Assessing the Impact of Comprehensive Chromosome Screening in Preimplantation Genetic Testing for Aneuploidy: A Meta-Analytical Review

Anindita Kundu[1]
Dr. Shubhi Mishra[2]
PhD Research scholar, Department Of School Of Science (Biotechnology), Sardar Pateluniversity, Balaghat(M.P.)India1
Assistant professor, Department Of School Of Science (Biotechnology),Sardar Patel university, Balaghat(M.P.)India[2]

Abstract

The utility of pre-implantation genetic testing (PGT-A) is controversial, with older meta-analyses demonstrating improved pregnancy outcomes, while newer trials have not shown benefit. Therefore, we performed a meta-analysis which aimed to evaluate the benefits of PGT-A using comprehensive chromosome screening (CCS) and its effects on in vitro fertilization (IVF) outcomes among randomized controlled trials (RCTs). We conducted a systematic search to identify RCTs comparing women undergoing PGT-A with CSS with women not undergoing PGT-A, from inception to December 2020. Random effects meta-analysis was utilized to calculate average odds ratios (OR) for clinical pregnancy rate (CPR), ongoing pregnancy rate (OPR), and miscarriage rate (MR). The heterogeneity of exposure was assessed using Forest plots and I2 statistics. Publication bias was evaluated using Egger's test. Among 1251 citations, seven RCTs met the inclusion criteria. Biopsies of embryos were carried out at various developmental stages, including polar body, day 3, and day 5-6 of culture. Data was analyzed as all studies and blastocyst only. Meta-analysis failed to show improvement in OPRs using PGT-A in the all ages, <35 years old and ≥ 35 years old age groups. There was also no significant difference in CPRs in any group. The MR decreased with the use of PGT-A (among all biopsy types and among blastocyst biopsies) in the all-ages group, but not when stratifying according to patient age, <35 and ≥ 35 years old. More data regarding the risks and advantages of PGT-A are needed to make a final decision on the value of this intervention in clinical practice. The exact magnitude of the benefit of PGT-A selection cannot be correctly determined until multiple standardized protocol IVF PGT-A trials are conducted.

Keywords: In Vitro Fertilization, Meta-Analysis, Preimplantation Genetic Screening

---

Introduction Aneuploidy is common in embryos generated using in vitro fertilization (IVF). While aneuploidy is largely age- and laboratory-dependent, even women <35 years may have high aneuploidy rates (1). Selection of competent euploid embryos for implantation is required for a viable pregnancy to develop, as transferring aneuploid embryos can cause miscarriages and lead to recurrent failures in IVF treatments (2). Several techniques have been developed to assess for chromosome number. Classically, embryos for transfer have been chosen based on morphological criteria alone; however, many embryos with favourable morphology are aneuploid. Moreover, even in the same cohort of embryos, some with poor morphology are euploid, while some with better morphology are aneuploid (3). Because of the unreliability of morphological markers, preimplantation genetic testing for aneuploidy (PGT-A) was developed.

In clinical practice, PGT-A was originally conducted with blastomere-stage biopsies, but this technique led to lower rates of live births (4). Currently, blastocyst-stage biopsies are used, because they are believed to have less detrimental effects on embryonic development than blastomere-stage biopsies do. Of note, extensive mosaicism has been detected in blastocysts, and 37% of trisomic embryos have been shown to reach the blastocyst stage (5). Clinically, PGT-A includes genetic analysis of the polar body, blastomere, or trophoblast cells. Genetic analysis was previously limited to the visualization of a small set of chromosomes using fluorescence-in-situ hybridization (FISH). Currently, comprehensive chromosome screening (CCS) is used to evaluate all chromosomes. Several

different methods of CCS are used including array comparative genomic hybridization (aCGH), quantitative polymerase chain reaction (PCR), single nucleotide poly morphisms, and next-generation sequencing.

The benefits of using PGT-A with CCS in IVF embryos are still uncertain (6, 7), and the evidence that PGT-A increases rates of pregnancy, implantation, and live birth is controversial (4, 8). Three systematic reviews from 2015 demonstrated higher rates of implantation, clinical pregnancy, and delivery with CCS-based PGT-A (9-11), with one review (11) only including trials of patients with good prognoses. Recently, the STAR trial, a large international multicentre randomized controlled trial (RCT), reported that PGT-A with CSS did not result in an improvement in overall pregnancy outcomes in their all-ages analysis, although there may be some benefit in certain age groups and miscarriage rates (12). In addition to the STAR trial, three other RCTs have been published since the 2015 me ta-analysis (13-15). Ozgur et al. (13) examined the efficacy of IVF with PGT-A (by CCS) using only best-scoring blastocysts (n=109) from young infertile patients (≤ 35 years) undergoing single blastocyst frozen embryo transfers. They demonstrated that in patients with at least two ≥ 2BB blastocysts (fair blastocysts, defined according to the Gardner scoring system, based on the morphological assessment of blastocyst expansion, inner cell mass (ICM), and trophectoderm [i.e., ≥2BB= expansion: ICM: trophectoderm]), blastocyst selection by PGT-A did not result in an increased live birth rate. Similarly, Verpoest et al. (14) examined whether, in women of advanced ma ternal age (36-40 years old) planning an ICSI cycle, PGT A by CCS of the first and second polar body to select embryos for transfer, increases the chance of achieving a live birth within one year compared to ICSI without chromosome analysis, and found comparable live birth rates (n=396 women, 24% live birth rate). Rubio et al. (15) examined PGT-A's clinical value in women of advanced maternal age (ages 38-41 years) (performing day 3 embryo biopsies using array comparative genomic hybridization). They found comparable cumulative delivery rates per patient, six months after study termination, with decreased miscarriage rates and a shorter time to pregnancy. In total, three of the four new trials showed no or minimal benefit with the use of PGT-A in IVF. In September 2020, a Cochrane meta-analysis was published evaluating the relationships of PGT-A with IVF outcomes. It concluded that there was an insufficient amount of high quality evidence to establish a significant difference in cumulative live birth rates, live birth rates after the initial embryo transfer, or miscarriage rates between IVF procedures conducted with and without PGT-A (16). Furthermore, there was a lack of available data regarding ongoing pregnancy rates in this context. However, the majority of included studies were day three biopsies using FISH, and as such are suspect of decreasing outcome rates. An updated meta-analysis only including prospective studies with CCS analysis, and an analysis of blastocyst biopsy solely, is lacking from the literature.

