Systematic Reviews

PROTOCOL

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Double versus single blastocyst biopsy and vitrification in preimplantation genetic testing (PGT) cycles: protocol for a systematic review and meta-analysis of clinical and neonatal outcomes

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Abstract

Background The number of re-biopsied blastocysts is widely increasing in IVF cycles and concerns regarding retesting, which involves double biopsy and vitrification-warming, have been raised. The re-biopsy intervention seems to significantly reduce the pregnancy potential of a blastocyst but the evidence is still restricted to retrospective observational studies reporting a low number of cycles with re-biopsied embryos. Additionally, the neonatal outcomes after the transfer of re-biopsied and re-vitrified embryos are poorly documented to date.

Methods A systematic review will be conducted, using PubMed/Medline, EMBASE, Cochrane Central Register of Controlled Trials, Scopus, Web of Science, and Google Scholar to identify all relevant randomized control trials (RCTs), cohort and case–control studies published until December 2024. The participants will include women undergoing preimplantation genetic testing and single euploid frozen embryo transfer (FET) cycles. The primary outcomes are live birth rate (LBR) and singleton birthweight, whereas secondary outcomes are post-warming embryo survival rate, clinical pregnancy (fetal heart pregnancies at 4.5 weeks), miscarriage rate (loss of pregnancy before the 20th week, and stillbirth), preterm birth (PB) rate, small-for-gestational age (SGA, < -1.28 SDS (standard deviation score)), large-for-gestational age (LGA, $> +1.28$ SDS), low birthweight (LBW; birthweight < 2500 g), preterm birth (gestation < 37 weeks), macrosomia (birthweight > 4000 g), pre-eclampsia, eclampsia, perinatal death, and major congenital malformations. Eligible studies will be selected according to pre-specified inclusion and exclusion criteria. Additionally, manual search will target other unpublished reports and supplementary data. At least two independent reviewers will be responsible for article screening, data extraction and bias assessment of eligible studies. A third reviewer will resolve any disagreements. The Newcastle–Ottawa scale (NOS) will be used to assess the quality of the included studies. Studies that receive a score of 7 or higher on the NOS will be considered to have high methodological quality. The extracted data will be pooled and a meta-analysis will be performed. To carry out the data synthesis, a random effects meta-analysis will be conducted using the RevMan software. Heterogeneity will be evaluated by Cochran's *Q* test and the I^2 statistics and the strength of evidence will be rated with reference to GRADE. The review and meta-analysis will be reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.

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Discussion The findings of this systematic review will be important to clinicians, embryologists, patients, and assisted reproductive service providers regarding the decision-making on retesting embryos for PGT in FET cycles.

Systematic review registration PROSPERO CRD42024498955.

Keywords Genetic testing/methods, PGT, Biopsy/adverse effects, Trophectoderm biopsy, Re-biopsy, Rewarming/adverse effects, Live birth rate, Birthweight, Neonatal outcomes, Systematic review

Background

Preimplantation genetic testing (PGT) can significantly enhance the success rate of assisted reproductive technologies (ART) and prevent the transmission of genetic disorders to offspring by eliminating embryos affected by a single gene mutation or mutations (PGT-M), structural rearrangements of chromosomes (PGT-SR), and aneuploidy (PGT-A) [1, 2]. The genetic analysis requires a trophectoderm biopsy (TE) from the embryo prior to transfer [3] and the current standard for sampling involves a blastocyst biopsy on days 5, 6, or 7 that extracts an average of five cells [4–7]. It was previously reported that TE biopsies containing a large number of cells were associated with a lower live birth rate, suggesting that TE cell number reduction, may affect clinical outcomes [5]. TE biopsy is performed prior to or following vitrification and the safety of double vitrification or even single vitrification remains controversial [8–13].

In FET cycles, the combination of blastocyst biopsy and vitrification involves a single vitrification–warming cycle. A second biopsy can be performed whether the results of the fresh biopsy are inconclusive, and a second vitrification round will be required if a patient with untested vitrified embryos decides to undergo PGT. The main causes of failure of PGT diagnosis are DNA amplification failure, data inconsistency, and non-concurrent results. Under these conditions, clinicians and patients face the dilemma of whether to transfer these "unscreened" embryos or to perform re-biopsy to obtain a PGT result. According to ESHRE PGT Consortium data, the rate of 'no result' embryos is estimated at 11% for PGT-M and 7% for PGT-SR whereas PGT-A fails to yield a diagnostic result in 0.86–3.8% of embryo biopsies [14].

