



The Effects of Preimplantation Genetic Testing for Aneuploidy (PGT-A) on Patient-Important Outcomes in Embryo Transfer Cases: A Meta-Analysis

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Abstract

Background: The aim of this study was to evaluate the effect of preimplantation genetic testing for aneuploidy (PGT-A) on patient-important reproductive outcomes after in vitro fertilization (IVF).

Methods: Randomized and non-randomized studies have been sought in Ovid, MEDLINE, EMBASE, Web of Science, Scopus, and Cochrane Central Register of Controlled Trials since each database's inception through May 2021. Main keywords used for the search strategy included "Embryo transfer", "In vitro fertilization", "DNA sequencing", and "Comparative genome hybridization". Studies were screened independently and in duplicate.

Results: Ten studies were finally analyzed, representing a total of 2630 embryo transfers. The pooled OR for live birth rates were 1.45 (95%CI 0.24-8.78, I² 96%) and 1.66 (95%PI 0.15-18.01, 95%CI 0.98-2.83, I² 81%) derived from the NRSIs and the RCTs, respectively, in which the miscarriage rate were 1.25 (95%CI 0.19-8.33, I² 70%) and 0.57 (95%PI 0.06-5.34, 95%CI 0.27-1.21, I² 53%), and clinical pregnancy rates were 3.08 (95%CI 2.22-4.29, I² 0%) and 1.43 (95%PI 0.38-5.42, 95%CI 0.96-2.13, I² 68%). Influence analyses showed a greater treatment effect when excluding studies without patients at advanced maternal age.

Conclusion: There seems to be no significant difference in reproductive outcomes when using PGT-A in the general population; however, the procedure seems advantageous for patients at advanced maternal age. Nevertheless, this warrants caution when recommending the procedure to all couples seeking ART, as the current possible benefits may not justify the additional costs for all groups of patients.

Keywords: Assisted reproductive techniques, Comparative genomic hybridization, Embryo transfer, In vitro fertilization, Preimplantation genetic diagnosis.

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Introduction

Assisted reproductive technology (ART) is an alternative for couples who are unable to conceive naturally. Despite the evolution of

applied techniques for the success of in vitro fertilization (IVF), the improvement in live birth rate (LBR) has not been substantial. The success rate

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is currently less than 50% with evidence showing decreasing rates as low as 11% in women over 40 years old compared to approximately 28% in the general US population (1-3).

This drastic reduction in success rates with advanced maternal age (AMA) is closely related to the incidence of aneuploidy, which is significantly more common in these women, along with implantation failure and recurrent pregnancy loss, as other common factors related to failure of ART (4-6).

Preimplantation genetic testing for aneuploidy (PGT-A) was proposed as a method to improve pregnancy rates in patients undergoing in vitro fertilization by eliminating the influence of aneuploidy on LBR (7). Using PGT-A, researchers sought to improve reproductive outcomes in patients with AMA, implantation failure, recurrent pregnancy loss, severe male factor infertility, and infertility of unknown origin. Proposed additional benefits included the reduction of time to achieve pregnancy as well as improving reproductive outcomes in IVF cycles with donated eggs (8, 9).

Multiple techniques have been proposed for PGT-A; scientists first began performing biopsies on cleavage stage embryos and studying blastomeres using Fluorescence in situ hybridization (FISH), but results were unsatisfactory (10). Additionally, the technique used to obtain cells for analysis has generated controversy as well; for example, a negative effect on reproductive success was reported regarding biopsies performed on day three embryos (11, 12).

Given the limitation of the initial PGT-A technologies, techniques were developed to evaluate all 24 chromosomes. These new techniques are now known as comprehensive chromosome screening (CCS) and include metaphase comparative genomic hybridization (mCGH), array comparative genomic hybridization (aCGH), next generation sequencing (NGS), single nucleotide polymorphism (SNP) genotyping method, and real-time quantitative polymerase chain reaction (q-PCR) (13-16). Following the introduction of CCS techniques, implantation rates increased, and abortion rates decreased. Additionally, the use of PGT-A increased the rates of single embryo transfers and reduced the risk of multiple pregnancy and premature birth associated with multiple embryo transfers (17).

Despite the promising results seen at the start of the chromosomal analysis era, inconsistent literature reports, increasing costs, systematic errors in

outcome evaluation, and the ethical dilemma when dealing with mosaic embryos have raised questions about the utility and generalized applicability of PGT-A for embryonic selection (18-21). A more in-depth analysis of the safety, risks, and benefits of these techniques in the real world is necessary; thus, this meta-analysis was performed to explore the impact of PGT-A before frozen-thawed (FET) or fresh embryo transfer (ET) on the most important reproductive outcomes of patients considering live birth rates and miscarriage rates as our main concern.

Methods

This meta-analysis was done in line with the guidelines of Preferred Reporting Items for Meta-Analyses (PRISMA), (22) and the PRISMA checklist can be found in supplementary file 1. Our protocol was registered in PROSPERO under CRD42020198866.

Eligibility criteria: In this study, randomized controlled trials (RCTs) and prospective non-randomized studies of interventions (NRSIs) were included which evaluated the effect of PGT-A with CCS on reproductive outcomes of couples undergoing FET or ET, compared to only morphological assessment of blastocysts before transfer. NRSIs were included in the current study as very few RCTs are available due to the fact that possible detrimental effects of PGT-A on birth rates is a relatively novel issue and the ethical concerns related to the procedure hinder the researchers to conduct RCTs.

The included studies comprised the research on couples, irrespective of the cause of infertility, who underwent in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) with transfers in the blastocyst stage and research reporting live birth rate (LBR), miscarriage rate, and/or clinical pregnancy (CP) per embryo transfer as their primary or secondary outcomes; moreover, in studies evaluating multiple IVF cycles, merely the first cycle results were considered eligible. Also, studies summarizing multiple cycles without reporting the first cycle results, using low-yield PGT-A techniques (e.g., FISH) and day three biopsy, assessing the effect of solely detecting mosaicism, or those without adequate reporting of outcomes of interest were excluded.

Information sources and search strategy: An experienced librarian, provided with input from the lead researcher, has designed and conducted the

search in Ovid, MEDLINE, EMBASE, Web of Science, Scopus, and Cochrane Central Register of Controlled Trials since each database's inception through May 2021; references from studies and reviews were also screened for eligibility. A general search strategy combining MeSH terms and free text was built, with the first group of terms referring to the possible techniques used for PGT-A (*e.g.*, single nucleotide polymorphism, genomic hybridization, DNA sequencing, *etc.*), the second to IVF and ICSI techniques, and the third to embryo transfer. No language restriction or design-specific filters were applied in the databases. The full search strategy used in Scopus is presented in supplementary file 2 as an example.

Study selection process: Four reviewers working independently and in duplicate assessed each manuscript's title, abstract, and full text for eligibility. Prior to each screening phase, pilots were conducted until achieving an acceptable level of inter-rater reliability (Fleiss' kappa >0.70) (23). Aiming for a high sensitivity in the title and abstract screening, disagreements between reviewers were solved by including the study in the next phase. Afterwards, the full text of included abstracts were obtained and the same procedure was followed to select the final studies for inclusion; disagreements at this level were solved by consensus or intervention of a third reviewer.

Data collection and outcomes of interest: A web-based extraction form was created and evaluated by all reviewers before data extraction. General information of the included studies (author, year, country, funding, and design) was extracted alongside information on the PGT-A technique used, studied subgroups (*e.g.* advanced maternal age, recurrent pregnancy loss), treatment cycles, embryo transfer techniques (fresh ET or FET), and number of embryos transferred.