Given the discrepancy between the results of the recent RCTs and the previous meta-analyses, we aimed to perform this meta-analysis to elucidate the effects of PGT-A with CCS on rates of clinical pregnancy, ongoing pregnancy, and miscarriage.

Materials and Methods

The systematic review and meta-analysis were conduct ed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guide lines (17).

Search strategy

A systematic search was conducted on the MEDLINE (Ovid), Embase (Ovid), Evidence-Based Medicine (EBM) Reviews (OvidB), CINAHL (EBSCO), and World Health Organization (WHO) Global Health Library databases from inception to December 2020. No language or date re strictions were applied. Search terms related to both 'preimplantation genetic screening' and 'IVF' were used. Search terms included: "in-vitro fertilization", "preimplantation genetic testing for aneuploidy", "comprehensive chromo some screening", "trophectoderm biopsy", "blastocyst biopsy", "clinical pregnancy rate", "ongoing pregnancy rate", "miscarriage rate", "randomized controlled trial".

A search for unpublished studies and ongoing clinical trials was also carried out using Biomed Central, ClinicalTrials.gov, WHO International Clinical Trials Registry Platform, and Thomson Center Watch. Furthermore, a manual search of published articles was performed to identify any additional relevant studies. Finally, reference lists of the primary studies and re view articles were searched to detect other potentially relevant studies.

Study selection

Title and abstract screening were conducted independently by two reviewers (JT and OT) using predeter mined inclusion and exclusion criteria. Those meeting the criteria underwent independent full text review. If an abstract was unavailable, the article moved on to full text review. Full texts were evaluated using the predetermined criteria, and eligible studies were included in the meta-analysis.

Inclusion and exclusion criteria

Included studies were restricted to RCTs which compared women undergoing IVF with PGT-A using CCS technology of biopsied trophectoderm cells (PGT-A-v2), day 3 embryos, or polar bodies, compared to women undergoing IVF without PGT-A. Studies were included if they reported one or more of the following outcomes: ongoing pregnancy rate (OPR, primary outcome), implantation rate, clinical pregnancy rate, and miscarriage rate (MR, secondary outcomes). Studies published in languages other than English were translated and included if they met the inclusion/exclusion criteria.

Studies were excluded if the participants had repeated implantation failure or repeated pregnancy loss, or if the study used PGT-A technology other than CCS. Abstracts and conference proceedings were excluded as well.

Data extraction and quality assessment

Systematic data extraction was conducted using a preformatted Excel spreadsheet. Data was collected regarding the study objectives, design, population, intervention, control, outcomes, and study quality.

Each of the studies underwent a quality assessment that encompassed various criteria, such as random sequence generation, allocation concealment, blinding, completeness of outcome data, selective outcome reporting, and other potential sources of bias (20). A judgment of 'Yes' signified a low risk of bias, 'No' indicated a high risk of bias, and 'Unclear' suggested an unclear or unknown risk of bias.

Statistical analysis

All analyses were carried out using "R software", version 3.4.1 (21). Eligible RCTs were included for quantitative comparison, and a random-effects model meta-analysis was utilized to calculate the estimated average mean difference as applied in the "R package 'metafor'" (22). Mean differences from studies were weighted by inverse variance, providing greater weight to larger and more precise studies. The heterogeneity of the exposure effects was visually assessed through the use of forest plots (23) and statistically evaluated using the I2 statistic to quantify het erogeneity across studies (24). Funnel plot analysis was performed to assess for publication bias, and publication bias was evaluated by visual inspection for asymmetry (25) and using Egger's test (26). To assess the impact of specific studies on the results and overall heterogeneity, we con ducted a sensitivity analysis by sequentially removing each study and comparing the model characteristics.

The outcome variables were ongoing pregnancy rate, clinical pregnancy rate, and MR among those with a pregnancy. Meta-analyses were performed overall and by subgroup comparisons by age: <35 years old and ≥ 35 years old. A random-effects model was utilized to estimate the average odds ratios (OR). For ongoing pregnancy, two separate analyses were conducted. The first analysis grouped all ages together from the included studies. The second analysis stratified patients by age: <35 years old and ≥ 35 years old.

Results

The search strategy yielded 1382 records, with three records identified from other sources (Fig.1). After the removal of duplicates, 1375 articles underwent title and abstract screening with 384 moving on to full text screening. Seven trials (1851 participants) were eligible for inclusion in the meta-analysis based on the pre-determined criteria (7, 12-15, 18, 27). In total, there were 905 participants in the PGT-A group and 946 in the control group. Three RCTs transferred fresh embryos (7, 15, 18), two used fresh/frozen embryos (14, 27), and the remaining two used frozen embryos (12, 13). Demographics, main characteristics, outcomes, and quality assessment of the seven eligible studies are presented in Table 1.

