Reproductive Biology and Endocrinology

REVIEW

Open Access

The diagnostic accuracy of preimplantation genetic testing (PGT) in assessing the genetic status of embryos: a systematic review and meta-analysis

Kexin Chen[1,2†], Zhixin Hu[1,2†], Yuxuan Lian[1,2†], Youzhen Han[1,2†], Xiaoting Zhou[1,2], Yonggang Li[1,2], Lifeng Xiang[1,2], Weiqun Jiang[1,2], Mingying Li[1,2], Peng Zeng[1,2], Manqin Zhang[1,2], Xi Luo[1,2], Yongfang Xu[1,2], Haishan Zheng[1,2], Mei Tian[1,2], Mei Wang[1,2], Rui Ma[1,2], Jichun Yang[1,2], Yun Bai[1,2], Ruiyu Du[1,2], Bo Deng[1,2], Ze Wu[1,2*], Yunxiu Li[1,2*] and Jiacong Yan[1,2*]

Abstract

Background Preimplantation genetic testing (PGT) is widely used in assisted reproduction to assess the genetic status of embryos. However, increasing evidence suggests that the trophectoderm (TE) may not fully reflect the genetic status of the inner cell mass (ICM), raising controversy about the accuracy of TE biopsy. Research in recent years has focused on cell-free DNA (cfDNA) found in blastocoel fluid (BF) and spent culture medium (SCM), as these may contain genetic information from both the TE and ICM. Therefore, further research and validation are essential to determine the reliability and clinical applicability of these diagnostic methods in PGT.

Methods Relevant studies published between January 2000 and August 2024 were identified through PubMed and Web of Science (WOS). Risk assessment and publication bias were evaluated using QUADAS-2 and Deek's test. Diagnostic meta-analysis was performed using a bivariate model to combine sensitivity and specificity, with results visualized through forest plots and summary receiver operating characteristic (SROC) curves.

Results Out of 6,407 initially screened records, 36 studies involving 4,230 embryos were included. TE biopsy was identified as the best method for diagnosing the genetic status of embryos (sensitivity: 0.839; specificity: 0.791, AUC: 0.878), while SCM had slightly lower accuracy (sensitivity: 0.874; specificity: 0.719, AUC: 0.869). The effectiveness of BF (AUC: 0.656) was significantly lower than that of TE biopsy and SCM. Despite this, TE biopsy has not yet achieved

†Kexin Chen, Zhixin Hu, Yuxuan Lian and Youzhen Han contributed equally to this work.

*Correspondence:
Ze Wu
wuzes2010@163.com
Yunxiu Li
lyrarshow@qq.com
Jiacong Yan
yan_jiacong@kust.edu.cn

Full list of author information is available at the end of the article

ideal diagnostic performance. However, TE biopsies demonstrate a high level of accuracy in diagnosing PGT-SR (AUC: 0.957). Additionally, multiple TE biopsies (AUC: 0.966) or TE biopsies combined with SCM (AUC: 0.927) can enhance the diagnostic efficiency of PGT.

Conclusion The findings of this study suggest that TE biopsy has yet to achieve optimal diagnostic accuracy, which may result in a significant number of missed embryo diagnoses and misdiagnoses. Our results confirm that SCM has the potential to serve as a supplementary test. Employing multiple biopsies or combining TE with SCM may enhance diagnostic efficiency and yield optimal results.

Keywords Trophectoderm (TE) biopsy, Spent culture medium (SCM), Blastocoel fluid (BF), Whole blastocyst (WB), Inner cell mass (ICM), Diagnostic accuracy

Introduction

Pre-implantation genetic testing (PGT) assesses the overall genetic status of an embryo through the biopsy of 3 to 10 trophectoderm (TE) cells [1–3]. In the absence of mosaicism, the diagnostic accuracy of TE-biopsy and whole blastocyst/inner cell mass (WB/ICM) analysis exceeds 95% [4–6]. Additionally, numerous studies have reported a high degree of consistency between the results of TE-biopsy and prenatal diagnosis [7, 8]. However, numerous studies have shown that mosaic embryos are prevalent among preimplantation embryos, with an incidence ranging from 2 to 40% [9–11]. Consequently, the results of TE-biopsy may not always align with those of WB/ICM analysis. In 2015, a study reported that embryos diagnosed as mosaic by TE-biopsy were successfully implanted, resulting in healthy live births [12]. A subsequent large-scale retrospective study confirmed these findings [13]. Additionally, TE-biopsy exhibits variability in detecting abnormal fragments, with 40% of these fragments being euploid upon re-biopsy [14]. These studies indicate that TE-biopsy does not fully represent the overall genetic status of the embryo, and there remains a degree of controversy regarding its accuracy.

Numerous clinical trials have reported significant improvements in patient outcomes following the implementation of PGT [15–18]. However, inconsistencies between TE-biopsy and the gold standard (WB/ICM) persist. Research by Gleicher et al. suggests that, assuming mosaicism is evenly distributed, at least 27 cells must be biopsied to achieve a comprehensive assessment of the embryo's genetic status [19]. Currently, the number of TE biopsied cells ranges from 3 to 10, leaving the extent of their representation of the embryo's full genetic status unclear.