Seeking to combine studies performing multiple and single embryo transfers, reproductive outcomes per embryo transfer, comprising live birth rates, miscarriage rates, and clinical pregnancy were considered as the main outcomes of interest. The definition of the outcomes given by the authors of each study was extracted to evaluate the possible heterogeneity derived from different classifications. For the studies not stating definitions, outcomes were identified according to the guide for the standardization of definitions and reports in infertility (24). All reviewers extracted the data independently and in duplicate and disagreements

were solved by consensus or intervention of a third reviewer.

Risk of bias in individual studies and certainty of evidence: Four reviewers, (LS, AFR, FDGC, PC) working independently and in duplicate, evaluated the risk of bias in individual RCTs using the Cochrane risk of bias tool 2.0 (RoB2.0). This tool evaluates six domains including bias arising from the randomization process, deviations from the intended intervention, missing outcome data, mis-measurement of the outcomes, and selection of the reported results. The overall risk of bias was classified as low, moderate (labeled "with some concerns" by the tool), or high, according to the tool's criteria (25).

For non-randomized studies of interventions (NRSIs), the risk of bias was evaluated using the Cochrane ROBINS-I scale, considering bias arising due to confounding variables, selection of participants, misclassification of interventions, deviations from the intended intervention, missing data, mismeasurement of outcomes, and selection of the reported outcomes. The overall risk of bias was classified as low, moderate, or serious according to the tool criteria (26). All decisions were based on the reproductive outcomes of the studies; if two outcomes had different risk of bias assessments, the outcome with the highest risk was considered for the final decision. Any disagreement between the reviewers was solved either by consensus or intervention of a third reviewer.

Certainty in the evidence for the primary outcomes was evaluated using the GRADE approach, considering the risk of bias of included studies, the inconsistency of effect estimates, the indirectness associated with the study design, the possibility of publication bias, and residual confounders (27). The results were divided according to the study designs (RCTs and NRSIs) and are summarized in a table of findings, generated using the GRADEpro GDT software (28).

Summary measures and synthesis of results: LBR, miscarriage, and CP rates were extracted alongside the embryo transfers and the treatment cycles. For the primary analysis, odds ratios (ORs) were calculated using the pregnancy events per embryo transfer; these ORs were pooled by DerSimonian and Laird approach (DL) for random effects meta-analyses. As expected, a considerable degree of clinical and methodological heterogeneity was derived from the different subgroups of patients seeking ART (*i.e.*, patients at an ad-

vanced maternal age, with recurrent pregnancy loss, *etc.*) and different stimulation and embryo transfer protocols.

Statistical heterogeneity of the pooled estimates was explored using the X^2 statistic with p-value threshold of <0.10 and the I^2 statistic, considering the thresholds described in the Cochrane handbook (29). Sensitivity analyses assessing the robustness of findings were planned, first by pooling LBR and CP events per cycle instead of per embryo transfer, and second, by pooling events per clinical pregnancies for the miscarriage rate. The use of fixed-effects meta-analyses depended on the changes of statistical heterogeneity when performing the sensitivity analyses. Subgroup analyses considering populations with advanced maternal age (AMA) and recurrent pregnancy loss (RPL) were planned; however, no formal subgroup effect assessment was possible due to lack of sufficient independent report for each subgroup among the included studies. Nonetheless, further exploration of heterogeneity was performed using influence analyses as described by Viechtbauer and Cheung (30) included in the "dmetar" package (31).

In order to consider how new studies could affect the obtained effect estimates, 95% prediction intervals (95% PI) for the pooled random-effects estimates were calculated through the method described by Higgins et al. (32) if four or more studies were synthesized in each meta-analysis. Statistical analyses were performed in R statistical software *vs.* 4.0.3 with R studio *vs.* 1.3.1056 using the packages "metaforest", "meta", and "dmetar".

Results

Our search strategy yielded 3649 studies after deduplication. From these, 212 full texts were selected for screening. Six RCTs and four NRSIs were included and analyzed, comprising a total of 2123 couples and 2630 embryo transfers. The study selection process and reasons for exclusion are summarized in figure 1. Most studies included patients with good prognosis with overall ages ranging from 25 to 42 years (8, 16, 33-40).

The technologies used for PGT-A included array-based comparative genomic hybridization (aCGH) in four studies (33, 36, 38, 39), next generation sequencing (NGS) in three studies, (34, 35, 37) real-time quantitative polymerase chain reaction (qPCR) in two studies, (8, 16) and single nucleotide polymorphism (SNP)-based microarray in one study (40). Most of the authors reported

performing frozen/thawed embryo transfer (FET) for both groups, except two studies where they reported a combination of fresh embryo transfer (ET) and FET (8, 38). One study reported only fresh embryo transfers (39). Regarding the number of embryos transferred per patient, five of the included studies performed a single transfer for all patients of both groups (35-39); the other studies varied in allowing up to two transfers (34, 38), not limiting the number of embryo transfers (33), and performing double transfers in the control group and single transfers in the PGT-A group (8, 40). The rest of the general characteristics of the included studies are summarized in table 1.

Live-birth rate per embryo transfer: Three out of four of the included NRSIs and four out of six RCTs reported that the LBR was associated with the interventions (16, 33-37, 40). Only two studies reported their definitions for LBR (34, 35). One defined the outcome as having at least one infant born alive which survived for at least one month, and the other explained the outcome as all infants born alive after 20 weeks. The pooled ORs were 1.45 (95%CI 0.24-8.78, I^2 96%) and 1.66 (95%PI 0.15-18.01, 95%CI 0.98-2.83, I^2 81%), derived from the estimates of the NRSIs (Figure 2A) and the RCTs (Figure 2B), respectively. The pooled estimates derived from RCTs and NRSIs had very low certainty, mainly due to considerable risk of bias, inconsistency, and imprecision.

Narrative assessment of the included studies showed that three studies indicated improvement in LBR by using PGT-A (16, 34, 40), while the rest of the studies showed no significant difference in LBR when using PGT-A or morphology alone (33, 35-37). In general, the population in the studies with no significant differences was older; moreover, greater proportion of female factors were found as causes of infertility among them and lower number of previous miscarriages were recorded.

Miscarriage rate per embryo transfer: Five of the RCTs (8, 16, 35, 39) and two of the NRSIs (34, 36) reported the miscarriage rate per ET of their patients and most studies considered miscarriage as fetal mortality after a gestational sac with fetal heartbeat was detected, or an infant born dead before 20 weeks of gestation. The pooled ORs were 1.25 (95%CI 0.19-8.33, I^2 70%) and 0.57 (95%PI 0.06-5.34, 95%CI 0.27-1.21, I^2 53%) derived from the NRSIs and the RCTs, respectively (Figures 3A and B). The certainty of pooled estimates was rated as very low, due to extremely small sample