As PGT has evolved in the setting of assisted reproductive technologies, an increasing number of embryos with undetermined results, yet potentially transferable, have emerged. Therefore, concerns regarding rebiopsy and retesting (double biopsy and double vitrification-warming) have been raised [2, 4, 15–19]. Approximately 2–6% of PGT embryos will require a second round of biopsy and vitrification [20], and a portion of these embryos will be transferred based on patient preferences. In this scenario, double biopsy and vitrification have been less investigated compared to single procedures (standard PGT), and no randomized controlled trials have been conducted on blastocyst rebiopsy and revitrification. The first report of blastocyst rebiopsy was published in 2017 [21], and to date, most small-sample observational studies on the association between blastocyst rebiopsy and pregnancy outcomes have reported an increased risk compared to single biopsy [6, 12, 17, 19]. In a study designed to isolate the effect of repeated TE biopsies, by controlling embryo exposure to double vitrification-warming, Sekhon and colleagues observed a 15% decrease in implantation rate in the double TE biopsy group [22]. Similarly, Zhuo and colleagues found that rebiopsied euploid embryos exhibit significantly lower odds of implantation and pregnancy compared to single-biopsied euploid embryos [16]. Since trophectoderm subsequently forms the placenta, it is proposed that multicellular TE biopsy is associated with adverse obstetrical or neonatal outcomes after a single frozen-warmed blastocyst transfer [1, 23–28]. Regarding repeated biopsies, obstetrical and neonatal outcomes have been underreported to date and vary between studies [12, 19, 20, 22, 29, 30]. This lack of evidence creates uncertainty and limits the guidance clinicians can provide to patients considering PGT testing for their previously biopsied embryos [17]. Recent studies have extracted DNA from blastocoel fluid and from the conditioned blastocyst culture medium in order to explore the clinical application of a noninvasive genetic screening [31–34]. However, current published data is not adequate in order to establish its application in clinical practice [35].

As with any assisted reproductive technology, blastocyst rebiopsy continues to evolve in FET cycles as a strategy to increase the number of embryos available for transfer, to optimize reproductive outcomes for the patient, and to limit the risk of transferring single gene disorders to offspring [2, 15]. There is great interest across the board in more evidence that could provide patients and In Vitro Fertilization (IVF) providers with reliable data about the risks of retesting embryos. The present study therefore aims to collect and analyze existing data in order to provide a comprehensive systematic review of IVF and neonatal outcomes from pregnancies conceived after retesting (an extra round of blastocyst biopsy and vitrification) compared to those derived from a single biopsy and vitrification in euploid FET cycles.

Methods/design

Research aim

The objective of this systematic review is to assess and synthesize pieces of evidence on the live birth and perinatal outcomes of singleton euploid blastocysts transferred after undergoing a second round of biopsy and vitrification-warming in comparison to embryos biopsied and vitrified-warmed once.

PICO—research question

How do rebiopsy and revitrification impact IVF and neonatal outcomes of women undergoing euploid FET cycles compared to an embryo biopsied and vitrified-warmed once?

Protocol and registration

The study protocol was registered with PROSPERO (identifier CRD42024498955—https://www.crd.york.ac.uk/PROSPERO/) and has been reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) [36].

Study eligibility criteria

The selection criteria will be described according to Patients, Intervention, Comparison and Outcomes (PICO) statements as previously stated. We will include only Randomized Controlled Trials (RCTs), cohort and case–control studies that compare the clinical outcomes between blastocysts biopsied and vitrified once and blastocyst retesting (biopsied and vitrified twice).

Setting

Single center and multicenter studies conducted in private fertility clinics and university-affiliated infertility practices addressing homologous and heterologous single embryo transfer (SET) cycles.

Participants

Inclusion: All transferred euploid embryos biopsied and vitrified-warmed twice compared to euploid embryos biopsied and vitrified once from women undergoing FET cycles. All embryos undergoing trophectoderm biopsy on day 5, 6, and 7 followed by vitrification and single embryo transfers. Since cleavage-stage embryo biopsy, which involved removing one or more cells (blastomeres), has been replaced by trophectoderm (TE) biopsy at the blastocyst stage in PGT cycles, studies on cleavage-stage biopsied embryos will be excluded from this systematic review. Instead, we will focus on the effect of double trophectoderm biopsy (performed only on blastocyst-stage embryos) on clinical outcomes, as its relevance and applicability are closely linked to the current PGT workflow in IVF clinics.