Since then, cell-free DNA (cfDNA) derived from blastocyst fluid (BF) has been considered as potentially containing genetic information from both TE and ICM, which may better represent the embryo's overall genetic profile [20]. However, multiple studies have demonstrated that the diagnostic efficiency of BF-based minimally invasive Preimplantation Genetic Testing (mi-PGT) is extremely low, rendering it unsuitable for clinical use [21, 22]. In contrast, SCM not only includes genetic information from both TE and ICM but also has a higher DNA concentration compared to BF, leading to significantly improved diagnostic accuracy [21]. Despite this, the current diagnostic efficiency of SCM remains below clinically acceptable standards due to potential maternal contamination and significant variability in results [23–25]. Therefore, the application value of SCM remains controversial.

Although PGT was introduced in 1990 and SCM-based ni-PGT in 2016, there remains a lack of evidence-based validation for the effectiveness of TE-biopsy and SCM in assessing embryonic genetic status [26, 27]. Further research is required to comprehensively evaluate the diagnostic performance of both TE-biopsy and SCM in assessing embryonic genetic status. The primary aim of this systematic review and meta-analysis was to evaluate the accuracy of TE-biopsy and SCM in diagnosing the overall genetic status of embryos (Fig. 1). Additionally, the secondary aim was to identify the most effective protocol for accurately assessing the overall genetic status of embryos.

Methods

Eligibility criteria

This study conducted a systematic quantitative and qualitative analysis based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [28]. The study protocol underwent assessment and was registered with PROSPERO (registration number: CRD42022310230).

Search strategy

From January 2000 to August 2024. PubMed, Web of Science were searched. The search process was carried out by two researchers (C.K. and H.Z.) independently to ensure accuracy and reliability. The retrieval process is mainly through the following medical subject heading (Mesh) terms and/or keywords: ((blastocoel fluid) AND ((trophectoderm) OR (whole blastocyst) OR (inner cell mass) OR (residual blastocyst) OR (remaining blastocyst) OR (whole embryo))); ((trophectoderm) AND ((whole