PRISMA 2009 flow diagram

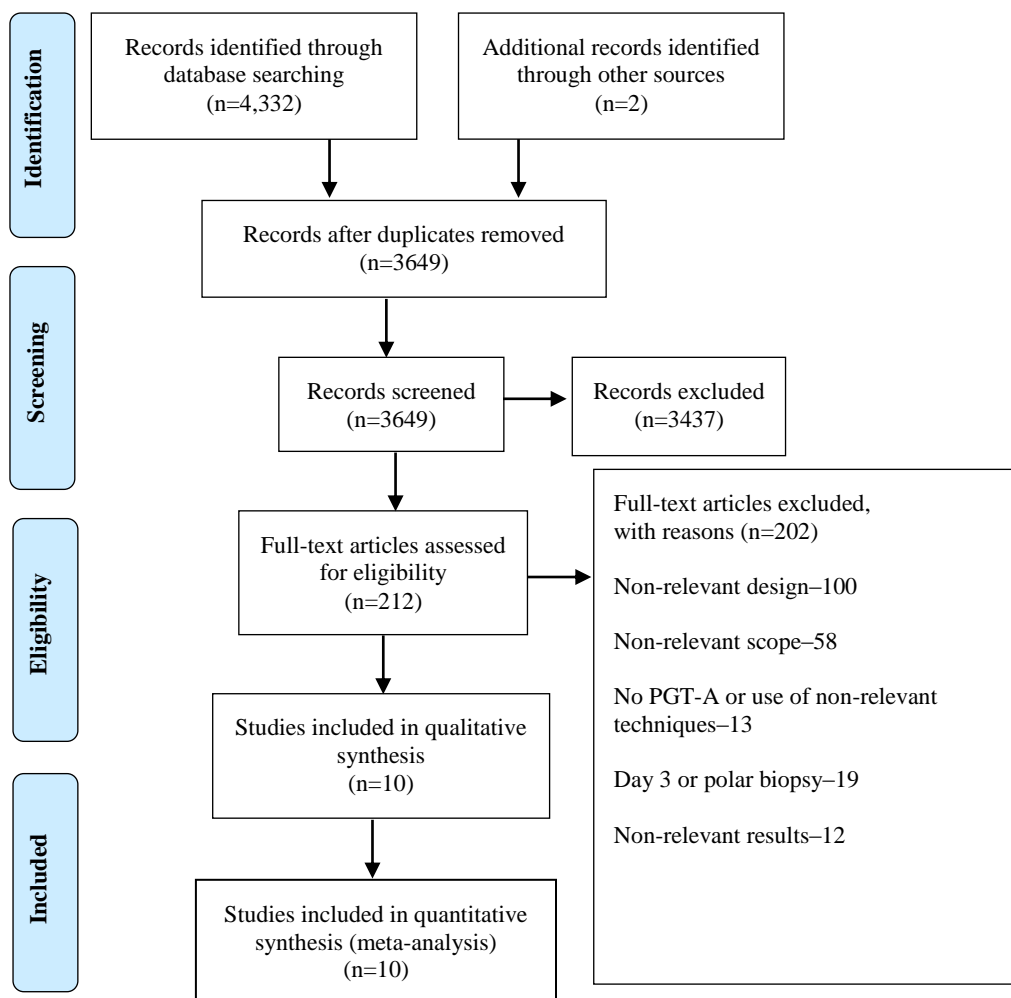


Figure 1. PRISMA flow diagram

Table 1. General characteristics of included studies

Study	Design	Country *	Funding	PGT-A technology	Mean age (SD)	Type of embryo transfer
Schoolcraft et al. 2010	NRSI	UK	Government	aCGH	37.7 (0.5)	Frozen/thawed
Yang et al. 2012	RCT	China, USA	NR	aCGH	31.4 (2.7)	Fresh
Scott Jr. et al. 2013	RCT	USA	Industry	qPCR	32.2 (0.5)	Frozen/thawed
Forman et al. 2013	RCT	USA	Industry	qPCR	34.8 (4.3)	Frozen/thawed Fresh
Greco et al. 2014	NRSI	Spain	NR	aCGH	32 (0.9)	Frozen/thawed Fresh
Liss et al. 2018	NRSI	Poland	Academic	NGS	35.8 (3.7)	Frozen/thawed
Ozgun et al. 2019	RCT	Turkey	Private	NGS	28.5 (3.71)	Frozen/thawed
Munné et al. 2019	RCT	USA	Industry	NGS	33.7 (3.5)	Frozen/thawed
Sato et al. 2019	NRSI	Japan	Government	aCGH	39.2 (2.1)	Frozen/thawed
Sui et al. 2020	RCT	China	Private	SNP microarray	35.8 (5.1)	Frozen/thawed

* Country was based on the setting of the study, or the reported country of the corresponding author if the setting was not reported.
 NRSI: Non-randomized study of intervention; NR: Non-reported; RCT: Randomized clinical trial; PGT-A: Preimplantation genetic testing for aneuploidy; aCGH: Array comparative genomic hybridization; qPCR: Real-time polymerase chain reaction; NGS: Next generation sequencing

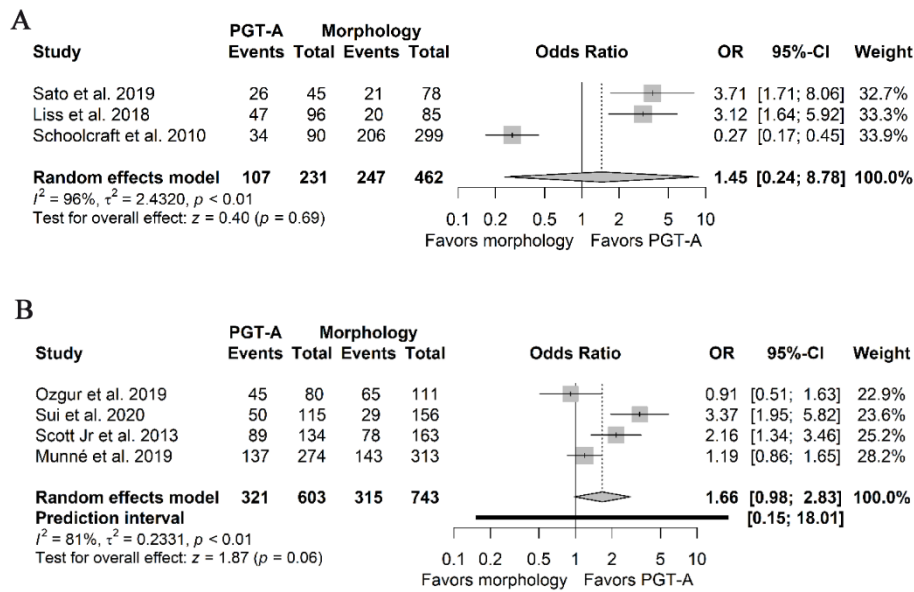


Figure 2. Forest plots of live-birth rate per embryo transfer. A) Pooled effect derived from NRSIs. B) Pooled effect derived from RCTs.

