sperm injection (ICSI).[10]

In the last few years, preimplantation genetic screening (PGS) has been more broadly performed before transfer of embryos in *in vitro* fertilization (IVF) programs. It helps to identify the aneuploid embryos, so as to reduce the implantation and miscarriage complications during the pregnancy. There have been multiple studies explaining the use of fluorescence *in situ* hybridization (FISH) for PGS. A meta-analysis compiling nine studies showed no usefulness of FISH attributed to the limited number of chromosomes that can be screened using FISH.[11] However, there has been a recent study with different conclusions, showing the usefulness of FISH for patients with AMA and RIF.[12]

Besides FISH, other techniques such as oligo arrays, single nucleotide polymorphism arrays, quantitative polymerase chain reaction (qPCR), and bacterial artificial chromosome arrays are also used for PGS.[13-16] However, the effectiveness of array comparative genomic hybridization (aCGH) technology in PGS has been reported by some studies.[17,18] In recently published reviews, aCGH was described as a reliable and accessible diagnostic approach to assess 24-chromosome aneuploidy.[19,20]

The present study is the first of its kind from India evaluating the reproductive outcomes of the IVF patients after performing PGS using aCGH for 24-chromosomes on the embryos to be transferred. It also highlights the impact of performing PGS using aCGH for raising the pregnancy rates (PRs) in couples undergoing IVF.

MATERIALS AND METHODS

Study design

This was a retrospective, multicenter study carried out at six different infertility centers, from September 2013 to June 2015. The patients were grouped as per their age as <35, 35–36, 37–38, 39–40, and >40 years. Clinical indications for PGS included RM (two or more miscarriages of unknown etiology), RIF (three or more previous IVF failures), MF (poor semen parameters), previous trisomic pregnancy (PTP) (couples with a PTP), and AMA (35 years of age or older).

All the patients underwent controlled ovarian stimulation from the second day of their periods on the flexible antagonist protocols. When at least two follicles reached a size of 17 mm in diameter, a trigger (250 mg human chorionic gonadotropin [hCG] or 0.2 mg triptorelin, if there was a risk of hyperstimulation) was administered, and oocyte retrieval was scheduled 35 h later. ICSI was performed in all the cases. Fertilization was assessed 17–20 h after microinjection, and embryo growth was recorded every 24 h. The comprehensive chromosomal screening cycles were performed in different IVF centers using two different culture protocols; wherein embryos were either grown sequentially in Vitrolife G-Plus Series IVF Medium (Vitrolife, Goteborg, Sweden) or COOK Culture System (COOK, Sydney, Australia) was used with tri-gas incubators.

The study was carried out as per the provisions of the Declaration of Helsinki.

Embryo biopsy performed on day 3

Embryos were placed on a droplet containing Ca^{2+}/Mg^{2+} free medium (G-PGD, Vitrolife, Goteborg, Sweden/LifeGlobal, Guilford, CT, USA), the zona pellucida was perforated using LASER technology (OCTAX, Herborn, Germany), and one/two blastomere was withdrawn from each embryo. Only embryos with five or more nucleated blastomeres and <25% fragmentation were biopsied. Individual blastomeres were placed in 0.2 ml PCR tubes containing 2 µl phosphate-buffered saline. For blastomere washing and handling, 1% polyvinylpyrrolidone was used.

Embryo transfer

The results of aCGH were available on day 5. If a fresh embryo transfer was planned, one or two properly developed euploid embryos were transferred on day 5. Luteal phase was supported by micronized progesterone 400 mg twice a day; β-hCG was tested 2 weeks later. In

patients at the risk of ovarian hyperstimulation syndrome where agonist trigger was used, well-developed euploid embryos were vitrified, and transferred in a subsequent hormone replacement therapy cycles.

DNA amplification and array comparative genomic hybridization protocol

A single cell from each embryo was amplified using the Sureplex DNA Amplification System (BlueGnome, Cambridge, UK). Amplicon quality was assured by gel electrophoresis (Lonza, Rockland, USA). Sample and control DNA were labeled with Cy3 and Cy5 fluorophores following the manufacturer's instructions. Labeling mixes were combined and hybridized on 24 sure arrays (V2 and V3, Illumina, USA) for 6–12 h. Each probe used was specific to a different chromosomal region and occupied a discrete spot on the slide.