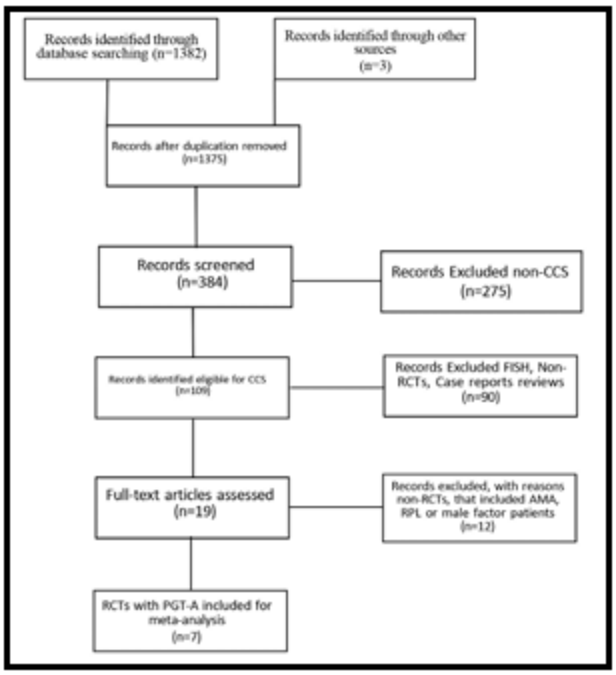

Fig.1: Preferred Reporting RCTs with PGT-A for systematic reviews and meta-analysis flow diagram showing the search for studies. RCTs; Randomized controlled trials and PGT-A; Preimplantation genetic testing for aneuploidy.

Two RCTs only included women < 35 years (13, 18), two RCTs only included women ≥ 35 years (14, 15), and one RCT reported outcomes for women < 35 years and ≥35 years (12). Two studies (7, 27) grouped women aged 21-42 with good prognosis. They did not report data stratified by age and were therefore only included in the all-ages analyses.

All RCTs, including participants <35 years old, were conducted using Trophectoderm biopsies, whereas studies including participants ≥35 years old were conducted using Trophectoderm biopsies (12, 13), day 3 embryo biopsies (15), or polar body biopsies (14). This meta-analysis reports analyses from RCTs, using all biopsy sources as well as Trophectoderm biopsies only.

The rationale behind including polar body and cleavage-stage biopsy was the fact that in many older women trophectoderm biopsy would not be possible because of poor embryo development.

Table 1: Characteristics of the studies included in the review

Study (Ref), Year	Number of patients PGS/controls	Randomization	PGS Method	Age (Y)	Inclusion	Exclusion	ART	Biopsy day	Outcomes	Quality
Yang et al. (18) , 2012	56/56	Prospective randomized controlled, single blind, by random number, MC, no power calculation	aCGH	<35	Good prognosis, <35 years; a history of regular ovulation; etiology of infertility was tubal factor or male factor (or both); no prior IVF treatment; no prior miscarriage; normal intrauterine contour; both ovaries intact; basal serum FSH<10 IU/l	Donor gametes or frozen/ thawed embryos	ICSI	D 5, Laser assisted hatching fresh cycles	Clinical pregnancy, ongoing pregnancy, missed abortion	Randomization by random number table; concealment of allocation not reported; single-blind; 2 centers; full paper; power calculation not reported; no intention to treat, and no study patient had embryos assigned to both laboratory groups
Forman et al. (19), 2013	89/86	Randomized open label, by computer not blinded, SC, non-inferiority trial with a margin of 20% assuming a baseline ongoing pregnancy rate of 60% in each group. 75 patients were required in each group	qPCR	<42	Good prognosis, <42 years; at most one prior failed IVF cycle; normal endometrial cavity; normal ovarian reverse (AMH>1.2 ng/ml, day 3 FSH <12 IU/l); at least 2 expanded blastocysts suitable for transfer or cryopreservation by day 6 of embryo development	Severe male infertility; anovulatory polycystic ovarian syndrome; BMI >30 kg/m^2	ICSI	D 5, Laser assisted hatching fresh/frozen	Sustained implantation, ongoing pregnancy, clinical miscarriage, multiple pregnancy rates	Randomization by computer; concealment of allocation achieved by sequentially numbered, opaque, sealed envelopes; not blinded; 1 center; full paper; power calculation; intention to treat; block randomization used for fresh and frozen cycles
Scott et al. (7), 2013	72/83	Prospective randomized controlled, by computer, Blinded? SC, designed to detect a 20% difference in sustained implantation rate with a power of 80%, assuming a baseline implantation rate of 40%	qPCR	21-42	21–42 years; no more than one prior failed IVF retrieval; normal endometrial cavity; basal FSH level <15IU/l; basal follicle cunt >8; available ejaculate sperm; willingness to limit transfer order to a maximum 2 embryos; 2 or more blastocysts by the afternoon of day 5	Less than 2 blastocysts by day 5	ICSI	D 5, Laser assisted hatching/ fresh	Implantation rate, ongoing pregnancy rate, delivery rate	Randomization by computer; concealment of allocation not reported; blind not reported; 1 center; full paper; power calculation; intention to treat; block randomization used for different age groups
Rubio et al. (15), 2017	100/105	Prospective randomized controlled, Blinded, Sample size was estimated at 120 patients per arm for 15 points absolute difference in the primary endpoint of delivery rate (α 5%, β 20%)	aCGH	38-41	Normal karyotypes, body mass index (BMI) <30 kg/m^2, Had 5 or more MII oocytes obtained from one or two cycles, and had sperm concentrations $\geq 2 \times 10^6$/mL	Endocrine/ systemic problems, prior PGD-A/PGD cycle, prior pregnancy or miscarriage due to chromosomal abnormalities	ICSI	D 3, fresh	Implantation, clinical pregnancy, ongoing implantation, delivery and live birth rates	Randomization by computer; concealment of allocation not reported; not blinded; 4 centers; power calculation; intention to treat; fresh (fresh and frozen cycles for cumulative)
Ozgur et al. (13), 2019	109/111	Prospective randomized controlled, Randomization 1:1, by computer, Blinded, SC The sample size estimated at 80% power) for an expected absolute increase in live birth of 20% from a reference rate of 55% to be 89 patients in each arm[r]	NGS	<35	$\leq$ 35 years, antral follicle count $\geq$ 5 (AFC; changed from $\geq$ 10), body mass index (BMI) $\geq$ 18 to $\leq$ 35 kg/m^2, and no intrauterine and or endometrial abnormalities	N/A	ICSI	D 5, frozen	Clinical pregnancy, miscarriage and live birth rate (after 20 weeks. of gestation)	Randomization by computer; concealment of allocation not reported; blinded; 1 center; power calculation; no intention to treat; frozen cycles