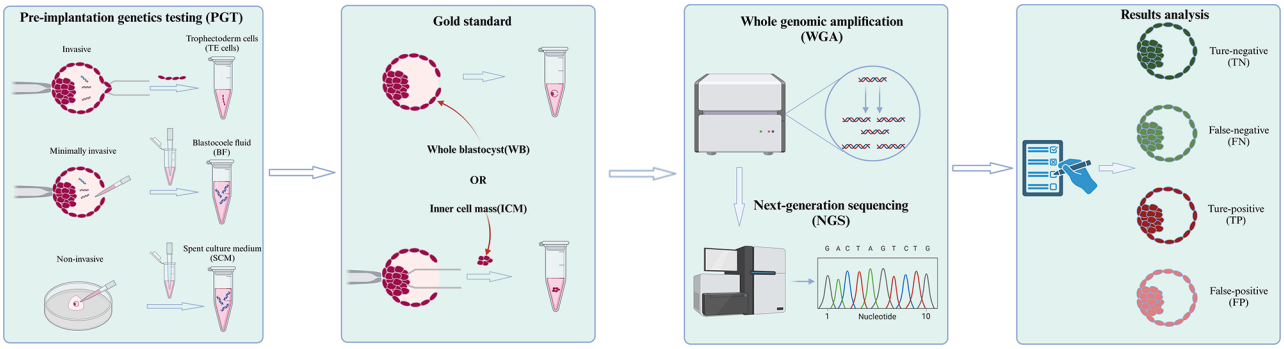

Fig. 1 Preimplantation genetic testing (PGT) workflow

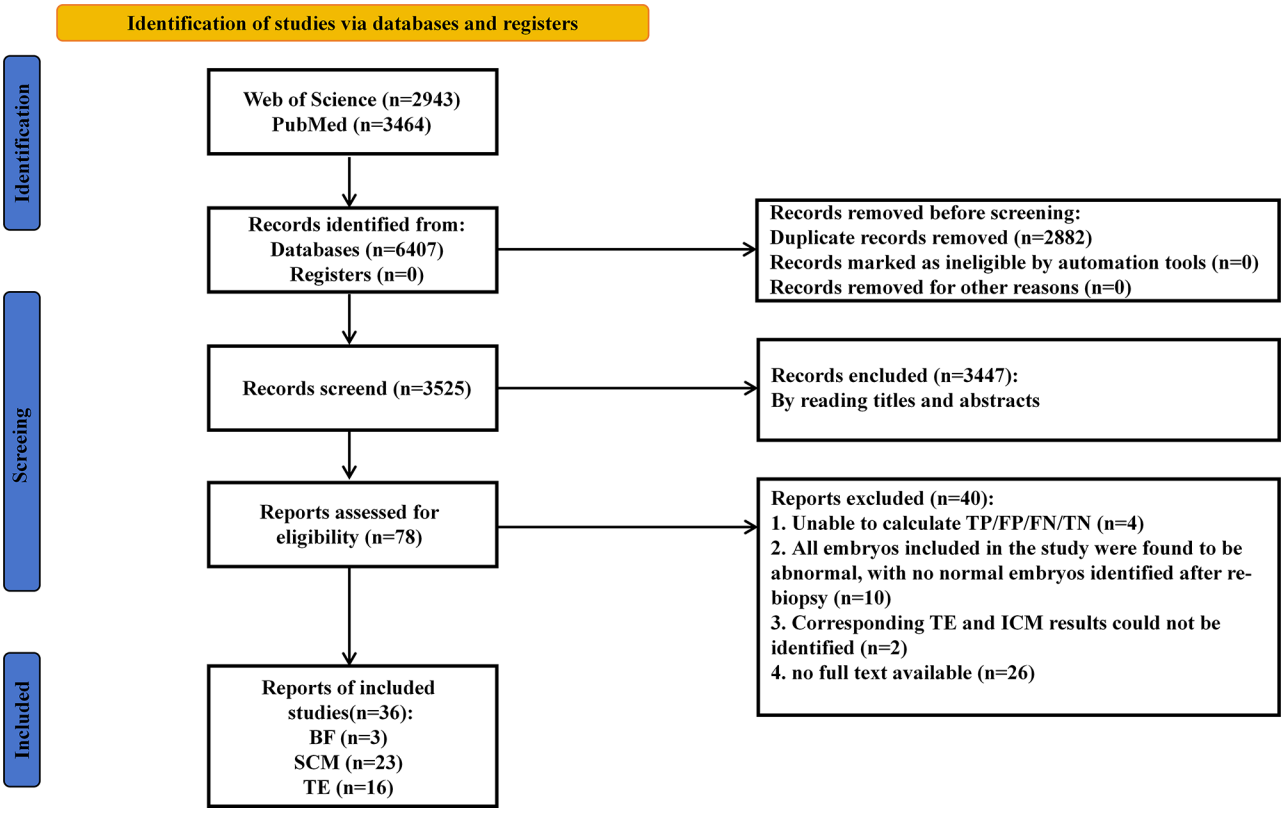

Fig. 2 Preferred reporting items for systematic review and meta-analyses (PRISMA) flow diagram of the systematic search and study selection process

blastocyst) OR (inner cell mass) OR (residual blastocyst) OR (remaining blastocyst) OR (whole embryo))); ((preimplantation genetic testing) AND ((whole blastocyst) OR (inner cell mass) OR (residual blastocyst) OR (remaining blastocyst) OR (whole embryo))); ((non-invasive preimplantation genetic testing) AND ((whole blastocyst) OR (inner cell mass) OR (residual blastocyst) OR (remaining blastocyst) OR (whole embryo))); ((spent culture medium) AND ((whole blastocyst) OR (inner cell mass) OR (residual blastocyst) OR (remaining blastocyst) OR (whole embryo))); ((blastocyst culture medium) AND ((whole blastocyst) OR (inner cell mass) OR (residual blastocyst) OR (remaining blastocyst) OR (whole embryo))); (((non-invasive preimplantation genetic testing) OR (spent culture medium) OR (blastocyst culture medium)) AND ((preimplantation genetic testing) OR (trophectoderm))). The process of literature retrieval and inclusion is shown in Fig. 2. After duplicates removal, a total 3525 studies are included, after preliminary of screen, 78 studies were conducted for full text reading. After retrieval and exclusion, 36 studies were included in this study for main result analysis and subgroup analysis to provide a basis for clinical application [3, 5, 6, 21–23, 25, 27, 29–56]. The process of retrieval, only English documents were searched.

Study selection

The inclusion and exclusion criteria of the literatures are formulated by reading and judge the relevance. In the process of literature screening, the literature is filtrated independently by four reviewers (C.K., H.Z., L.Yu., and H.Y.), and the controversial articles are judged by the fifth reviewer (Y.Jia.). The initial step involves two reviewers conducting a preliminary screening. The reviewers then re-screens the title and abstract, scrutinizing them carefully. Finally, the reviewers read the full text to identify the literature that meets the inclusion criteria.

The inclusion criteria for this study are as follows: (i) Trophectoderm and WB/ICM from the same blastocyst were analyzed. (ii) Trophectoderm, SCM, and WB/ICM from the same blastocyst were collected for analysis. (iii) Trophectoderm, BF, and WB/ICM from the same blastocyst were collected for analysis. (iv) The trophectoderm and SCM from the same blastocyst were analyzed. Studies will be included in the analysis if they satisfy at least one of the inclusion criteria. The ICM develops into the fetus, and some studies use ICM as the gold standard for evaluation. Other studies use WB, which include the ICM, as the gold standard. Consequently, this study adopts WB/ICM as the gold standard. Using WB/ICM as the gold standard, the studies included in the analysis were categorized and assessed to evaluate the diagnostic efficacy of TE-biopsy, SCM, and BF. Additionally, TE-biopsy served as a control to determine the diagnostic efficacy of SCM.