Chromosomal loss or gain was revealed by the color adopted by each spot after hybridization. The technique involved the competitive hybridization of differentially labeled test and reference DNA samples. Fluorescence intensity was detected using a laser scanner (Power Scanner, TECAN, Mannedorf, Switzerland), and BlueFuse Multi Software was used for data processing (BlueGnome, Cambridge, UK). The 24sure microarray product description (February 8, 2012, document version 2.3 and model number 408501-00) describes 10 Mb effective resolution for 24sure using BlueFuse software, the minimum size specified for segmental aneuploidies. The entire protocol was completed in <24 h and therefore, embryo transfer and vitrification of surplus euploid embryos were scheduled for day 5.

Pregnancy outcomes

The outcomes of the study, i.e., PR per transfer (defined as the percentage of clinical pregnancies with a fetal heartbeat); IR (defined as the percentage of embryos transferred resulting in an implanted gestational sac); and miscarriage rate (defined as the percentage of clinical pregnancies that were spontaneously miscarried before week 12 of pregnancy) were calculated.

Statistics analysis

Chi-square test was used for comparisons between study groups with respect to percentages. $P < 0.05$ was considered to be statistically significant.

RESULTS

Overall, 235 PGS cycles were included in the study. When data were to be analyzed as per the female age groups, a total of five cases were excluded from the study as embryo transfer had yet to be planned for them. However, while analyzing the data as per indication groups, three more cases (out of 230) were excluded as PGS was carried out for them due to patients' choice (unspecific indication). The parameters included in the PGS analysis are given in Table 1.

The mean age of the women included in the analysis was 35.3 years. A total of 1401 embryos were analyzed and in 1342 (95.8%) of them, amplification and further analysis were successful. The details of the parameters according to the age groups are given in Table 2.

In 160 cycles, at least one euploid embryo was available for transfer, with a PR of 51.4% per transfer, and 36.9% per cycle. The overall miscarriage rate was found to be 15% in the different age groups. The mean number of embryos analyzed was similar for all the indication groups. The percentage of aneuploid embryos was comparatively lower for all the indications below 35 years of age (67.6% in RM, 69.7% in RIF, 81.5% in PTP, and 75.3% in MF) as compared to the AMA group (79.8%).

These results had an impact on the percentage of cycles with at least one euploid embryo for transfer, making it lower for the AMA group (61.2%) compared to the other indications. However, once embryo transfer was achieved, the chances of successful pregnancy and implantation were similar for all the mentioned indications, with a range between 30.8% and 46.2% for PRs per transfer and between 33.3% and 42.9% for IRs.

Table 1: Parameters of the cycles included in preimplantation genetic screening

Study features	*n* (%)
Number of cycles*	235
Patients waiting for transfer	5
Cycles included in the study	230
Mean age (range), year	35.3±4.3 (22-47)
Mean number of embryos analyzed (range)	6.1±3.17 (1-22)
Number of informative embryos	1342
Total number of aneuploid embryos	1009 (75.18)
Number of embryos with a chaotic pattern†	243 (18.12)
Number of embryos with >1 aneuploidy	307 (22.57)
Number of cycles with all abnormal embryos	70 (43.75)
Number of cycle with embryo transfers	160 (69.56)
Number of embryo transfers‡	173
Mean number of embryos transferred (mean±SD)	1.53±0.75
Number of pregnancies/transfer	89/173 (51.45)
Number of pregnancy/patients with euploid embryos	85/160 (53.12)
Number of pregnancies/cycle	85/230 (36.95)
Implantation rate	111/246 (45.12)
Miscarriage rate	15/89 (16.85)

*Number of OPU cycles, †Chaotic pattern (aneuploidy embryos) can be due to imbalances in various, and often each of the chromosomes tested, ‡Number of transfers performed with euploid embryos (fresh+frozen embryo transfers). OPU=Oocyte pickup

However, the PR per PGS cycle was lower for the RM group (31.3%) compared to rest of the groups.

Interestingly, Table 3 shows a different distribution of chromosomal abnormality types according to the indication. The chromosome distribution showed a chaotic pattern which was relatively homogenous among indications. The most remarkable difference was observed for the percentage of embryos with aneuploidy for more than one chromosome, which was comparatively higher in the AMA and MF groups (34.5% for both) as compared to the other indications (range: 19.7%–26.2%).

This percentage increased with maternal age, ranging from 32.1% in 40 years to 55.8% in 42 years of age. Therefore, the overall incidence of aneuploidy ranged from 63.4% to 95.6%.