Table 1: Continued

Study	Patients PGS/Controls	Randomization	PGS Method	Age (Y)	Inclusion	Exclusion	ART	Biopsy day	Outcomes	Quality
Verpoest et al. (14), 2018	205/191	Prospective randomized controlled, Web block randomization, Blinded, MC, Assuming a 20% live birth rate at 1 year in the control group, a group size of 266 participants per study arm was aimed for to have a power of more than 90% in detecting an absolute increase of 15 points or more in the live birth rate	aCGH	36-40	BMI between 18 and 30 kg/m², patients prepared to accept the transfer of up to two embryos absence of any type of hereditary condition in the patient's or partner's personal and family history	Infertility treatment involving the use of donor oocytes; menstrual cycle irregularity (< 24 days and >35 days) three or more previous failed IVF or ICSI cycles with the present partner; poor ovarian response. In previous IVF or ICSI cycles; partner requiring surgical sperm retrieval; partner asthenozoospermi, macrozoospermia globozoospermia, 3. or more clinical miscarriages; and (chronic use of antipsychotics, anxiolytics or continuous use of non-steroidal anti-inflammatoy drugs (NSAID)	ICSI	Polar body, fresh/ frozen, paternal contribution is not tested and that mosaicism occurring during later stages is missed	Clinical pregnancy, miscarriage rates, live births	Web block randomization; allocation not reported; blinded; 7 centers; power calculation; no intention to treat; online collection of data monitoring; fresh and frozen cycles
Munne et al. (12), 2019	274/314(total) 152/169(<35 Y) 122/145(>35) 42 no euploid for transfer, mosaics not transferred 16.8% embryos tested	Randomized, controlled, randomized 1:1, blinded, MC, the intention-to-treat (ITT) population was defined as patients undergoing randomization	NGS	25-40	Female age 25–40 years undergoing IVF with autologous oocytes with at least two blastocysts of sufficient quality for biopsy and vitrification by day 6	Severe male factor, diminished ovarian reserve, using donor oocyte or gestational carrier, undergoing PGS, diagnosis outside of this study, more than two previous failed IVF-ETs, more than one miscarriage	ICSI	D5, frozen	Clinical pregnancy, ongoing pregnancy, miscarriage rates	

#; Due to high cost of procedure and social preferences possible high bias of patient selection/randomization, SC; Single center, MC; Multi center, aCGH; Array comparative genomic hybridization, qPCR; Quantitative polymerase chain reaction, ICSI; Intracytoplasmic sperm injection, AMH; Anti-müllerian hormone, FSH; Follicle-stimulating hormone, IVF; *In vitro* fertilization, PGD; Pre-implantation genetic diagnosis, NGS; Next generation sequencing, and PGS; Preimplantation genetic screening.

Ongoing pregnancy rate Comparing the ongoing pregnancy rates in all ages, there was no difference between groups undergoing PGT-A using all biopsy types versus controls (OR=1.38, 95% CI=0.98, 1.95; P=0.07). There was significant heterogeneity among the studies (I^2=62.5%, P=0.03), but the funnel plot did not show any signs of bias. Egger's test for asymmetry was not significant (P=0.08); however, power to detect asymmetry is limited due to the small number of trials (Fig.2A).

The OPR in participants ≥35 years old was comparable in subjects undergoing PGT-A using all biopsy types and in controls (OR=1.49, 95% CI=1.00, 2.22, P=0.05). Of note, the ongoing pregnancy rates in participants ≥35 years old cannot be relied upon due to high level of het erogeneity as only one out of the three studies in this group performed