Exclusion: Blastomere biopsy performed on cleavage-stage embryos (day 3) and embryos cryopreserved by the slow freezing method will not be included.

Intervention

Re-biopsied and re-vitrified blastocysts from patients undergoing single euploid FET cycles.

Comparator

Blastocysts biopsied and vitrified-warmed once from patients undergoing single euploid FET cycles.

Main outcome(s)

The primary outcomes are live birth rate (LBR) and singleton birthweight. Live birth will be assessed as live births per embryo transferred. Birthweight will be assessed at the time of delivery after 37–42 weeks of gestation. Low birthweight was defined as a birthweight of < 2500 g, and macrosomia was defined as a birthweight of > 4000 g.

Secondary outcomes

Embryo survival, clinical pregnancy rate (calculated as fetal heart pregnancies at 4.5 weeks per blastocyst transfers in the selected studies), miscarriage (clinical pregnancies that did not result in live births in the first 20 weeks of pregnancy, including stillbirths), preterm birth (PB), small-for-gestational age (SGA, < -1.28 SDS), large-for-gestational age (LGA, $> +1.28$ SDS), preterm birth (gestation < 37 weeks), pre-eclampsia, eclampsia, perinatal death, and major congenital malformations. To eliminate the confounding factors resulting from multiple pregnancies, we only included single euploid embryo FET cycles if provided in the publications.

Search strategy and literature search

We will search the following electronic bibliographic databases: PubMed (MEDLINE), Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, Google Scholar, and Web of Science (science and social science citation index) according to expert recommendations [37] for biomedical systematic reviews. The search strategy was developed according to P-I-C components of PICO [38]. The search strategy developed for PubMed/MEDLINE is shown in Additional File 1. The search terms were adapted for use with other bibliographic databases. Controlled vocabulary terms, text words and medical subject headers (MeSH) will be searched. Search strategy peer review was performed by the authors through PRESS Checklist. Databases syntax and thesaurus were extensively reviewed as well as proximity operators, truncation, subject headings (function explode/noexp), search fields (ti,ab), limits, and filters.

We also considered alternative spellings for keywords and surveyed the grey literature for non-reported negative studies of other Internet resources, conference proceedings, and contact with experts. A systematic search on OpenGrey, medRxiv, ProQuest, Google, and ClinicalTrials.gov will be performed [39]. For completeness, we will check the reference lists of all eligible studies and review articles to assess additional references. If there are errors or corrections of studies included with a complete text, we will report the date on which they occurred. The searches in databases and grey literature will be re-run immediately prior to analysis to ensure that the most current information is presented in the review. We will not be retrieving or including any unpublished data.

Study screening and selection

Titles and/or abstracts of studies retrieved using the search strategy and those from additional sources will be screened independently by two review authors to identify studies that potentially meet the inclusion criteria outlined above. To make a decision, two members of the review team will perform full-text screenings of these potentially eligible studies independently. Any disagreements between them over the eligibility of particular studies will be resolved through discussion with a third reviewer.

Data extraction

Before starting data extraction, we will pilot the process to ensure reliability in the interpretation and use of the inclusion criteria. Two unblinded review authors will extract data independently, discrepancies will be identified and resolved through discussion with a third author when is necessary. Upon completion of the data extraction template, the reviewers will extract the data and reasons for exclusion will be listed. Data extracted will include demographic information, methodology, intervention details, and all reported patient-important outcomes. More detailed information will be extracted such as: last name of the first author; year of publication; study setting; study population and participant baseline characteristics; type of control used; study design; statistical methods implemented and main results (e.g., odds ratios), relative risks; information for the assessment of the risk of bias. Categorical data will be extracted as a frequency from the number of events observed at the endpoint (*n*, *N*, and CI) whereas continuous data will be assessed as mean ± SD or median, IQ.