Figure 1 represents the relationship between aneuploidy rate and the maternal age. It represents the rate of aneuploidy among different age groups with three different types of aneuploidy, namely, aneuploid embryos, embryos with chaotic pattern, and embryos with >1 aneuploidy.

The present data, given in Table 4, showed much better results for all the age groups in terms of PRs and IRs compared with the patients who did not get PGS done for the euploid embryo selection during the same period at our clinics.

Table 2: Clinical outcomes of 230 comprehensive chromosome screening cycles according to the different age groups

Observations	Age group of the patients[21]				
	<35	35-36	37-38	39-40	>40
Number of cycles	101	38	37	27	27
Age, mean±SD	31.5±2.52	35.5±0.51	37.5±0.51	39.4±0.51	42.5±1.76
Embryos analyzed, mean±SD	6.4±3.31	6.0±2.83	6.4±2.75	4.3±2.15	6.4±4.02
Number of informative embryos	618 (95)	222 (97)	226 (95)	111 (96)	168 (96)
Total number of aneuploid embryos (%)	432 (70)	150 (68)	180 (80)	99 (89)	151 (87)
Number of embryos with a chaotic pattern (%)	104 (24)	33 (22)	46 (26)	26 (26)	30 (20)
Number of embryos with >1 aneuploidy (%)	103 (24)	41 (19)	47 (21)	34 (29)	78 (44)
Number of cycles with all abnormal embryos, *n* (%)	20 (20)	7 (18)	11 (30)	18 (67)	14 (52)
Number of patients with embryo transfers (%)	81 (80)	31 (82)	26 (70)	9 (33)	13 (48)
Mean number of embryos transferred, mean±SD	1.6±0.89	1.5±0.51	1.3±0.64	1.2±0.44	1.2±0.59
Number of pregnancies/transfer (%)	44/92 (48)	19/31 (59)	15/28 (54)	5/9 (55)	6/13 (46)
Number of pregnancy/patients with euploid embryos	44/81 (54)	19/31 (61)	15/26 (58)	5/9 (55)	6/13 (46)
Number of pregnancies/cycle (%)	44/101 (44)	19/38 (50)	15/37 (41)	5/27 (19)	6/27 (22)
Implantation rate (%)	40.9	56.3	47.4	54.5	37.5
Miscarriage rate (%)	10.9	6.4	7.7	0.0	7.6

No statistically significant difference was observed between any of the groups. SD=Standard deviation

Table 3: Clinical outcomes of 227 comprehensive chromosome screening cycles according to the different indications

Observations	Indications (age in years: <35)				
	RM	RIF	PTP	MF	AMA
Number of cycles	22	70	13	13	129
Age, mean±SD	32.2±2.28	31.7±2.25	29.8±3.10	29.4±3.48	38.4±2.69
Embryos analyzed, mean±SD	7.4±3.72	6.2±3.00	6.4±4.01	6.6±3.33	5.9±3.05
Number of informative embryos (%)	148 (91)	416 (95)	81 (97)	85 (99)	727 (96)
Total number of aneuploid embryos (%)	100 (67.6)	290 (69.7)	66 (81.5)	64 (75.3)	580 (79.8)
Number of embryos with a chaotic pattern (%)	19 (19.0)	70 (24.1)	15 (22.7)	15 (23.4)	135 (23.3)
Number of embryos with>1 aneuploidy (%)	20 (20.0)	76 (26.2)	13 (19.7)	22 (34.4)	200 (34.5)
Number of cycles with all abnormal embryos, *n* (%)	1 (4.5)	14 (20.0)	5 (38.5)	3 (23.1)	50 (38.8)
Number of patients with embryo transfers (%)	21 (95.5)	56 (80.0)	8 (61.5)	10 (76.9)	79 (61.2)
Mean number of embryos transferred, mean±SD	1.5±0.75	1.5±1.01	1.9±0.51	1.3±0.51	1.4±0.57
Number of pregnancy/patients with euploid embryos	7/21 (33.3)	32/65 (49.2)	4/8 (50.0)	6/11 (54.5)	45/82 (54.9)
Number of pregnancies/transfer (%)	7/22 (31.8)	32/70 (45.7)	4/13 (30.8)	6/13 (46.2)	45/129 (34.9)
Number of pregnancies/cycle (%)	7/21 (31.3)	29/56 (51.8)	4/8 (50.0)	6/10 (60.0)	45/79 (57.0)
Implantation rate (%)	34.4	39.8	33.3	42.9	40.7
Miscarriage rate (%)	9.1	10.8	0	9.1	6.1