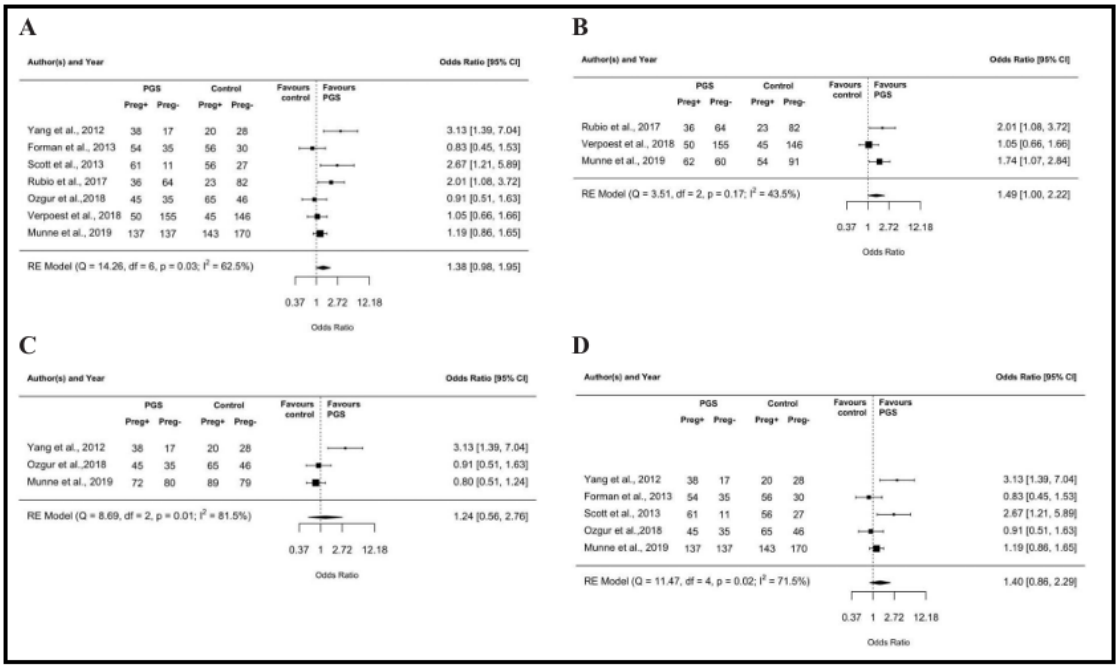

Fig.2: Forest plots showing the results of meta-analysis on ongoing pregnancy comparing the effect of CCS-based PGT-A and traditional morphological method after IVF/ICSI. **A.** Forest plot of pooled RR on ongoing pregnancy of RCTs, all ages, **B.** Forest plot of pooled RR on ongoing pregnancy of RCTs, older participants (age≥35 years), **C.** Forest plot of pooled RR on ongoing pregnancy of RCTs, younger participants (< 35 years), and **D.** Forest plot of pooled RR on ongoing pregnancy of RCTs, all ages (D3/polar body CCS excluded). CCS; Comprehensive chromosome screening, PGT-A; Pre-implantation genetic testing for aneuploidy, IVF; *In vitro* fertilization, ICSI; Intracytoplasmic sperm injection, RCTs; Randomized controlled trials, PGS; Preimplantation genetic screening, and RR; Relative risk.

PGT-A using trophectoderm biopsies, while the other two studies used day 3 and polar body biopsies. The funnel plot did not show any signs of bias. Only three studies were included, so it was not possible to formally test for asymmetry (Fig.2B).

Comparing the OPR in participants < 35 years old, there was also no difference between groups undergoing PGT-A (all studies used blastocyst biopsies) versus controls (OR=1.24, 95% CI=0.56, 2.76, P=0.59). There was again significant heterogeneity among the studies A C (I^2= 81.47%, P=0.01), but the funnel plot did not show any signs of bias. With only three studies, it was not possible to formally test for asymmetry (Fig.2C).

When restricting the analysis to studies using only trophectoderm biopsies in all ages (Fig.2D), there was no difference between groups undergoing PGT-A using blastocyst biopsies versus controls (OR=1.40, 95% CI=0.86, 2.29, P=0.18); however, there was significant heterogeneity amongst the studies (I^2=71.5%, P=0.02). The funnel plot did not show any signs of bias, and Egger's test for asymmetry was not significant (P=0.18).

A meta-analysis could not be performed comparing PGT-A using only blastocyst biopsies versus controls in women ≥35 years old, as only a single study (12) evaluated these groups.

Clinical pregnancy rate

Comparing the clinical pregnancy rate in all ages, there was no difference between groups undergoing PGT-A using all biopsy types versus controls (OR=1.02, 95% CI=0.69, 1.51, P=0.92). There was significant heterogeneity among the studies (I^2=68.81%, P=0.011). The funnel plot did not show any signs of bias, and Egger's test for asymmetry was not significant (P=0.25); however, with seven studies the power to detect asymmetry is limited (Fig.3A).

For participants ≥35 years old, there was no significant Taskin et al. difference between women who underwent PGT-A using all biopsy types versus controls (OR=1.02, 95% CI=0.65, 16.2, P=0.92). There was sub-significant heterogeneity among the studies (I^2=62.54%, P=0.065). The funnel plot did not show any signs of bias; however, with only three studies, it was not possible to formally test for asymmetry (Fig.3B).

The clinical pregnancy rate in participants < 35 years old was not significantly different between groups undergoing PGT-A (all with trophectoderm biopsy) versus controls (OR=1.18, 95% CI=0.55, 2.55, P=0.67). There was significant heterogeneity among the studies (I^2=79.11%, P=0.022). The funnel plot did not show any signs of bias; however, with only three studies, it was not possible to formally test for asymmetry (Fig.3C).

Restricting the analysis for all ages to include only studies using PGT-A with trophectoderm biopsies (Fig.3D), there was no significant difference in clinical pregnancy rate between the intervention and control groups (OR=1.16, 95% CI=0.63, 2.13, P=0.63). There was significant heterogeneity among the studies (I^2=77.6%, P=0.007). There was no indication of bias in the funnel plot, and Egger's test for asymmetry was not significant (P=0.43), however with five studies, there was little power to detect asymmetry.

Miscarriage rate

Comparing the MR in all ages, there were significantly fewer miscarriages in the PGT-A group using all biopsy types than in the control group (OR=0.47, 95% CI=0.29, 0.77, P=0.003). There was no significant heterogeneity among the studies (I^2=35.66%, P=0.11). The funnel plot did not show any signs of bias, but Egger's test for asymmetry was significant (P=0.01). However, with seven studies we had little power to detect asymmetry (Fig.4A).

The MR for participants ≥ 35 years old was comparable between those who underwent PGT-A using all biopsy types and controls (OR=0.32, 95% CI=0.10, 1.01, P=0.05). The miscarriage rates in participants ≥ 35 years old could not be relied upon due to a high level of heterogeneity, as only one out of the three studies in this group performed PGT-A using trophectoderm biopsies, while the other two studies used day three and polar body biopsies. The funnel plot did not show any signs of bias; however, with only three studies, it was not possible to formally test for asymmetry (Fig.4B).