NRSIs: Non-randomized studies of interventions; RCTs: Randomized clinical trials; PGT-A: Preimplantation genetic testing for aneuploidy; OR: Odds ratio; 95%CI: 95% confidence interval

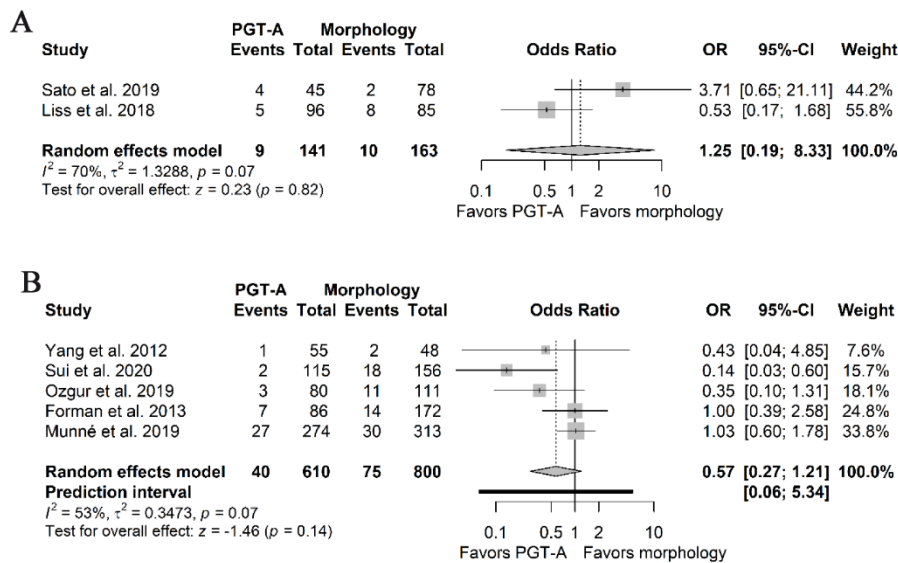


Figure 3. Forest plots of miscarriage rate per embryo transfer. A) Pooled effect derived from NRSIs. B) Pooled effect derived from RCTs.

NRSIs: Non-randomized studies of interventions; RCTs: Randomized clinical trials; PGT-A: Preimplantation genetic testing for aneuploidy; OR: Odds ratio; 95%CI: 95% confidence interval

sizes, considerable risk of bias, and inconsistency in both cases. Most studies considered miscarriage rates as secondary outcomes.

Clinical pregnancy per embryo transfer: Five RCTs and all NRSIs reported the CP rate. Most studies considered this outcome as ultrasonographic evidence of a fetal heartbeat at 4-6 weeks of gesta-

tion. The pooled ORs were 3.08 (95%CI 2.22-4.29, I^2 0%) and 1.43 (95%PI 0.38-5.42, 95%CI 0.96-2.13, I^2 68%), derived from the estimates of the NRSIs (Figure 4A) and RCTs (Figure 4B), respectively. The certainty of pooled estimates was rated as very low, due to serious risk of bias and inconsistency for the RCTs, and due to a very

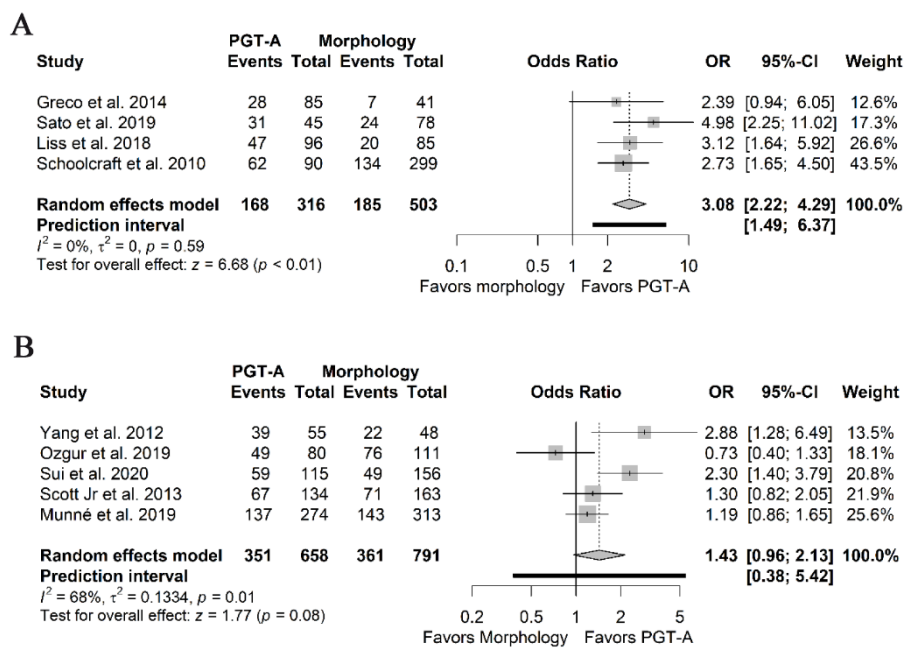


Figure 4. Forest plots of clinical pregnancy per embryo transfer. A) Pooled effect derived from NRSIs. B) Pooled effect derived from RCTs.

NRSIs: Non-randomized studies of interventions; RCTs: Randomized clinical trials; PGT-A: Preimplantation genetic testing for aneuploidy; OR: Odds ratio; 95%CI: 95% confidence interval

serious risk of bias and a plausible residual confounding which could have spuriously favored the use of PGT-A for the NRSIs. The main difference between the RCTs and NRSIs stemmed from the effect of maternal age on the PGT-A effectiveness estimation, as most RCTs controlled for this factor, either by excluding patients with AMA (35, 39) or by stratifying the randomization based on maternal age blocks (8, 16, 37).

Subgroup and sensitivity analyses

Change of outcome measure: Changing the outcome measure from embryo transfer to cycles had a significant impact on the estimates for LBR and CP of the NRSIs (Supplementary Files 4A and 5A). The pooled odds ratios for LBR changed significantly favoring the use of PGT-A (1.78 95%CI 1.18-2.59, I^2 0%) and the effect estimates for CP remained significant, favoring PGT-A (1.68 95%CI 1.16-2.44, I^2 3%). In both cases, the statistical heterogeneity was reduced, possibly because the difference between the number of embryos transferred between the groups across the studies was not considered in this calculation. Regarding the effect estimates of LBR and CP from the RCTs (Supplementary Files 4B and 5B), both remained non-significant (1.27 95%PI 0.05-31.02, 95%CI 0.63-2.25, I^2 86% and 1.20 95%PI

0.12-11.59, 95%CI 0.63-2.25, I^2 83%, respectively). Additionally, statistical heterogeneity increased in both estimates.

Pooled effects for miscarriage rates remained non-significant when changing the outcome measure from embryo transfer to clinical pregnancies (Supplementary File 6A and 6B), in both NRSIs and RCTs (0.49 95%CI 0.06-4.31, I^2 74% and 0.42 95%PI 0.03-6.84, 95%CI 0.17-1.04, I^2 59%, respectively). Statistical heterogeneity was relatively unaffected for the estimate derived from the NRSIs and was slightly increased for the one derived from the RCTs.

Advanced maternal age: Subgroup analyses for assessing the intervention effect on patients with AMA (defined as >35 years) were not possible as most studies included patients with a wide age range varying from 25 to 42 years. Only four studies were fit for indirect assessment of the effect of maternal age on reproductive outcomes, two which excluded patients with AMA (35, 39), and two with stratification of the population by maternal age (8, 37); however, only one study reported reproductive outcomes for each age group (37). Individual OR derived from the single study reporting the LBR and miscarriage rates per ET for patients with and without AMA showed a

clear difference between subgroups, 1.74 (1.07-2.84) vs. 0.86 (0.56-1.34) for LBR in patients 35 years or older, and younger than 35 years, respectively and 0.71 (0.31-1.65) vs. 1.38 (0.66-2.92) for miscarriage rate in the same populations.

Through influence analyses for RCTs in pooling the LBR and CP outcomes, a significant contribution to heterogeneity and a large treatment effect were demonstrated in studies which excluded patients with AMA (8, 35). On the other hand, the influence of these studies was not as strong for the miscarriage rate estimate.

Excluding both influential studies from the LBR and CP meta-analyses resulted in estimates trending towards a superiority of PGT-A, though the estimates remained non-significant.