No statistically significant difference was observed between any of the groups. RM=Recurrent miscarriage, RIF=Repetitive implantation failure, PTP=Previous trisomic pregnancy, MF=Male factor, AMA=Advanced maternal age, SD=Standard deviation

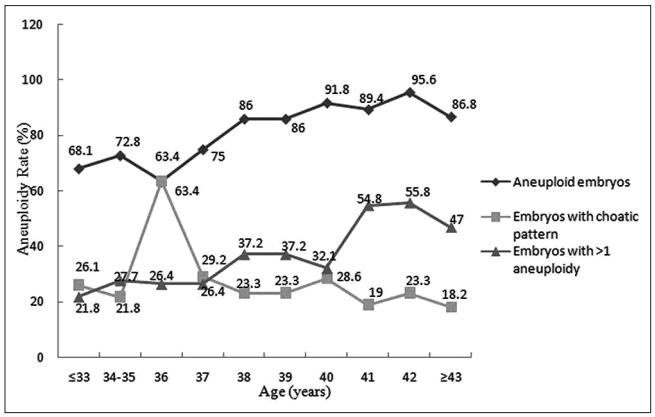

Figure 1: Relationship between aneuploidy rates and maternal age

Table 4: Clinical outcome comparison of patients with or without preimplantation genetic screening

Age groups (year)	PR (%)		IR (%)	
	With PGS	Without PGS	With PGS	Without PGS
<35	54.3	48.2	40.9	28.8
35-36	61.3	41.3	56.3	21.6
37-38	57.7	43.4	47.4	21.8
39-40	55.5	14.6	54.5	8.6
>40	46.2	24.0	37.5	13.6

PGS=Preimplantation genetic screening, IR=Implantation rate, PR=Pregnancy rate

DISCUSSION

Errors in the meiotic divisions in human cells cause various anomalies in chromosomal content in the embryos, namely, aneuploidy. It leads to a reduction in PRs by reducing IRs, increasing miscarriage rates, and other complications during pregnancy.[16,22,23]

Interestingly, the euploid and the aneuploid embryos cannot be distinguished from one another by regular morphological evaluation methods.[24] Hence, there is a need of a reliable method to assess aneuploidy in the embryos. Independent of the type of platform used, the technique selected for screening all the 24 chromosomes should offer reliable and timely results, and should only be applied in clinical programs after validation with an already well-established technique.[25]

PGS through various techniques such as FISH, qPCR, or aCGH can be performed to monitor the chromosomal anomalies in the embryos. However, aCGH technique offers a 100% exhibition of aneuploidy in embryos by evaluation of all the 24 chromosomes and eases out on cumbersome steps involved in FISH, such as fixation on microscopic slides.[25] The current study confirmed the benefits of PGS using aCGH to screen the aneuploidy in the embryos to be used in the IVF processes. Baart *et al*., 2006 evaluated the efficiency of PGS in detecting the aneuploidy and mosaicism in the embryos by reanalyzing them on day 3 and day 5 stage in the younger females (<38 years). They concluded that mosaicism and aneuploidy impacted the results of PGS, but the latter is still an effective and reliable tool in analyzing the aneuploidy in the embryos before transferring them into females during IVF. The conclusions by Baart *et al*. have shown that rates of mosaicism and aneuploidy in the embryos from young IVF patients were similar to those published for older women by Staessen *et al*.[26,27] However, in the present study, the incidence of aneuploidy rate increased as the age increased which supported the previous reports where similar trend was observed for patients having more than one aneuploid embryo.[9,28,29]

The high aneuploidy rate of 75% observed in the present study is comparable to the results in the study by Rabinowitz *et al*., where they found 72.3% aneuploidy rate with the cleavage stage embryos.[9,30]

Franasiak *et al*. in 2014 concluded that the higher rates of aneuploidy specifically trisomies and monosomies were obtained in elder women with an age more than 40 years.[31] Similarly, Gutiérrez-Mateo *et al*. observed 63.2% aneuploidy rate for cleavage stage embryos which was found to be increasing with the maternal age.[32] They found aCGH to be a robust and specific (with low error rates of 1.2%) approach to assess the aneuploidy in the embryos to be used in IVF, thus leading to improved rates of implantation and pregnancy success. aCGH was found to detect about 42% more abnormalities and 13% more abnormal embryos than other PGS techniques such as FISH.[32]