For participants <35 years, there was no difference between groups undergoing PGT-A using trophectoderm biopsies versus controls (OR=0.70, 95% CI=0.24, 2.06, P=0.52). There was no significant heterogeneity among the studies (I^2 =48.30%, P=0.156). The funnel plot did not show any signs of bias; however, with only three studies, it was not possible to formally test for asymmetry (Fig.4C).

Limiting the all-ages analysis to studies that used PGT A with only trophectoderm biopsies (Fig.4D), the lower rate of miscarriage in the intervention group remained significant (OR=0.58, 95% CI=0.34, 0.97, P=0.04). There was no significant heterogeneity among the studies (I^2=24.1%, P=0.41). There was no indication of bias in the funnel plot, and Egger's test for asymmetry was not significant (P=0.08).

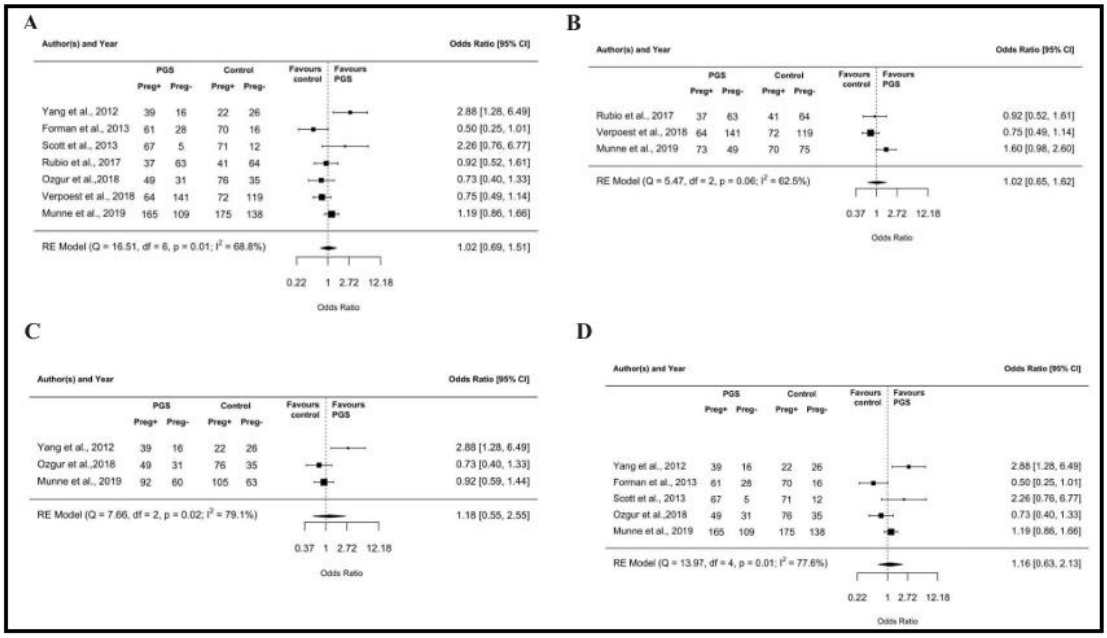

Fig.3: Forest plots showing the results of meta-analysis on clinical pregnancy rates comparing the effect of CCS-based PGT-A and traditional morphological method after IVF/ICSI. **A.** Forest plot of pooled RR on clinical pregnancy rates of RCTs, all ages, **B.** Forest plot of pooled RR on clinical pregnancy rates of RCTs, older participants (age≥35 years), **C.** Forest plot of pooled RR on clinical pregnancy rates of RCTs, younger participants (< 35 years), and **D.** Forest plot of pooled RR on clinical pregnancy rates of RCTs, all ages (D3/polar body CCS excluded). PGT-A; Pre-implantation genetic testing for aneuploidy, IVF; In vitro fertilization, ICSI; Intracytoplasmic sperm injection, RCTs; Randomized controlled trials, PGS; Preimplantation genetic screening, and RR; Relative risk.