Recurrent pregnancy loss: Only one NRSI (36) and one RCT (40) reported reproductive outcomes in patients with recurrent pregnancy loss; thus, no clear conclusions on the possible modifying effect of this factor can be reached. Both studies found significant advantages of PGT-A in LBR and CP when applied in patients with RPL; having adjusted OR, the obtained values from NRSI for LBR and CP were 3.89 (1.16-13.1) and 5.14 (1.52-17.3), respectively. Additionally, adjusted risk ratios (RRs) reported by the RCT were 2.34 (1.59-3.45) and 1.63 (1.22-2.19) for LBR and CP, respectively. However, only the RCT found a significant effect of PGT-A on miscarriage rate, reporting an adjusted RR of 0.15 (0.04-0.64).

Finally, influence analysis of the pooled estimates from the RCTs demonstrated that the study by Sui et al., which only included patients with RPL, was the main influence on the overall heterogeneity of the models for LBR, CP, and miscarriage rate; therefore, excluding this study from the meta-analysis resulted in overall effect estimates trending towards no difference between the use of PGT-A and morphology alone.

Risk of bias and GRADE assessment: The results of the risk of bias assessment are summarized in figures 5A and 5B; one of the included RCTs was at a low risk of bias, (8) two had some concerns, (16, 35) and two were at high risk of bias (37, 39); the main areas of concerns were about randomization process, deviation from the intended interventions, and possible selection of reported results. Regarding the NRSI, two of the studies demonstrated a critical risk of bias, (33, 38) one showed the moderate risk, (36) and the other serious risk (34). The main affected domains across the stud

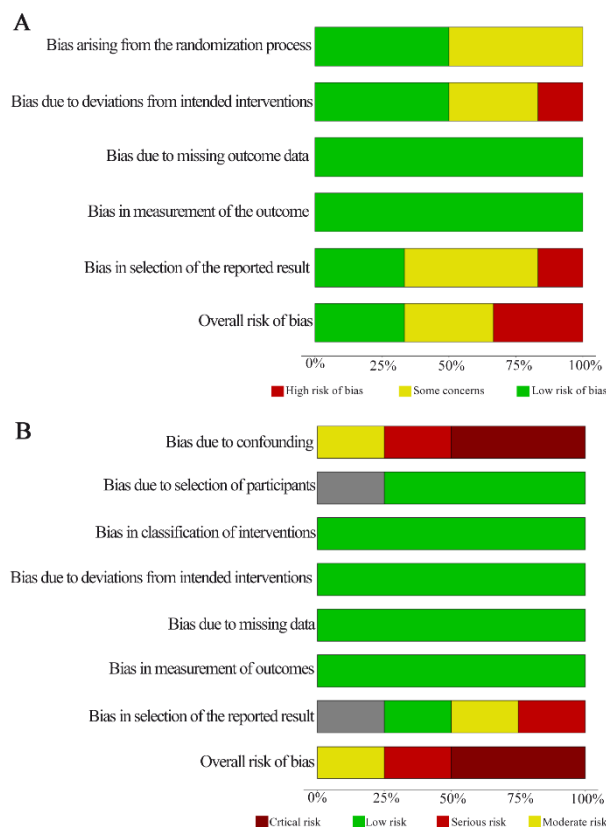


Figure 5. Graph summaries of risk of bias. A) ROB 2.0 results for RCTs. B) ROBINS-I results for NRSIs. RCTs: Randomized controlled trials; NRSIs: Non-randomized studies of interventions

ies were the lack of statistical or methodological control for critical sources of bias and the evidence of selection of reported results derived from multiple analyses. The table of findings summarizing the conclusions and rationale behind the GRADE assessment for each outcome can be found in the supplementary file 3.

Discussion

Findings: In our study, no significant difference was found in reproductive outcomes after ART between women undergoing PGT-A for the selection of embryos versus morphology alone. This is a significant finding considering that the present study focused on patient-important reproductive outcomes. Interestingly, when analyzing the possible influence of AMA and a history of RPL on the pooled estimates through sensitivity and influence analyses, it was found that the possible reason behind the heterogeneity of our estimates could have been related to the inclusion of studies where couples of AMA were excluded. The ten-

dency towards PGT-A superiority when excluding these studies indicates a greater benefit of chromosomal screening in patients with AMA, which relates to the increased incidence of aneuploidy in these patients (41, 42). This finding calls for further exploration of the effectiveness of PGT-A as it is related to maternal age.

Comparison with current evidence: The initial studies that evaluated the usefulness of selecting embryos according to their level of euploidy generated great expectations regarding the improvement of reproductive results (38). However, many of these studies are now being questioned due to methodological flaws in their outcome assessment (35, 43).

In 2015, a systematic review and meta-analysis concluded that embryo selection through CCS improves implantation rates in patients with normal ovarian reserve (44). That same year, another systematic review found a possible beneficial effect of embryo selection, using PGT-A, on implantation and clinical pregnancy rates in young patients (45). Five years later, a review argued the need for limiting the use of PGT-A in day-to-day clinical practice and leaving these techniques only for research purposes until their safety is well established (20). Our study reached a similar conclusion, which further supports the idea that the techniques used for PGT-A should only be used in certain situations where there is a clear beneficial potential. Additionally, the selection of our eligibility criteria compared with the criteria used in past reviews may be a stronger indicator of the lack of a true beneficial effect of the use of PGT-A in the general population.

A systematic review and network meta-analysis was published in 2021 with the purpose of identifying which groups of patients could benefit the most from PGT-A; however, only two of their eleven references were about live-birth rate and most of them used implantation rates as a measure of effectiveness of ART, which is a practice that has been currently questioned (46, 47). In addition, the clinical pregnancy rate does not represent the final result that every couple who is treated with ART seeks to take a healthy newborn home. This, coupled with the small number and low quality of the RCTs pooled by previous systematic reviews, warrants caution in accepting PGT-A as a beneficial procedure.

Several reviews have been published since 2020, where authors called for taking a step back in the

use of these tools in day-to-day clinical practice and instead leaving these techniques only for research purposes until their safety is well established (20, 48, 49). As shown by our findings, when pooled estimates are focused on patient-important outcomes, the effect of using PGT-A is less impactful, either in women with a good prognosis, (35, 37) patients of AMA (33) with a history of implantation failure, or women with RPL (36, 38).

One of the RCTs included in our synthesis used more modern technologies as compared with previous studies. In addition, their authors considered LBR as the measure for effectiveness. (35). However, they did not find improvement in LBR or a lower abortion rate when PGT-A was used. On the other hand, the results of another RCT published in the same year showed no difference in LBR or abortion rates with the use of PGT-A in patients with a history of implantation failure or those with a history of RPL. Notably, an improvement in LBR was found when they reported the results obtained per patient (36).

The results of other included RCTs showed no difference in the ongoing pregnancy and abortion rate, when selecting an embryo to transfer via NGS (37). Nonetheless, the authors reported better ongoing pregnancy rates in a group of women between 35 and 40 years when evaluating the results per embryo transfer. This further supports the plausible spurious advantage of PGT-A derived from previous inadequate analyses.

Strengths: Strengths of this meta-analysis include a strict inclusion criterion, and the inclusion of both experimental and observational designs which allowed a broad exploration of current evidence. The most important outcomes for couples were analyzed, which truly represent the objectives of ART and are in accordance with the expectations of our patients. The decisions behind our exclusions were based on recent findings which have identified the most useful techniques and procedures, as well as the harms caused by other techniques. Moreover, the selected studies were confined to those in which embryo biopsy was performed in the blastocyst stage as this is the most efficient and least harmful way to obtain enough cells for the correct chromosomal analysis (50, 51). In addition, the incidence of aneuploidy is lower in embryos in the blastocyst stage than in earlier stage embryos (52).