Risk of bias assessment

The Newcastle–Ottawa scale (NOS) will be used to assess the quality of the included articles. Attributing one point to each answer marked with an asterisk below scores the NOS quality instrument. Possible total points are 4 points for Selection, 2 points for Comparability, and 3 points for Outcomes. Studies that receive a score of 7 or above on the NOS will be considered high quality [40, 41]. The Cochrane Risk of Bias 2 (RoB 2) tool will be used to assess quality of potential RCTs. Whenever possible, grey literature will be evaluated using the same standards as traditional studies. As part of our critical appraisal approach, we will apply the AACODS checklist for the domains authority, precision, coverage, objectivity, date, and significance, where relevant [42, 43]. Two authors will check quality assessment independently, and any disagreements solved by a third reviewer until a consensus is reached.

Data synthesis

We will provide a narrative synthesis of the findings from the included studies, structured around the type of intervention, baseline characteristics, type of outcome and intervention content. Where studies have used the same type of intervention and comparator, with the same outcome measure, a meta-analysis will be performed [44–46]. Where most of the studies are retrospective cohort studies, dichotomous outcomes will be pooled to determine the odds ratio (OR) or risk ratio (RR) with 95% confidence intervals (CIs). Data from the continuous outcomes will be pooled using the mean difference (MD) will be calculated between the groups to determine the effect size [44]. The I^2 statistic will be used to quantify heterogeneity. A random-effects model will then be used to pool the estimates in a forest plot [46]. Where information is missing to calculate a common effect metric, additional information will be requested by contacting the authors. The funnel plot will be used to assess potential publication bias following the Cochrane recommendations on testing for funnel plot asymmetry. Sensitivity analysis will be performed for the outcomes with funnel plot asymmetry to assess the leverage of the studies on the results [44]. Potential heterogeneity sources will be examined through subgroup analysis [47]. When sample size permits, data will be grouped by maternal age or embryo development stage at rebiopsy (day 5/6 embryos). The sources of heterogeneity will be explored and appropriated quantified to avoid compromising interpretability of the results of the meta-analysis. The strength of evidence will be rated with reference to GRADE. The Review Manager (RevMan Version 7.2.0. Software, available at https://revman.cochrane.org) will be used for statistical analysis.

Data management

Search results from bibliometric databases were imported to the web-based software Covidence (https://www.covidence.org/) and de-duplicated. Results from grey literature searching will be into Sciwheel (Sciwheel, Reference Manager and Generator, Harvard, APA) and de-duplicated. All results from grey literature and the second round of databases search will be then imported into the Covidence for title/abstract screening, full-text screening as well as data extraction [48]. All data extracted will be exported to RevMan (ReviewManager, Cochrane) for quantitative analysis.

Reporting

To allow for transparency and reproducibility of the findings, the methods and results of this systematic review and meta-analysis will be reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [49–52].

Timeline for systematic review

Data extraction started in September 2024 and will be completed by December 2024. A draft manuscript will be completed by January 2025.

Discussion

Potential re-biopsy-related damage to the blastocyst and the impact on live birth and neonatal outcomes are still debatable. It is paramount to evaluate whether blastocyst retesting (double biopsy and vitrification) poses additional IVF, obstetric and/or neonatal risks compared with euploid embryos undergoing a single biopsy and vitrification [1, 2, 6, 12, 17, 18, 53]. Therefore, this systematic review and meta-analysis will assess and analyze the current clinical outcomes of blastocyst re-biopsy compared with single biopsy and vitrification in single euploid FET cycles. This study can contribute to clinicians' decision-making and assist providers in supporting patients by thoroughly weighing the risks and benefits of embryo re-biopsy. The strengths and limitations of the evidence will be considered, and findings will be discussed in context with related studies. The results of this SR will summarize the existing evidence of the impact of embryo retesting on clinical outcomes and help to identify gaps in knowledge where further research is required. It is also expected that the findings will be useful for the development of additional guidelines on PGT practice.

Protocol amendments

Any amendment that is made to the protocol whilst conducting the systematic review will be detailed clearly in the published article and will be updated on PROSPERO.

Abbreviations

IVF	In vitro fertilization
FET	Frozen transfer cycles
PGT	Preimplantation genetic testing
PGT-A	Preimplantation genetic testing for aneuploidy
PGT-M	Preimplantation genetic testing for monogenic/single genes defects
PGT-SR	Preimplantation genetic testing for structural rearrangements

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13643-025-02846-8.

Supplementary Material 1.

Acknowledgements

Not applicable.