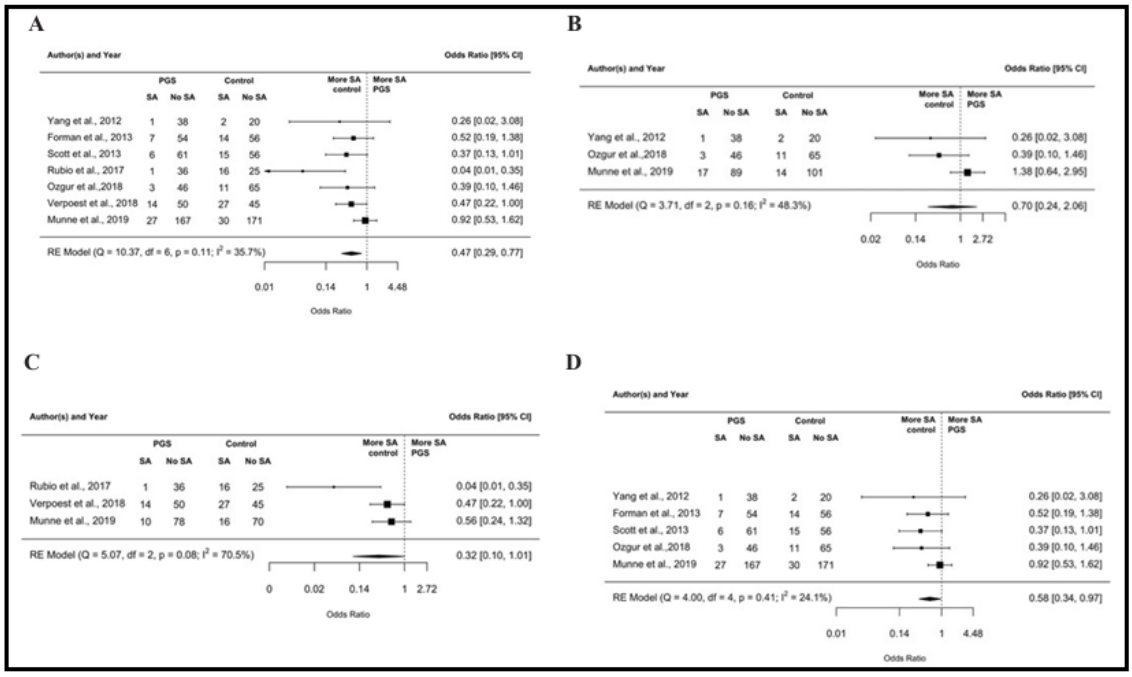

Fig.4: Forest plots showing the results of meta-analysis on spontaneous abortion rates comparing the effect of CCS-based PGT-A and traditional morphological method after IVF/ICSI. **A.** Forest plot of pooled RR on spontaneous abortion rates of RCTs, all ages, **B.** Forest plot of pooled RR on spontaneous abortion rates of RCTs, older participants (age≥35 years), **C.** Forest plot of pooled RR on spontaneous abortion rates of RCTs, younger participants (< 35 years), and **D.** Forest plot of pooled RR on spontaneous abortion rates of RCTs, all ages (D3/polar body CCS excluded). CCS; Comprehensive chromosome screening, PGT-A; Pre-implantation genetic testing for aneuploidy, IVF; *In vitro* fertilization, ICSI; Intracytoplasmic sperm injection, RCTs; Randomized controlled trials, PGS; Preimplantation genetic screening, and RR; Relative risk.

Discussion

This meta-analysis was conducted in order to clarify the role of PGT-A in IVF procedures, given the publication of new RCTs, including the STAR trial (12). The results of our meta-analysis demonstrated that PGT-A does not improve ongoing pregnancy rates when considering women of all age groups generally, nor when stratifying according to age <35 and ≥ 35 years. These results support the findings in the STAR trial. However, they did not replicate the results of previous RCTs (13, 28) or the meta-analysis (11), which did show a benefit, albeit based on fewer studies.

This meta-analysis also identified a MR of approximately 10% in both the PGT-A (11.2% at ≥ 35 years old) and control (8.3% at <35 years old, 11.0% at ≥ 35 years old) arms; however, there were significantly fewer miscarriages in the PGT-A groups for all ages combined, using all biopsy types or trophectoderm biopsies. Thus, PGT-A with IVF may reduce the potentially high emotional burden associated with miscarriages and consequent possible withdrawal from infertility treatment.

Several aspects surrounding the use of PGT-A in IVF remain unknown. Identification of mosaic embryos and the implications for outcomes are still evolving (29, 30). It has now become evident that a notable proportion of embryos with only mosaic aneuploidies, especially those with a limited number of affected chromosomes and low percentages, have the potential for viability and can result in live births following their transfer (31, 32). It is difficult to assess the effect of mosaicism in our meta-analysis, as the STAR trial excluded mosaic embryos, which consisted of 16.8% of tested embryos, and other RCTs may be similarly affected. In addition, it is uncertain why the all-age group and women <35 and ≥ 35 years old do not benefit from PGT-A according to the pregnancy rate per embryo transfer. It is possible that trophectoderm biopsy may injure the embryos and affect implantation. If the

embryo biopsy results in development out of phase with the endometrium, large RCTs using frozen embryos only may show an increase in the benefits of PGT-A.

Limitations of this meta-analysis include the inability to evaluate a group of subjects 35-39 years of age. In addition, the inclusion of a relatively small number of trials in the analysis restricts the statistical power to detect differences between the groups. Strengths include the inclusion of prospective RCTs only and the inclusion of 133% more studies than the last meta-analysis in 2015.

Several issues should be noted as related to the articles which were included. In spite of these issues, it should be noted that this is the data we have currently and need to make clinical decisions based on this data. Forman et al. (19) compared the use of single embryo transfer (SET) in the PGT-A group versus double embryo transfer (DET) in the control group, while Yang et al. (18) and Scott et al. (7) only reported on women who had an embryo transfer and therefore this study was not based on intention to treat. In IVF with PGT-A, there is a notable proportion of women who will not undergo embryo transfer compared to IVF without PGT-A. This occurs because all of the embryos are deemed aneuploid or unsuitable for transfer. If a study includes only women who had an embryo transfer or only reports on outcomes per embryo transfer, it will inherently bias the study in favor of PGT-A. This bias occurs because the study excludes women who did not achieve pregnancy, as they did not have an embryo transfer, thus affecting the overall evaluation in favor of PGT-A. Such studies impose limitations on drawing any definitive conclusions regarding the impact of PGT-A on the effective ness of IVF, where ideally treatment outcomes should be calculated per woman (including all women going for treatment) or per started treatment cycle (including all started treatments). In Rubio et al.'s (15) study, multiple ovum pickups were allowed to collect oocytes before an attempted transfer. The provided data did not enable the authors to compute the exact numbers related to the first ovum pick-up. Multiple ovum-pickups per woman, before performing PGT-A, complicates the assessment of PGT A's impact on IVF treatment outcomes. This is because it artificially inflates the number of available oocytes, potentially providing a greater advantage to the PGT-A group compared to the control group, especially when outcomes are reported exclusively for the first embryo transfer. Given the provided data, it was not possible to correct for this strategy. Ozgur et al. (13) performed blastocyst stage biopsy, only on the embryos with the highest morphological scores, in the event that a participant was randomized to the PGT-A group. When available, euploid embryos were selected for transfer; otherwise, a non-biopsied embryo was chosen for transfer. As a result, it was not possible to employ the ideal unit of analysis (e.g. per woman or per ovum pick-up) to evaluate the effectiveness of PGT-A. We wish to caution physicians on the use of PGT-A in women from age groups without proven benefits based on the updated studies, while advantages may have been present based on the 2015 meta-analyses. The risk of discarding mosaic embryos with the potential for live birth and of damaging embryos at biopsy should be considered.