Information extraction

The extraction of 36 included literature information was completed by four reviewers (C.K., H.Z., L.Yu. and H.Y.). The specific information is shown in Table 1, which mainly includes author information, magazine type, ART, number of embryos, DNA amplification system and sequencing platform, number of biopsy cells and so on.

Risk of bias and applicability assessment

This study is a diagnostic meta-analysis, employing Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) for risk assessment. The QUADAS tool, which is currently the most recommended for evaluating diagnostic accuracy, is endorsed by the Cochrane collaboration [57]. It assesses various components, including case selection, trials, gold standards, case flow, and progress, with regard to bias risk. The quality assessment of the literature was done independently by four reviewers (C.K., H.Z., L.Yu. and H.Y.). Ambiguous documents are resolved by a fifth reviewer (Y.Jia.).

Data extraction

In accordance with the inclusion criteria, data were extracted from the included studies, encompassing: (i) name of the first author; (ii) year of publication; (iii) gold standard; (iv) patient population; (v) number of true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN). Missing or unclear data prompted attempts to contact the corresponding author via email for clarification. Data extraction was conducted independently by four researchers and reviewed by all authors to identify any potential errors.

Statistical analysis

Data statistics were computed from the TP, FP, FN, and TN reported in the included studies to derive sensitivity, specificity, and area under the curve (AUC) values. When studies included TE, SCM, and gold standard assessments simultaneously, they were grouped separately for meta-analysis. This study employed a bivariate model to estimate the combined sensitivity and specificity along with their 95% confidence intervals. This approach utilizes random effects (RE) to directly model the heterogeneity and correlation of sensitivity and specificity across studies (Reitsma et al. 2005). The bivariate model also calculated additional diagnostic indicators, such as the diagnostic odds ratio (DOR) and likelihood ratio (LR). The DOR reflects how much more likely a positive test result is compared to a negative result. The LR measures the ratio of the probability of a test result in patients to the probability of the same result in non-patients, and is categorized into the positive likelihood ratio (LR+) and the negative likelihood ratio (LR−). A higher LR + value indicates greater accuracy of the positive result, while a lower LR − value signifies greater accuracy of the negative result. To illustrate between-study heterogeneity, we constructed forest plots and AUC curves with 95% confidence intervals for the bivariate model meta-analysis. Since meta-analysis of diagnostic test accuracy require bivariate data in terms of sensitivity and specificity, we avoided using statistical methods typically applied in systematic reviews of interventions, such as Cochran's Q and I^2 statistics. Subgroup analyses were conducted using bivariate models to explore the impact of: (i) patient age; (ii) trophectoderm quality; (iii) patient population characteristics; and (iv) SCM source. Publication bias was assessed using Deeks' funnel plot, with the P value from the statistical test indicating whether publication bias was significant [58]. A P value of < 0.05 was considered significant; otherwise, it was deemed not significant. All analyses were performed using Review Manager (RevMan) 5.4, Stata 16.0, R version 4.4.1, Rstudio version 1.4.555, and the mvmeta, ggplot2, mada, metafor, and matedat packages (C.K., H.Z., L.Yu. and H.Y.). The Fig. 1 was created with BioRender.com (https://www.biorender.com/), and it is assigned the agreement number CS27TZCUD2.