Authors' contributions

All authors were involved in the conception and design of the protocol. AV developed the search strategy and drafted the protocol. VS, TB and MB critically revised the protocol. JK conceived the study, critically revised the manuscript and gave final approval for the manuscript to be published, and agree to be accountable and guarantor for all aspects of the review. All authors read, provided feedback, and approved the final manuscript.

Data availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors report no financial or commercial conflicts of interest.

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References

1. Mao D, Xu J, Sun L. Impact of trophectoderm biopsy for preimplantation genetic testing on obstetric and neonatal outcomes: a meta-analysis. Am J Obstet Gynecol. 2024;230:199-212.e5.
2. Verpoest W. Refreezing and rebiopsy – are these worth it? Hum Reprod. 2023;38(1) https://doi.org/10.1093/humrep/dead093.061.
3. Kokkali G, Traeger-Synodinos J, Vrettou C, Stavrou D, Jones GM, Cram DS, Makrakis E, Trounson AO, Kanavakis E, Pantos K. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of beta-thalassaemia: a pilot study. Hum Reprod. 2007;22:1443–9.
4. Guzman L, Nuñez D, López R, et al. The number of biopsied trophectoderm cells may affect pregnancy outcomes. J Assist Reprod Genet. 2019;36:145–51.
5. Neal SA, Franasiak JM, Forman EJ, Werner MD, Morin SJ, Tao X, Treff NR, Scott RT Jr. High relative deoxyribonucleic acid content of trophectoderm biopsy adversely affects pregnancy outcomes. Fertil Steril. 2017;107 Suppl 3:731-736.e1.
6. Aluko A, Vaughan DA, Modest AM, Penzias AS, Hacker MR, Thornton K, Sakkas D. Multiple cryopreservation-warming cycles, coupled with