Conclusion

This meta-analysis did not demonstrate a significant improvement in OPR using PGT-A in the all ages, <35 years old and ≥35 years old age groups. There was also no significant difference in clinical pregnancy rates in any group. The MR decreased with the use of PGT-A in the all-ages group, but not when stratifying according to patient age <35 and ≥35 years old. The exact magnitude of the benefit of PGT-A cannot be correctly determined until multiple standardized protocols of IVF PGT-A trials are conducted (for example, SET of only blastocysts with Trophectoderm biopsy (day 5/6) and frozen embryo transfer). Accurate information, including the risks and benefits of PGT-A, is needed for patients and healthcare professionals to make informed choices regarding the use of this technology.

References

1. Irani M, Canon C, Robles A, Maddy B, Gunnala V, Qin X, et al. No effect of ovarian stimulation and oocyte yield on euploidy and live birth rates: an analysis of 12 298 trophectoderm biopsies. Hum Reported. 2020; 35(5): 1082-1089.

2. Kahraman S, Sahin Y, Yelke H, Kumtepe Y, Tufekci MA, Yapan CC, et al. High rates of aneuploidy, mosaicism and abnormal morpho kinetic development in cases with low sperm concentration. J Assist Reported Genet. 2020; 37(3): 629-640.

3. Barash OO, Ivani KA, Willman SP, Rosenbluth EM, Wachs DS, Hinckley MD, et al. Association between growth dynamics, morphological parameters, the chromosomal status of the blastocysts, and clinical outcomes in IVF PGS cycles with single embryo trans fer. J Assist Reprod Genet. 2017; 34(8): 1007-1016.

4. Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplanta tion genetic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update. 2011; 17(4): 454-466.

5. Sandalinas M, Sadowy S, Alikani M, Calderon G, Cohen J, Munné S. Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage. Hum Reprod. 2001; 16(9): 1954-1958.

6. Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, et al. Diminished effect of maternal age on implantation after pre implantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril. 2013; 100(6): 1695-1703.

7. Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screen ing and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril. 2013; 100(3): 697-703.

8. Hardarson T, Hanson C, Lundin K, Hillensjö T, Nilsson L, Stevic J, et al. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a ran domized controlled trial. Hum Reprod. 2008; 23(12): 2806-2812.

9. Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effec tiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Hum Reprod. 2015; 30(2): 473-483.

10. Chen M, Wei S, Hu J, Quan S. Can comprehensive chromosome screening technology improve IVF/ICSI outcomes? A meta-analysis. PLoS One. 2015; 10(10): e0140779.

11. Dahdouh EM, Balayla J, García-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(3): 281 289.

12. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril. 2019; 112(6): 1071-1079. e7.

13. Ozgur K, Berkkanoglu M, Bulut H, Yoruk GDA, Candurmaz NN, Coetzee K. Single best euploid versus single best unknown-ploidy blastocyst frozen embryo transfers: a randomized controlled trial. J Assist Reprod Genet. 2019; 36(4): 629-636.

14. Verpoest W, Staessen C, Bossuyt PM, Goossens V, Altarescu G, Bonduelle M, et al. Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial. Hum Reprod. 2018; 33(9): 1767-1776.

15. Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017; 107(5): 1122-1129.

16. Cornelisse S, Zagers M, Kostova E, Fleischer K, van Wely M, Mas tenbroek S. Preimplantation genetic testing for aneuploidies (ab normal number of chromosomes) in in vitro fertilisation. Cochrane Database Syst Rev. 2020; 9(9): CD005291.

17. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannid is JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interven tions: explanation and elaboration. BMJ. 2009; 339: b2700.

18. 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(1): 24.

19. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril. 2013; 100(1): 100-107. e1.

20. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The cochrane collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011; 343: d5928.

21. The R project for statistical computing. Available from: https:// www.r-project.org (8 Mar 2023).

22. Viechtbauer W. Conducting meta-analyses in R with the metafor Package. J Stat Softw. 2010; 36(3): 1-48.

23. Lewis S, Clarke M. Forest plots: trying to see the wood and the trees. BMJ. 2001; 322(7300): 1479-1480. 194

24. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring in consistency in meta-analyses. BMJ. 2003; 327(7414): 557-560.

25. Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ. 2011; 343: d4002.

26. Egger M, Davey Smith G, Schneider M, Minder C. Bias in me ta-analysis detected by a simple, graphical test. BMJ. 1997; 315(7109): 629-634.

27. Forman EJ, Upham KM, Cheng M, Zhao T, Hong KH, Treff NR, et al. Comprehensive chromosome screening alters traditional mor phology-based embryo selection: a prospective study of 100 consecutive cycles of planned fresh euploid blastocyst transfer. Fertil Steril. 2013; 100(3): 718-724.

28. Yang L, Gao J, Zeng L, Weng Z, Luo S. Systematic review and meta-analysis of single-port versus conventional laparoscopic hysterectomy. Int J Gynaecol Obstet. 2016; 133(1): 9-16.

29. Scott RT Jr, Galliano D. The challenge of embryonic mosaicism in preimplantation genetic screening. Fertil Steril. 2016; 105(5): 1150 1152.

30. Taylor TH, Gitlin SA, Patrick JL, Crain JL, Wilson JM, Griffin DK. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. Hum Reprod Update. 2014; 20(4): 571-581.

31. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N Engl J Med. 2015; 373(21): 2089-2090.

32. Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK, et al. One hundred mosaic embryos transferred prospectively in a single clinic: exploring when and why they result in healthy pregnancies. Fertil Steril. 2019; 111(2): 280-293.