Table 1 Characteristics of included studies

First author, year of publication	Participants/*N*	Sample/*N*	Diagnostic sample					Country	Age	Sequencing methods	Type of PGT	Embryo quality	Whether apply AH	Frozen/ Fresh embryo
			Sample type	TP	FP	FN	TN							
Sialakouma et al.,2021	13	40	TE-SCM	16	1	5	11	Greece	35.3 ± 4.2	NGS	PGT-A	≥BB	YES	Frozen
Shitara et al.,2021	12	20	WB-TE	7	1	2	6	Japan	35.6 ± 3.2	NGS	PGT-A	≥ 3BB	NO	Frozen
			WB-SCM	7	0	1	8							
			TE-SCM	7	1	1	8							
Gui et al.,2016	13	51	ICM-TE	25	0	1	11	China	/	NGS	PGT-SR	/	NO	Frozen
Lledo et al.,2021	29	184	TE-SCM	79	23	12	43	Spain	41.3 ± 3.4	NGS	PGT-A	≥BB	YES	Fresh
Sun et al.,2023	58	278	TE-SCM	112	57	8	65	China	/	NGS	PGT-A	/	YES	Fresh
	43	214		112	36	6	38				PGT-SR			
Hanson et al.,2021	35	166	TE-SCM	68	43	13	21	USA	38.5 ± 1.2	NGS	PGT-A	≥ 4CC	YES	Fresh
Lei et al.,2022	67	113	TE-SCM	56	11	9	35	China	33.83 ± 5.03	NGS	PGT	/	YES	Fresh
Rubio et al.,2020	371	1301	TE-SCM	410	137	105	456	Spain	36.4 ± 5.2	NGS	PGT-A	≥CC	NO	Fresh
Xu et al.,2023	23	35	WB-TE	8	2	3	16	China	34.1 ± 5.4	NGS	PGT-A	> CC	NO	Frozen
			ICM-TE	8	4	5	15							
			WB-SCM	6	8	0	7							
			ICM-SCM	9	7	1	7							
			TE-SCM	8	11	0	7							
First author, year of publication	Participants/N	Sample/N	Diagnostic sample					Country	Age	Sequencing methods	Type of PGT	Embryo quality	Whether apply AH	Frozen/ Fresh embryo
			Sample type	TP	FP	FN	TN							
Griffin et al.,2022	144	174	ICM-TE	64	27	11	72	USA	32 ± 4.2	NGS	PGT-A	/	/	Fresh
Chavli et al.,2022	/	46	ICM-TE	20	4	10	12	Netherlands	/	NGS	PGT-A	/	/	Frozen
Zhao et al.,2022	/	85	TE-SCM	17	14	4	50	China	/	NGS	PGT-A	> CC	YES	Fresh
Takahashi et al.,2021	11	29	WB-TE	16	1	1	11	Japan	34.7 ± 2.7	NGS	PGT-A	≥ 3BB	/	Frozen
Ho et al.,2018	20	27	WB-TE	6	2	0	19	USA	/	NGS	PGT-A	/	YES	Frozen
Jiao et al.,2019	8	21	WB-TE	6	3	0	12	China	/	NGS	PGT-A	≥BB	YES	Frozen
			WB-SCM	6	2	0	13							
			TE-SCM	8	0	1	12							
Zhang et al.,2019	/	27	WB-SCM	3	4	2	18	China	29.6 ± 2.8	NGS	/	≥CC	YES	Frozen
Xu et al.,2016	17	42	TE-SCM	15	4	2	21	China	/	NGS	PGT-A	/	/	Frozen
Tobler et al.,2015	/	60	WB-BF	14	19	2	25	USA	/	aCGH	/	/	/	Frozen
Wu et al.,2021	70	101	WB-TE	38	5	6	44	China	/	NGS	PGT	≥ 3BC	/	Frozen
			ICM-TE	36	5	4	46							
Huang et al.,2019	13	52	WB-TE	32	9	0	9	USA	35.9	NGS	PGT-A, PGT-SR	≥BB	YES	Frozen
			WB-SCM	33	7	0	12							
			TE-SCM	37	2	4	7							

Table 1 (continued)

First author, year of publication	Participants/*N*	Sample/*N*	Diagnostic sample					Country	Age	Sequencing methods	Type of PGT	Embryo quality	Whether apply AH	Frozen/ Fresh embryo
			Sample type	TP	FP	FN	TN							
First author, year of publication	Participants/N	Sample/N	Diagnostic sample					Country	Age	Sequencing methods	Type of PGT	Embryo quality	Whether apply AH	Frozen/ Fresh embryo
			Sample type	TP	FP	FN	TN							
Chen et al.,2021	/	256	WB-TE	86	32	10	128	China	24–39	NGS	PGT-A	CC	/	Fresh
			WB-SCM	83	43	13	117							
			TE-SCM	89	37	29	101							
Popovic et al.,2018	25	34	ICM-TE	10	4	1	16	Belgian	23–39	NGS	PGT-A	≥BC	/	Frozen
Vera-Rodriguez et al.,2018	42	56	TE-SCM	20	2	23	6	Spain	/	NGS	PGT-A	/	YES	Fresh
Kulmann et al.,2021	1	7	TE-SCM	4	1	1	1	Brazil	35	NGS	PGT-A	/	YES	Fresh
Tšuiko et al.,2018	/	14	ICM-TE	5	1	0	8	Estonia	33.8 ± 5.7	NGS	PGT-A	≥ 3BC	YES	Frozen
			ICM-BF	4	3	0	3							
Li et al.,2018	/	40	WB-TE	17	5	2	14	China	/	NGS	PGT-A	/	YES	Frozen
			WB-SCM	17	6	2	13							
			TE-SCM	17	5	4	12							
Xie et al.,2022	25	161	TE-SCM	66	49	5	24	China	35.7 ± 4.1	NGS	/	≥ 4BC	YES	Fresh, Frozen
	37	122	TE-SCM	56	30	9	23		35.4 ± 5.1	NGS	PGT	≥ 4BC	YES	Fresh, Frozen
Yeung et al.,2019	37	168	TE-SCM	71	15	16	14	China	36.8 ± 3.9	NGS	PGT-A, PGT-SR	≥CC	YES	Fresh
Chuang et al.,2018	12	33	ICM-TE	19	1	1	8	China	34.4	NGS	PGT-A	≥BC	/	Frozen
Kuznyetsov et al.,2018	35	47	TE-SCM	10	1	2	6	Canada	38.9 ± 3.2	NGS	PGT-A	/	YES	Fresh
Kuznyetsov et al.,2020	28	90	TE-SCM	10	1	1	37	Canada	36.8 ± 3	NGS	PGT-A	≥BB	YES	Fresh
			TE-SCM	15	1	0	26					< BB		
Liu et al.,2016	7	56	TE-SCM	8	2	3	18	China	/	NGS	PGT	/	/	Fresh
Shi et al.,2022	40	212	WB-SCM	37	19	3	64	China	/	NGS	/	> CC	NO	Frozen
			WB-BF	16	15	2	26							
			ICM-SCM	27	9	2	51							
			ICM-BF	25	24	4	36							
Kang et al.,2024	12	68	TE-SCM	21	15	6	15	China	/	NGS	PGT	/	YES	Fresh