blastocyst biopsy, negatively affect IVF outcomes. Reprod Biomed Online. 2021;42:572–8.
7. Zhang S, Luo K, Cheng D, et al. Number of biopsied trophectoderm cells is likely to affect the implantation potential of blastocysts with poor trophectoderm quality. Fertil Steril. 2016;105:1222-1227.e4.
8. Li X, Li W, Jia H, Gao Y, Shi W, Bai H. Double vitrification-warming cycles, coupled with blastocyst biopsy, impair live birth but do not affect neonatal outcomes. Int J Gynaecol Obstet. 2023;160:806–13.
9. Makieva S, Sachs MK, Xie M, Velasco A, El-Hadad S, Kalaitzopoulos DR, Dedes I, Stiller R, Leeners B. Double vitrification and warming does not compromise the chance of live birth after single unbiopsied blastocyst transfer. Hum Reprod Open. 2023;Suppl 4:hoad037.
10. Shen X, Ding M, Yan Y, Huang C, Wang S, Zhou J, Xing J. Correction: Perinatal outcomes of singletons following double vitrification-warming procedures: a retrospective study using propensity score analysis. BMC Pregnancy Childbirth. 2023;23(1):108. https://doi.org/10.1186/s12884-023-05413-y. Erratum for: BMC Pregnancy Childbirth. 2023;23(1):30. https://doi.org/10.1186/s12884-023-05369-z.
11. Wang X, Mao R, Wang M, Long R, Jin L, Zhu L. The effect of recryopreservation on embryo viability and outcomes of in vitro fertilization: a systematic review and meta-analysis. Fertil Steril. 2023;120:321–32.
12. De Vos A, Van Landuyt L, De Rycke M, Verdyck P, Verheyen G, Buysse A, Belva F, Keymolen K, Tournaye H, Verpoest W. Multiple vitrification-warming and biopsy procedures on human embryos: clinical outcome and neonatal follow-up of children. Hum Reprod. 2020;35:2488–96.
13. Kwan HCK. Reconsideration of the safety and effectiveness of human oocyte cryopreservation. Reprod Biol Endocrinol. 2023;21:22.
14. Spinella F, Bronet F, Carvalho F, Coonen E, De Rycke M, Rubio C, Goossens V, Van Montfoort A. ESHRE PGT consortium data collection XXI: PGT analyses in 2018. Hum Reprod Open. 2023;Suppl 2:hoad010.
15. Nohales M, Coello A, Martin A, Insua F, Meseguer M, de Los Santos MJ. Should embryo rebiopsy be considered a regular strategy to increase the number of embryos available for transfer? J Assist Reprod Genet. 2023;40:1905–13.
16. Zhuo R, Estevez SL, Ghofranian A, Hernandez-Nieto C, Baird M, Gounko D, Lee J, Copperman AB, Danis RB. Comparison of pregnancy outcomes between single-biopsied and rebiopsied euploid embryos. Fertil Steril. 2023;120: e51.
17. Al Hashimi B, Linara-Demakakou E, Harvey SC, Harvey KE, Griffin DK, Ahuja K, Macklon NS. Double vitrification and warming of blastocysts does not affect pregnancy, miscarriage or live birth rates. Reprod Biomed Online. 2024;49: 104103.
18. Theodorou E, Chronopoulou E, Ozturk O, Brunetti X, Serhal P, Ben-Nagi J. Impact of double trophectoderm biopsy on reproductive outcomes following single euploid blastocyst transfer. Eur J Obstet Gynecol Reprod Biol. 2024;298:35–40.
19. Bradley CK, Livingstone M, Traversa MV, McArthur SJ. Impact of multiple blastocyst biopsy and vitrification-warming procedures on pregnancy outcomes. Fertil Steril. 2017;108:999–1006.
20. Cimadomo D, Rienzi L, Romanelli V, Alviggi E, Levi-Setti PE, Albani E, Dusi L, Papini L, Livi C, Benini F, Smeraldi A, Patassini C, Ubaldi FM, Capalbo A. Inconclusive chromosomal assessment after blastocyst biopsy: prevalence, causative factors and outcomes after re-biopsy and re-vitrification. A multicenter experience. Hum Reprod. 2018;33:1839–46.
21. Bhadarka H, Patel NH, Jadeja YD, Patel KB, Patel MN. Int J Infertility and Fetal Medicine. 2017;8:120–4.
22. Sekhon L, MacAvey B, Lee J, Duke M, Flisser E, Copperman AB. Evaluating IVF and perinatal outcomes following repeat trophectoderm biopsy. Fertil Steril. 2018;110:E77–8.
23. Ji H, Zhang MQ, Zhou Q, Zhang S, Dong L, Li XL, Zhao C, Ding H, Ling XF. Trophectoderm biopsy is associated with adverse obstetric outcomes rather than neonatal outcomes. BMC Pregnancy Childbirth. 2023;23:141.
24. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, Sun X, Ling X, Li H, Hao C, Tan J, Yang J, Zhu Y, Liu F, Chen D, Wei D, Lu J, Ni T, Zhou W, Wu K, Gao Y, Shi Y, Lu Y, Zhang T, Wu W, Ma X, Ma H, Fu J, Zhang J, Meng Q, Zhang H, Legro RS, Chen ZJ. Live birth with or without preimplantation genetic testing for aneuploidy. N Engl J Med. 2021;385(22):2047–58.
25. Van Heertum K, DeVilbiss EA, Goldfarb J, Mumford SL, Weinerman R. Does embryo biopsy, independent of vitrification, impact perinatal outcomes? An analysis of perinatal outcomes following preimplantation genetic testing biopsy in fresh and frozen embryo transfer cycles. Fertil Steril. 2024;5:47–54.
26. Hao Y, Long X, Kong F, Chen L, Chi H, Zhu X, Kuo Y, Zhu Y, Jia J, Yan L, Li R, Liu P, Wang Y, Qiao J. Maternal and neonatal outcomes following blastocyst biopsy for PGT in single vitrified-warmed embryo transfer cycles. Reprod Biomed Online. 2022;44:151–62.