Limitations: Limitations of this review include

the considerable risk of bias of included studies and small number of studies, which limited our capability to strongly recommend the use of PGT-A for embryo selection. As shown by our wide prediction intervals, future estimates are expected to differ significantly as more high-quality studies become available. Furthermore, the use of PGT-A has been related to the possibility of reducing the number of multiple pregnancies. However, it could not be possible to determine the effect of PGT-A on the rate of multiple pregnancy due to lack of reporting.

Finally, the actual efficiency of PGT-A may be affected by the wide variety of available CCS technologies. Combining the results derived from different available techniques may have contributed significantly to the heterogeneity, reducing the true effect of PGT-A. Nonetheless, a formal analysis regarding the influence of CCS on our outcomes of interest was not possible due to the lack of well-reported studies exploring this possibility.

Conclusion

The lack of evidence pointing to a clear advantage of selecting embryos for transfer through PGT-A and its effect on LBR, CP, and miscarriage rates, compared to morphological assessment alone warrants caution when recommending this procedure for all couples seeking ART, as the current possible benefits may not justify the additional costs and risk linked to the procedures. Identifying the characteristics of patients who benefit from the use of PGT-A is paramount for a cost-effective application of new technologies. The findings of this meta-analysis suggest a selective positive effect of PGT-A on reproductive outcomes of patients with AMA and RPL. Further studies exploring the association of these maternal factors and PGT-A efficacy are needed to confirm the findings.

Acknowledgement

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Conflict of Interest

Authors declare no conflict of interest.

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Supplementary

Supplementary File 1. PRISMA checklist

Section and topic	Item #	Checklist item	Location where item is reported
Title			
Title	1	Identify the report as a systematic review.	1
Abstract			
Abstract	2	See the PRISMA 2020 for abstracts checklist.	3
Introduction			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	5
Methods			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5-6
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	6
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	7
Data items	10 ^a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (<i>e.g.</i> for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	7
	10 ^b	List and define all other variables for which data were sought (<i>e.g.</i> participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	7-8
Effect measures	12	Specify for each outcome the effect measure(s) (<i>e.g.</i> risk ratio, mean difference) used in the synthesis or presentation of results.	8
	13 ^a	Describe the processes used to decide which studies were eligible for each synthesis (<i>e.g.</i> tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	8
	13 ^b	Describe any methods required to prepare the data for presentation or synthesis, such as handling missing summary statistics, or data conversions.	8
	13 ^c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	8
	13 ^d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	8
	13 ^e	Describe any methods used to explore possible causes of heterogeneity among study results (<i>e.g.</i> subgroup analysis, meta-regression).	9
Reporting bias assessment	13 ^f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	9
	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	NR
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	7-8

Contd. Supplementary File 1. PRISMA checklist

Section and topic	Item #	Checklist item	Location where item is reported
Results			
Study selection	16 ^a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	9
	16 ^b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	9
Study characteristics	17	Cite each included study and present its characteristics.	9
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	13
Results of Individual studies	19	For all outcomes, present: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (<i>e.g.</i> confidence/credible interval), ideally using structured tables or plots for each study.	10-13
Results of syntheses	20 ^a	For each synthesis, briefly summarize the characteristics and risk of bias among contributing studies.	10-13
	20 ^b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (<i>e.g.</i> confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	10-13
	20 ^c	Present results of all investigations of possible causes of heterogeneity among study results.	10-13
	20 ^d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	11-13
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NR
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	10-13
Discussion			
Discussion	23 ^a	Provide a general interpretation of the results in the context of other evidence.	14
	23 ^b	Discuss any limitations of the evidence in the review.	17
	23 ^c	Discuss any limitations of the review processes.	17
	23 ^d	Discuss implications of the results for practice, policy, and future research.	17-18
Other information			
Registration and protocol	24 ^a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	5
	24 ^b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	5
	24 ^c	Describe and explain any amendments to information provided at registration or in the protocol.	NR
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	19
Competing interests	26	Declare any competing interests of review authors.	19
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms, data extracted from included studies, data used for all analyses, analytic code, and any other materials used in the review.	NR

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From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; 372: n71. doi: 10.1136/bmj.n71

For more information, visit: <http://www.prisma-statement.org/>

Supplementary File 2. Full search strategy

# 4	207	#3 AND #2 AND #1 Indexes=SCI-EXPANDED, ESCI Timespan=All years
# 3	17, 632	TS = ("single embryo transfer" or "embryo transfer" or "embryo transplantation" or "transfer, embryo" or "blastocyst transfer" or "embryo transfers" or "transfers, embryo" or "tubal embryo stage transfer" or "tubal embryo transfer") Indexes=SCI-EXPANDED, ESCI Timespan=All years
# 2	42, 094	TS = ("sperm injections, intracytoplasmic " or "injection, intracytoplasmic sperm" or "injections, intracytoplasmic sperm" or "injections, sperm, intracytoplasmic" or "intracytoplasmic sperm injection" or "intracytoplasmic sperm injections" or "sperm injection, intracytoplasmic" or "intracytoplasmic sperm injection" or "ICSI" or "intra cytoplasmic sperm injection" or "babies, test-tube" or "baby, test-tube" or "fertilization, test-tube" or "fertilizations in vitro" or "fertilization in vitro" or "fertilizations, test-tube" or "extracorporeal fertilization" or "in vitro fertilization" or "in vitro fertilization" or "IVF (in vitro fertilization)" or "in vitro fertilizations" or "test tube babies" or "test tube fertilization" or "test-tube babies" or "test-tube baby" or "test-tube fertilization" or "test-tube fertilizations") Indexes=SCI-EXPANDED, ESCI Timespan=All years
# 1	242, 623	TS = ("polymorphism, single nucleotide" or "nucleotide polymorphism, single" or "nucleotide polymorphisms, single" or "polymorphisms, single nucleotide" or "SNPs" or "single nucleotide polymorphism" or "single nucleotide polymorphisms" or "single nucleotid polymorphism" or "array based comparative genomic hybridization" or "array comparative genomic hybridization" or "array-based comparative genomic hybridization" or "comparative genome hybridization" or "comparative genome hybridizations" or "comparative genomic hybridizations" or "genome hybridization, comparative" or "genome hybridizations, comparative" or "genomic hybridization, comparative" or "genomic hybridizations, comparative" or "hybridization, comparative genome" or "hybridization, comparative genomic" or "hybridizations, comparative genome" or "hybridizations, comparative genomic" or "comparative genomic hybridization" or "metaphase comparative genomic hybridization" or "next generation secuencing" or "DNA sequencing, high-throughput" or "deep sequencing" or "deep sequencings" or "high throughput DNA sequencing" or "high throughput nucleotide sequencing" or "high throughput RNA sequencing" or "high-throughput DNA sequencing" or "high-throughput RNA sequencing" or "Illumina sequencing" or "Ion proton sequencing" or "Ion torrent sequencing" or "massively parallel sequencing" or "massively-parallel sequencing" or "next generation sequencing" or "next-generation sequencing" or "nucleotide sequencing, high-throughput" or "pyrosequencing" or "RNA sequencing, high-throughput" or "sequencing, deep" or "sequencing, high-throughput DNA" or "sequencing, high-throughput nucleotide" or "sequencing, high-throughput RNA" or "sequencing, Illumina" or "sequencing, Ion proton" or "sequencing, Ion torrent" or "sequencing, massively-parallel" or "sequencing, next-generation" or "high-throughput nucleotide sequencing" or "quantitative PCR" or "quantitative polymerase chain reaction" or "quantitative polymerase chain reaction") Indexes=SCI-EXPANDED, ESCI Timespan=All years