Table 1 (continued)

First author, year of publication	Participants/*N*	Sample/*N*	Diagnostic sample					Country	Age	Sequencing methods	Type of PGT	Embryo quality	Whether apply AH	Frozen/Fresh embryo
			Sample type	TP	FP	FN	TN							
Li et al.,2021	12	22	WB-TE	4	5	0	12	China	/	NGS	PGT-A	/	YES	Frozen
			WB-SCM	4	6	0	10							
			TE-SCM	6	4	3	7							
	10	19	WB-TE	2	2	0	14				PGT-SR			
			WB-SCM	2	4	0	13							
			TE-SCM	3	3	1	11							
Hu et al.,2023	54	260	TE-SCM	36	27	9	9	China	/	NGS	PGT-A	/	YES	Fresh
			TE-SCM	118	35	16	10				PGT-SR			

WB: whole blastocyst; ICM: inner cell mass; TE: trophectoderm; SCM: spent culture media; BF: blastocyst fluid; PGT-SR: preimplantation genetic testing for structural rearrangements; PGT-A: preimplantation genetic testing for aneuploidy; AH: assisted hatching; TP: true positive; FP: false positive; FN: false negative; TN: true negative; NGS: next-generation sequencing; aCGH: array-based comparative genomic hybridization

Results

Search results and characteristics of included studies

A systematic search of PubMed and Web of Science databases identified 6,407 records, with 3,464 from PubMed and 2,943 from Web of Science. After removing 2,882 duplicate records, 3,447 records were excluded based on title and abstract screening. An additional 40 records were excluded after a full-text review (Fig. 2). Ultimately, 36 studies met the inclusion criteria, providing test results from blastocyst fluid (BF = 3), spent culture media (SCM = 23), and trophectoderm (TE = 16) biopsies. The general characteristics of the included studies are summarized in Table 1.

Risk of bias and applicability assessments

The QUADAS-2 assessment results for risk of bias and applicability are presented in Fig. 3. Among the studies using WB/ICM as the gold standard, only two studies (L. Wu 2021 and Baoheng Gui 2016) had a high risk of bias in patient selection, while the remaining studies were rated as low risk. The index test domain consistently showed a low risk of bias across all studies. In the reference standard domain, most studies exhibited a low risk of bias, indicating high confidence in the findings based on validated standards. The flow and timing domains also showed consistent trends, with overall low applicability concerns across all three domains for WB/ICM.

Among the studies using TE as a control, six were rated as having a high risk of bias, while the remaining studies were classified as low or medium risk. Although some studies in the index test domain displayed a high risk of bias, most were within the low to moderate risk range. The applicability assessment revealed partially high applicability in this patient population, likely due to the consistency of clinical settings and the relevance of patient characteristics. Although the risk of bias in studies using TE as a control was slightly higher compared to those using WB/ICM, the validity and generalizability of TE in specific populations were well supported. These findings suggest that patient selection in both study groups was relatively randomized, with strict protocols followed during implementation to ensure the objectivity and reproducibility of the results (Supplementary Figure S1).

Deek's test was employed to assess publication bias and evaluate heterogeneity in diagnostic accuracy within the review. The p-value for Deek's test exceeded 0.05 across all outcomes, indicating the absence of heterogeneity (Supplementary Figure S2).

TE biopsy falls short of ideal diagnostic accuracy

Using WB/ICM as the gold standard, the diagnostic sensitivity and specificity of TE biopsy (PGT), SCM (ni-PGT), and BF (mi-PGT) were illustrated through forest plots (Fig. 4), with their diagnostic metrics summarized