27. Sites S. The impact of embryo biopsy on obstetric and neonatal outcomes. Human Reprod. 2023;38:dead093 .060.
28. Elias FTS, Weber-Adrian D, Pudwell J, Carter J, Walker M, Gaudet L, Smith G, Velez MP. Neonatal outcomes in singleton pregnancies conceived by fresh or frozen embryo transfer compared to spontaneous conceptions: a systematic review and meta-analysis. Arch Gynecol Obstet. 2020;302:31–45.
29. Neal SA, Morin SJ, Tiegs AW, Hong KH, Werner MD, Scott RT. Repeat biopsy for preimplantation genetic screening (PGS) reanalysis does not adversely impact obstetrical outcomes. Fertil Steril. 2018;109: e41.
30. Kim JG, Jalas C, Zhan Y, Hanson BM, Herlihy NS, Klimczak AM, Margolis CK, Roberts LM, Hong K, Seli E, Scott RT. Neonatal outcomes are not impacted by a second trophectoderm biopsy. Fertil Steril. 2021;116: e288.
31. Navarro-Sánchez L, García-Pascual C, Rubio C, Simón C. Non-invasive preimplantation genetic testing for aneuploidies: an update. Reprod Biomed Online. 2022;44:817–28.
32. Simon C. Noninvasive preimplantation genetic testing for aneuploidy in spent blastocyst media will substitute for trophectoderm biopsy. Fertil Steril. 2021;115:840.
33. Kuznyetsov V, Madjunkova S, Antes R, Abramov R, Motamedi G, Ibarrientos Z, et al. Evaluation of a novel non-invasive preimplantation genetic screening approach. PLoS One. 2018;13: e0197262.
34. Kuznyetsov V, Madjunkova S, Abramov R. Minimally invasive cell-free human embryo aneuploidy testing (miPGT-A) utilizing combined spent embryo culture medium and blastocoel fluid - towards development of a clinical assay. Sci Rep. 2020;10:7244.
35. Cinnioglu C, Glessner H, Jordan A, Bunshaft S. A systematic review of noninvasive preimplantation genetic testing for aneuploidy. Fertil Steril. 2023;120:235–9.
36. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4:1.
37. Bramer WM, Rethlefsen ML, Kleijnen J, Franco OH. Optimal database combinations for literature searches in systematic reviews: a prospective exploratory study. Syst Rev. 2017;6:245.
38. Frandsen TF, Bruun Nielsen MF, Lindhardt CL, Eriksen MB. Using the full PICO model as a search tool for systematic reviews resulted in lower recall for some PICO elements. J Clin Epidemiol. 2020;127:69–75.
39. Godin K, Stapleton J, Kirkpatrick SI, Hanning RM, Leatherdale ST. Applying systematic review search methods to the grey literature: a case study examining guidelines for school-based breakfast programs in Canada. Syst Rev. 2015;4:138.
40. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses. 2012. Available from: http://www.ohrica/programs/clinical_epidemiology/oxfordasp.
41. Zhang Y, Huang L, Wang D, Ren P, Hong Q, Kang D. The ROBINS-I and the NOS had similar reliability but differed in applicability: a random sampling observational studies of systematic reviews/meta-analysis. J Evid Based Med. 2021;14:112–22.
42. Tyndall J. How low can you go? Toward a hierarchy of grey literature. In: Australian Library and Information Association Biennial Conference. Alice Springs; 2008.
43. Tyndall J. AACODS checklist for appraising grey literature. Adelaide: Flinders University; 2010.
44. Higgins JP, Whitehead A, Turner RM, Omar RZ, Thompson SG. Meta-analysis of continuous outcome data from individual patients. Stat Med. 2001;20:2219–41.
45. Lensen S. When to pool data in a meta-analysis (and when not to)? Fertil Steril. 2023;119:902–3.
46. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Synth Methods 2010;1:97–111.

47. Sun X, Ioannidis JP, Agoritsas T, Agoritsas T, Alba AC, Guyatt G. How to use a subgroup analysis: users' guide to the medical literature. JAMA. 2014;311:405–11.
48. Harrison H, Griffin SJ, Kuhn I, Usher-Smith JA. Software tools to support title and abstract screening for systematic reviews in healthcare: an evaluation. BMC Med Res Methodol. 2020;20:7.
49. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and MetaAnalyses: The PRISMA Statement. PLoS Med. 2009;6(6):e1000097. https://doi.org/10.1371/journal.pmed1000097.
50. Moher D, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4: 1.
51. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015;350:g7647 Erratum in: BMJ. 2016 Jul 21;354:i4086.
52. 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.
53. Guarneri C, Reschini M, Pinna M, Perego L, Sanzani E, Somigliana E, Sorrentino U, Cassina M, Zuccarello D, Ciaffaglione M. The impact of a second embryo biopsy for preimplantation genetic testing for monogenic diseases (PGT-M) with inconclusive results on pregnancy potential: results from a matched case-control study. J Assist Reprod Genet. 2024;41:1173–9.

Publisher's Note

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