Aneuploidy is also considered to be one of the main causes leading to implantation failures in the patients undergoing IVF treatment.[16,24,25] The same was established through this study since the embryos screened to be having normal chromosomal numbers were successfully implanted, resulting in pregnancy. The similar conclusion was drawn by Rodrigo *et al*., who conducted similar study with the objective to evaluate the usefulness of PGS by aCGH to identify normal embryos from the aneuploid ones.[9]

It has been observed in the current study that the IRs were higher (40.7%) and miscarriage rates were lower (6.1%) in the AMA groups. Understandably, the patients in the higher age group had more chances of no embryos available for the transfer, but the patients who had embryo transfer possible exhibited the similar pregnancy and IRs compared to the younger age group patients. Younger age group patients showed an increase in the miscarriage rate, which could be due to other uterine factors.[9] Donoso *et al*. concluded the same in their study in 2007.[28]

In addition, the percentage of aneuploid embryos was the highest in AMA than all the other indications, in both the

present study (79.8%) and the study conducted by Rodrigo *et al*. (85.3%). Rodrigo *et al*. observed a range of 31.3%–60% in the rates of pregnancy/cycle with the highest percentage in MF and the lowest in RM. However, this range was 19.3%–52% in the current study with the highest rate in MF and the lowest in RM.[9]

In PTP couples, published data described an increased risk of recurrent aneuploid conceptions, particularly in women under 37 years of age.[33] However, the current study indicates comparatively low conception rate in this group when day 3 biopsy is performed. This can be due to low sample number in the group.

The best clinical results after PGS are observed in MF couples (PR 60.0% and IR 42.9%). This type of 24-chromosome PGS seems to be a very promising indication for this patient group.

CONCLUSION

A 24-chromosome PGS using aCGH is a robust method to assess the aneuploidy in the embryos. PGS can further lead to transfer of good quality euploid embryos, resulting in an improved implantation and pregnancy/transfer rate and reduced miscarriage rates.