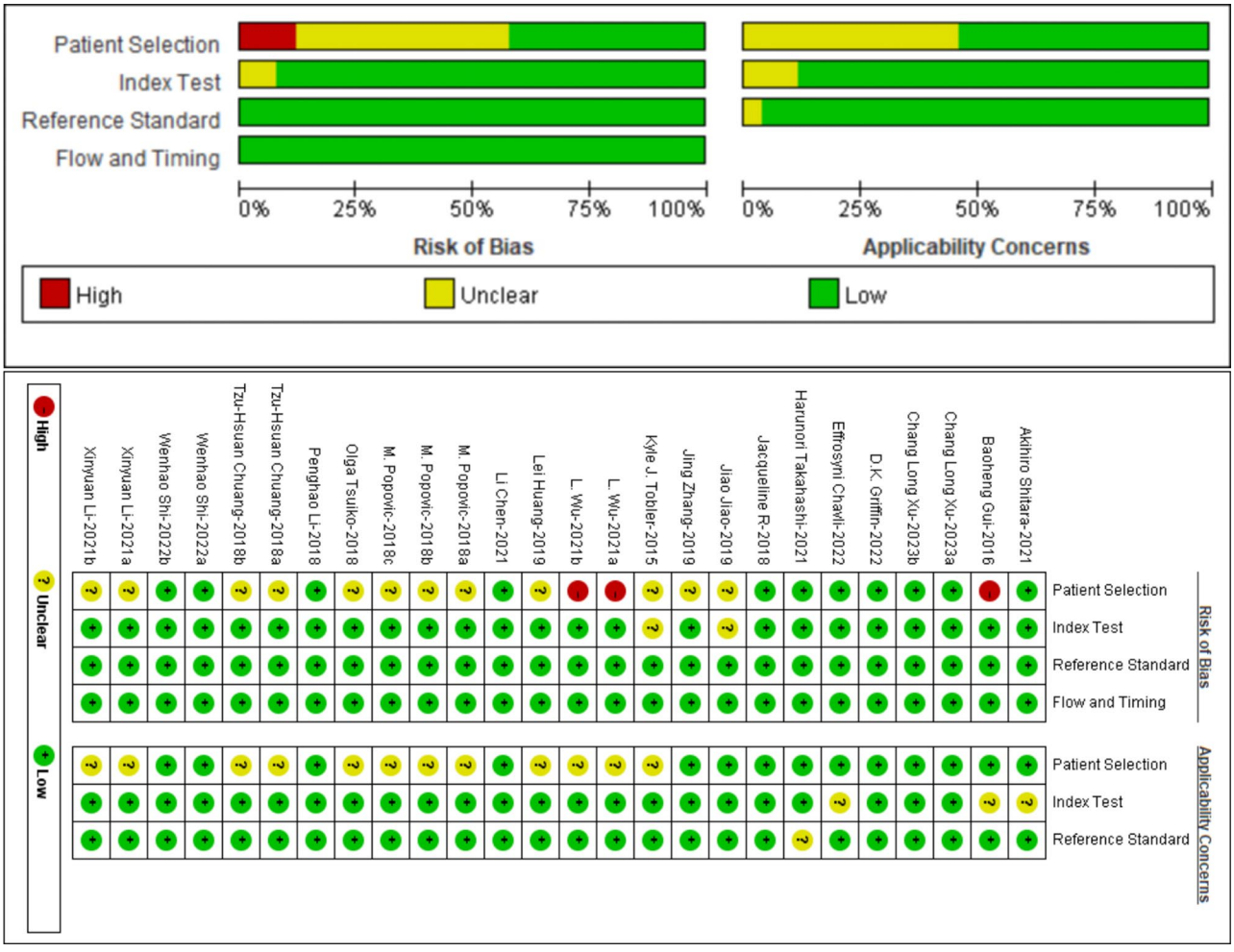

Fig. 3 Risk of bias and applicability concerns graph/summary (gold standard: WB/ICM)

in Table 2. While the sensitivities of the three diagnostic methods were comparable, the specificity of TE was higher than that of SCM and BF. The improved pooled specificity for TE contributed to its higher diagnostic accuracy compared to SCM and BF as defined by its AUC value when visualizing all markers on SROC curves (Fig. 5). Additionally, TE biopsy exhibited the highest AUC value at 0.878, surpassing SCM's 0.869 and BF's 0.656. The diagnostic odds ratio (DOR) of TE was also superior to those of SCM and BF. Positive likelihood ratios (LR+) and negative likelihood ratios (LR-) further confirmed that TE biopsy outperformed SCM and BF in diagnostic efficiency.

TE biopsy subgroup analysis

The diagnostic AUC value of TE biopsy is below 0.9, indicating that it has not yet achieved the desired level of diagnostic accuracy. To ensure an accurate and unbiased evaluation of PGT's diagnostic efficiency, data from prospective studies were analyzed. The results revealed that the AUC for prospective PGT was only 0.807, lower than the overall diagnostic efficiency of PGT (0.878), with both its sensitivity and specificity also falling short of the overall efficiency of TE (Table 2). This suggests that the diagnostic efficacy of PGT is influenced by variations in population characteristics, embryo types, and patient profiles.

PGT's diagnostic outcomes are closely tied to population characteristics, yet its diagnostic value across different populations remains unclear. In analyzing two distinct populations, the AUC for PGT-SR was 0.957, higher than the 0.806 for PGT-A, with TE biopsy demonstrating higher diagnostic sensitivity and specificity in the PGT-SR population compared to the PGT-A population (Table 3). The diagnostic efficiency of TE biopsy was exceptionally high in the PGT-SR group, with an AUC exceeding 0.9, achievement of diagnostic level. However, in the PGT-A population, the AUC of TE biopsy was only 0.806, Far from reaching the diagnostic threshold.