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1	("polymorphism, single nucleotide" or "nucleotide polymorphism, single" or "nucleotide polymorphisms, single" or "polymorphisms, single nucleotide" or "SNPs" or "single nucleotide polymorphism" or "single nucleotide polymorphisms" or "single nucleotide polymorphisms" or "array based comparative genomic hybridization" or "comparative genome hybridization" or "comparative genome hybridizations" or "comparative genomic hybridizations" or "genome hybridization, comparative" or "genome hybridizations, comparative" or "genomic hybridization, comparative" or "genomic hybridizations, comparative" or "hybridization, comparative genome" or "hybridization, comparative genomic" or "hybridizations, comparative genome" or "hybridizations, comparative genomic" or "comparative genomic hybridization" or "metaphase comparative genomic hybridization" or "next generation sequencing" or "DNA sequencing, high-throughput" or "deep sequencing" or "deep sequencings" or "high throughput DNA sequencing" or "high throughput nucleotide sequencing" or "high throughput RNA sequencing" or "high-throughput DNA sequencing" or "high-throughput RNA sequencing" or "Illumina sequencing" or "Ion proton sequencing" or "Ion torrent sequencing" or "massively parallel sequencing" or "massively-parallel sequencing" or "next generation sequencing" or "next-generation sequencing" or "nucleotide sequencing, high-throughput" or "pyrosequencing" or "RNA sequencing, high-throughput" or "sequencing, deep" or "sequencing, high-throughput DNA" or "sequencing, high-throughput nucleotide" or "sequencing, high-throughput RNA" or "sequencing, Illumina" or "sequencing, Ion proton" or "sequencing, Ion torrent" or "sequencing, massively-parallel" or "sequencing, next-generation" or "high-throughput nucleotide sequencing" or "quantitative PCR" or "quantitative polymerase chain reaction" or "quantitative polymerase chain reaction"). mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	661499
2	("sperm injections, intracytoplasmic" or "injection, intracytoplasmic sperm" or "injections, intracytoplasmic sperm" or "injections, sperm, intracytoplasmic" or "intracytoplasmic sperm injection" or "intracytoplasmic sperm injections" or "sperm injection, intracytoplasmic" or "intracytoplasmic sperm injection" or "ICSI" or "intra cytoplasmic sperm injection" or "babies, test-tube" or "baby, test-tube" or "fertilization, test-tube" or "fertilizations in vitro" or "fertilization in vitro" or "fertilizations, test-tube" or "extracorporeal fertilization" or "in vitro fertilization" or "in vitro fertilization" or "IVF (in vitro fertilization)" or "in vitro fertilizations" or "test tube babies" or "test tube fertilization" or "test-tube babies" or "test-tube baby" or "test-tube fertilization" or "test-tube fertilizations"). mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	118432
3	("single embryo transfer" or "embryo transfer" or "embryo transplantation" or "transfer, embryo" or "blastocyst transfer" or "embryo transfers" or "transfers, embryo" or "tubal embryo stage transfer" or "tubal embryo transfer").mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, ox, px, rx, an, ui, sy]	55491
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[("polymorphism, single nucleotide" or "nucleotide polymorphism, single" or "nucleotide polymorphisms, single" or "polymorphisms, single nucleotide" or "SNPs" or "single nucleotide polymorphism" or "single nucleotide polymorphisms" or "single nucleotide polymorphisms" or "array based comparative genomic hybridization" or "array comparative genomic hybridization" or "array-based comparative genomic hybridization" or "comparative genome hybridization" or "comparative genome hybridizations" or "comparative genomic hybridizations" or "genome hybridization, comparative" or "genome hybridizations, comparative" or "genomic hybridization, comparative" or "genomic hybridizations, comparative" or "hybridization, comparative genome" or "hybridization, comparative genomic" or "hybridizations, comparative genome" or "hybridizations, comparative genomic" or "comparative genomic hybridization" or "metaphase comparative genomic hybridization" or "next generation sequencing" or "DNA sequencing, high-throughput" or "deep sequencing" or "deep sequencings" or "high throughput DNA sequencing" or "high throughput nucleotide sequencing" or "high throughput RNA sequencing" or "high-throughput DNA sequencing" or "high-throughput RNA sequencing" or "Illumina sequencing" or "Ion proton sequencing" or "Ion torrent sequencing" or "massively parallel sequencing" or "massively-parallel sequencing" or "next generation sequencing" or "next-generation sequencing" or "nucleotide sequencing, high-through-

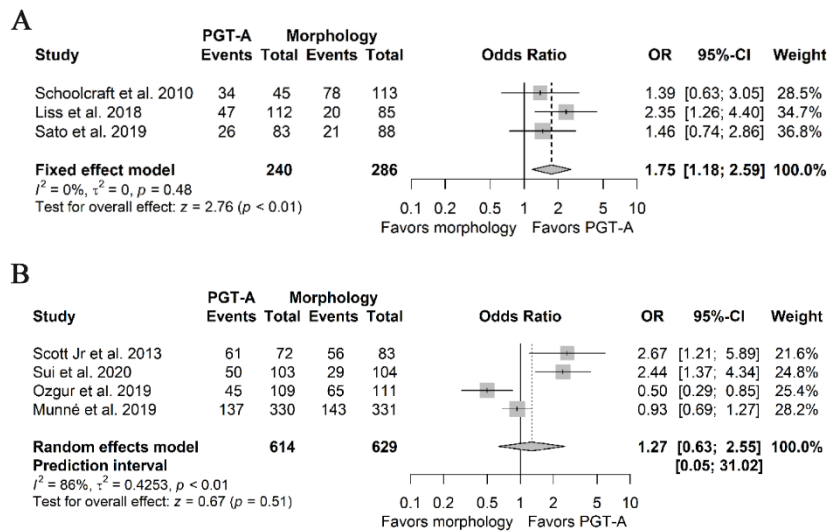
put" or "pyrosequencing" or "RNA sequencing, high-throughput" or "sequencing, deep" or "sequencing, high-throughput DNA" or "sequencing, high-throughput nucleotide" or "sequencing, high-throughput RNA" or "sequencing, Illumina" or "sequencing, Ion proton" or "sequencing, Ion torrent" or "sequencing, massively-parallel" or "sequencing, next-generation" or "high-throughput nucleotide sequencing" or "quantitative PCR" or "quantitative polymerase chain reaction" or "quantitative polymerase chain reaction")] and [{"sperm injections, intracytoplasmic" or "injection, intracytoplasmic sperm" or "injections, intracytoplasmic sperm" or "injections, sperm, intracytoplasmic" or "intracytoplasmic sperm injection" or "intracytoplasmic sperm injections" or "sperm injection, intracytoplasmic" or "intracytoplasmic sperm injection" or "ICSI" or "intra cytoplasmic sperm injection" or "babies, test-tube" or "baby, test-tube" or "fertilization, test-tube" or "fertilizations in vitro" or "fertilization in vitro" or "fertilizations, test-tube" or "extracorporeal fertilization" or "in vitro fertilization" or "in vitro fertilization" or "IVF (in vitro fertilization)" or "in vitro fertilizations" or "test tube babies" or "test tube fertilization" or "test-tube babies" or "test-tube baby" or "test-tube fertilization" or "test-tube fertilizations"}] and [{"single embryo transfer" or "embryo transfer" or "embryo transplantation" or "transfer, embryo" or "blastocyst transfer" or "embryo transfers" or "transfers, embryo" or "tubal embryo stage transfer" or "tubal embryo transfer"}]