Acknowledgments

We would like to thank all the clinicians and embryologists at the *in vitro* fertilization units included in this study. They would also like to thank Carmen Rubio Lluesa (Igenomix, Valencia, Spain), Juan Antonio Garcia (Director IVI Madrid, IVI Director for India and GCC), and Irene Rubio Palacios (Co-head Embryology Program, NIF, India) for their support and guidance. The authors also acknowledge Knowledge Isotopes (www.knowledgeisotopes.com) for the writing support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Rajagopalan H, Lengauer C. Aneuploidy and cancer. Nature 2004;432:338-41.
2. Nagaoka SI, Hassold TJ, Hunt PA. Human aneuploidy: Mechanisms and new insights into an age-old problem. Nat Rev Genet 2012;13:493-504.
3. Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, *et al*. A cytogenetic study of 1000 spontaneous abortions. Ann Hum Genet 1980;44(Pt 2):151-78.
4. Lamb NE, Freeman SB, Savage-Austin A, Pettay D, Taft L, Hersey J, *et al*. Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. Nat Genet 1996;14:400-5.
5. Sugiura-Ogasawara M, Ozaki Y, Katano K, Suzumori N, Kitaori T, Mizutani E. Abnormal embryonic karyotype is the most frequent cause of recurrent miscarriage. Hum Reprod 2012;27:2297-303.
6. 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 randomized pilot study. Mol Cytogenet 2012;5:24.
7. Margalioth EJ, Ben-Chetrit A, Gal M, Eldar-Geva T. Investigation and treatment of repeated implantation failure following IVF-ET. Hum Reprod 2006;21:3036-43.
8. Wong EC, Ferguson KA, Chow V, Ma S. Sperm aneuploidy and meiotic sex chromosome configurations in an infertile XYY male. Hum Reprod 2008;23:374-8.
9. Rodrigo L, Peinado V, Mateu E, Remohí J, Pellicer A, Simón C, *et al*. Impact of different patterns of sperm chromosomal abnormalities on the chromosomal constitution of preimplantation embryos. Fertil Steril 2010;94:1380-6.
10. Kim JW, Lee WS, Yoon TK, Seok HH, Cho JH, Kim YS, *et al*. Chromosomal abnormalities in spontaneous abortion after assisted reproductive treatment. BMC Med Genet 2010;11:153.
11. Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: A systematic review and meta-analysis of RCTs. Hum Reprod Update 2011;17:454-66.
12. Rubio C, Bellver J, Rodrigo L, Bosch E, Mercader A, Vidal C, *et al*. Preimplantation genetic screening using fluorescence *in situ* hybridization in patients with repetitive implantation failure and advanced maternal age: Two randomized trials. Fertil Steril 2013;99:1400-7.
13. Scott RT Jr., Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, *et al*. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases *in vitro* fertilization implantation and delivery rates: A randomized controlled trial. Fertil Steril 2013;100:697-703.
14. Hellani A, Abu-Amero K, Azouri J, El-Akoum S. Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening. Reprod Biomed Online 2008;17:841-7.
15. Treff NR, Levy B, Su J, Northrop LE, Tao X, Scott RT Jr. SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. Mol Hum Reprod 2010;16:583-9.
16. 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.
17. Wilton L, Voullaire L, Sargeant P, Williamson R, McBain J. Preimplantation aneuploidy screening using comparative genomic hybridization or fluorescence *in situ* hybridization of embryos from patients with recurrent implantation failure. Fertil Steril 2003;80:860-8.
18. Fragouli E, Wells D, Whalley KM, Mills JA, Faed MJ, Delhanty JD. Increased susceptibility to maternal aneuploidy demonstrated by comparative genomic hybridization analysis of human MII oocytes and first polar bodies. Cytogenet Genome Res 2006;114:30-8.
19. Simpson JL. Preimplantation genetic diagnosis to improve pregnancy outcomes in subfertility. Best Pract Res Clin Obstet Gynaecol 2012;26:805-15.
20. Handyside AH. 24-chromosome copy number analysis: A comparison of available technologies. Fertil Steril 2013;100:595-602.
21. Chawla M, Fakih M, Shunnar A, Bayram A, Hellani A, Perumal V, *et al*. Morphokinetic analysis of cleavage stage embryos and its relationship to aneuploidy in a retrospective time-lapse imaging study. J Assist Reprod Genet 2015;32:69-75.
22. Chavez SL, Loewke KE, Han J, Moussavi F, Colls P, Munne S, *et al*. Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. Nat Commun 2012;3:1251.

23. Mir P, Mateu E, Mercader A, Herrer R, Rodrigo L, Vera M, *et al.* Confirmation rates of array-CGH in day-3 embryo and blastocyst biopsies for preimplantation genetic screening. J Assist Reprod Genet 2016;33:59-66.
24. Wells D, Alfarawati S, Fragouli E. Use of comprehensive chromosomal screening for embryo assessment: Microarrays and CGH. Mol Hum Reprod 2008;14:703-10.
25. Schoolcraft WB, Fragouli E, Stevens J, Munne S, Katz-Jaffe MG, Wells D. Clinical application of comprehensive chromosomal screening at the blastocyst stage. Fertil Steril 2010;94:1700-6.
26. Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, *et al.* Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: A prospective randomized controlled trial. Hum Reprod 2004;19:2849-58.
27. Baart EB, Martini E, van den Berg I, Macklon NS, Galjaard RJ, Fauser BC, *et al.* Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. Hum Reprod 2006;21:223-33.
28. Donoso P, Staessen C, Fauser BC, Devroey P. Current value of preimplantation genetic aneuploidy screening in IVF. Hum Reprod Update 2007;13:15-25.
29. Fragouli E, Katz-Jaffe M, Alfarawati S, Stevens J, Colls P, Goodall NN, *et al.* Comprehensive chromosome screening of polar bodies and blastocysts from couples experiencing repeated implantation failure. Fertil Steril 2010;94:875-87.
30. Rabinowitz M, Ryan A, Gemelos G, Hill M, Baner J, Cinnioglu C, *et al.* Origins and rates of aneuploidy in human blastomeres. Fertil Steril 2012;97:395-401.
31. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, *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.e1.
32. Gutiérrez-Mateo C, Colls P, Sánchez-García J, Escudero T, Prates R, Ketterson K, *et al.* Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. Fertil Steril 2011;95:953-8.
33. De Souza E, Halliday J, Chan A, Bower C, Morris JK. Recurrence risks for trisomies 13, 18, and 21. Am J Med Genet A 2009;149A: 2716-22.