Supplementary File 3. GRADE table of findings

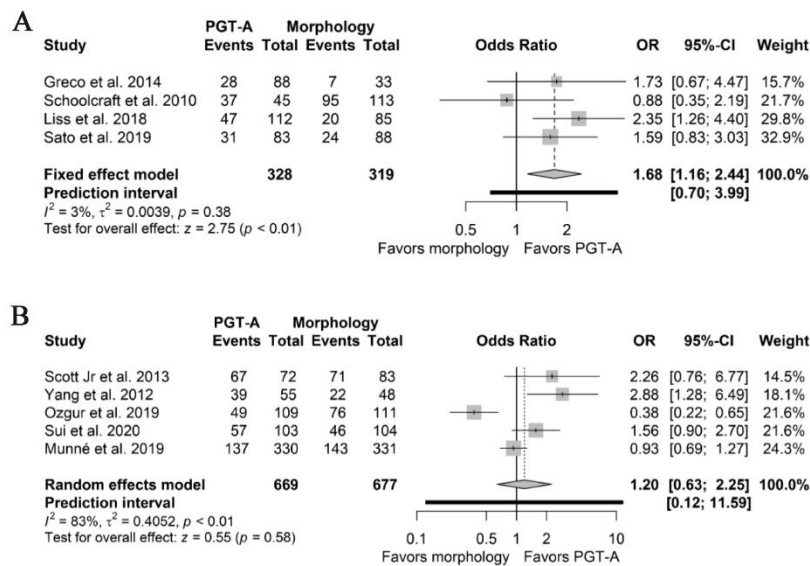
No. of studies	Study design	Certainty assessment					Other considerations	No of patients		Effect		Certainty	Importance
		Risk of bias	Inconsistency	Indirectness	Imprecision			Preimplantation genetic diagnosis	Morphology assessment alone	Relative (95% CI)	Absolute (95% CI)		
Live birth rate per embryo transfer													
5	Randomized trials	Serious ^a	Serious ^b	Not serious	Serious ^c	None	321/603 (53.2%)	315/743 (42.4%)	OR 1.66 (0.98 to 2.83)	13 more per 100 (from 0 fewer to 25 more)	⊕○○○ Very low	Critical	
Live birth rate per embryo transfer													
3	Observational studies	Very serious ^d	Serious ^b	Not serious	Serious ^e	All plausible residual confounding would suggest spurious effect, while no effect was observed	107/231 (46.3%)	247/462 (53.5%)	OR 1.45 (0.24 to 8.78)	9 more per 100 (from 32 fewer to 38 more)	⊕○○○ Very low	Critical	
Clinical pregnancy per embryo transfer													
5	Randomized trials	Serious ^a	Serious ^f	Not serious	Not serious	None	351/658 (53.1%)	361/791 (45.6%)	OR 1.43 (0.96 to 2.13)	9 more per 100 (from 1 fewer to 18 more)	⊕⊕○○ Low	Important	
Clinical pregnancy per embryo transfer													
4	Observational studies	Very serious ^d	Not serious	Not serious	Serious ^c	All plausible residual confounding would suggest spurious effect, while no effect was observed	168/316 (53.2%)	185/503 (36.8%)	OR 3.08 (2.22 to 4.29)	27 more per 100 (from 20 more to 35 more)	⊕⊕○○ low	Important	
Miscarriage rate per embryo transfer													
5	Randomized trials	Serious ^a	Serious ^f	Not serious	Serious ^e	None	40/610 (6.6%)	75/800 (9.4%)	OR 0.57 (0.27 to 1.21)	4 fewer per 100 (from 7 fewer to 2 more)	⊕○○○ very low	Important	
Miscarriage rate per embryo transfer													
2	Observational studies	Very serious ^d	Very serious ^b	Not serious	Very serious ^e	None	9/141 (6.4%)	10/163 (6.1%)	OR 1.25 (0.19 to 8.33)	1 more per 100 (from 5 fewer to 29 more)	⊕○○○ very low	Important	

CI: Confidence interval; OR: Odds ratio

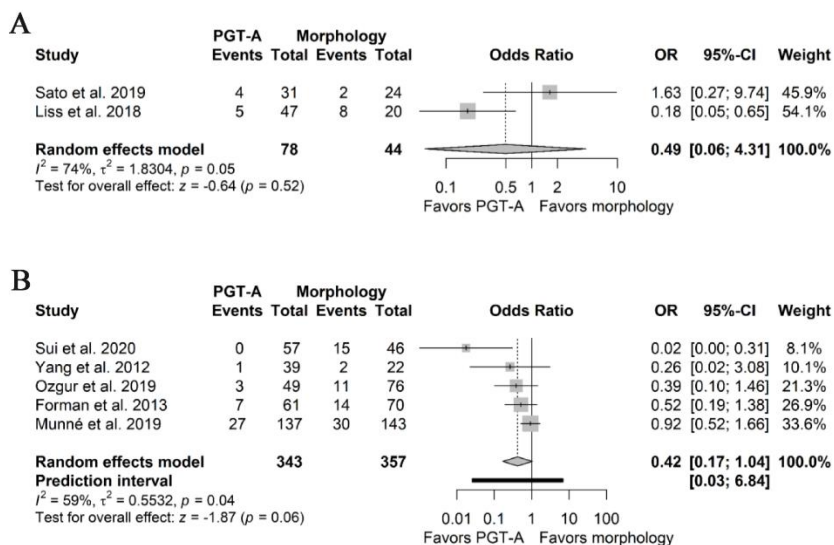
a: Most included studies were between some concerns and high risk of bias; b: There is a high level of heterogeneity and poor overlap of 95% CI; c: There is a small sample of embryo transfer, which makes the estimate imprecise; d: Included studies were all between moderate and critical risk of bias; e: Imprecise 95% CI; f: Poor overlap of 95% CI



Supplementary File 4. Forest plots of live birth rate per cycle. A) Pooled effect derived from NRSIs. B) Pooled effect derived from RCTs. NRSIs: Non-randomized studies of interventions; RCTs: Randomized clinical trials; PGT-A: Preimplantation genetic testing for aneuploidy; OR: Odds ratio; 95%CI: 95% confidence interval



Supplementary File 5. Forest plots of clinical pregnancy per cycle. A) Pooled effect derived from NRSIs. B) Pooled effect derived from RCTs. NRSIs: Non-randomized studies of interventions; RCTs: Randomized clinical trials; PGT-A: Preimplantation genetic testing for aneuploidy; OR: Odds ratio; 95%CI: 95% confidence interval



Supplementary File 6. Forest plots of miscarriage rate per clinical pregnancy. A) Pooled effect derived from NRSIs. B) Pooled effect derived from RCTs.

NRSIs: Non-randomized studies of interventions; RCTs: Randomized clinical trials; PGT-A: Preimplantation genetic testing for aneuploidy; OR: Odds ratio; 95%CI: 95% confidence interval