Furthermore, PGT's diagnostic performance is correlated with patient characteristics, The risk of chromosome number and structural abnormalities in embryos

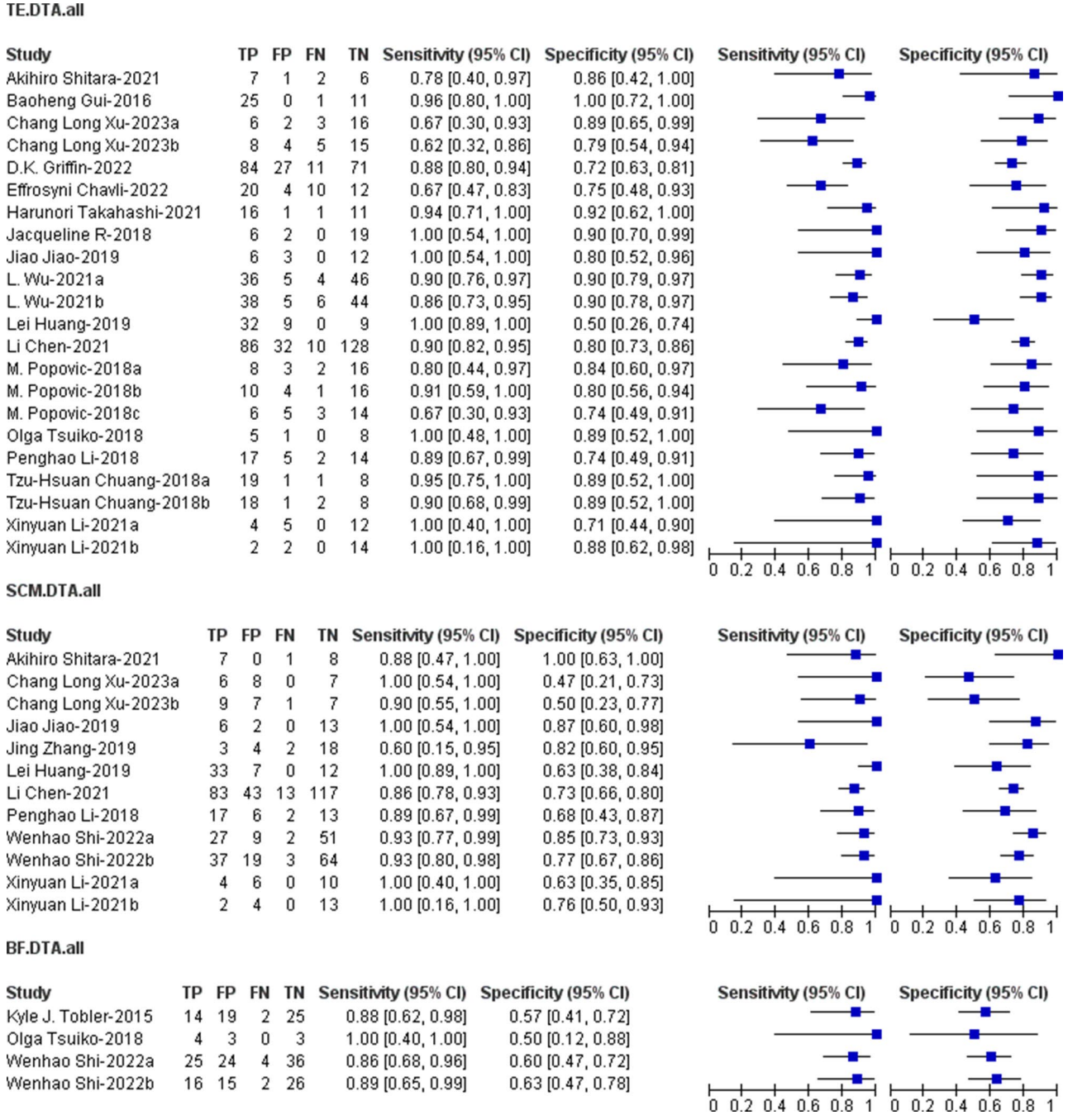

Fig. 4 Sensitivity and specificity forest plots of in the PGT diagnosis

Table 2 Summary diagnostic accuracy measures of preimplantation genetic testing (PGT)

Index Test	*N*	Sensitivity % (95% CI)	Specificity % (95% CI)	AUC	DOR (97.5% CI)	Positive LR (97.5% CI)	Negative LR (97.5% CI)
TE(WB/ICM)	16	0.839(0.790–0.879)	0.791(0.746–0.830)	0.878	20.300(12.700–30.800)	4.030(3.240–4.980)	0.205(0.151–0.270)
SCM(WB/ICM)	9	0.874(0.825–0.911)	0.719(0.650–0.780)	0.869	18.400(10.800–29.300)	3.140(2.480–3.970)	0.178(0.123–0.247)
BF(WB/ICM)	3	0.859(0.757–0.922)	0.593(0.514–0.668)	0.656	9.520(4.220–18.600)	2.120(1.700–2.620)	0.248(0.130–0.418)

WB: whole blastocyst; ICM: inner cell mass; TE: trophectoderm; SCM: spent culture media; BF: blastocyst fluid; DOR: diagnostic odds ratio; LR: likelihood ratio

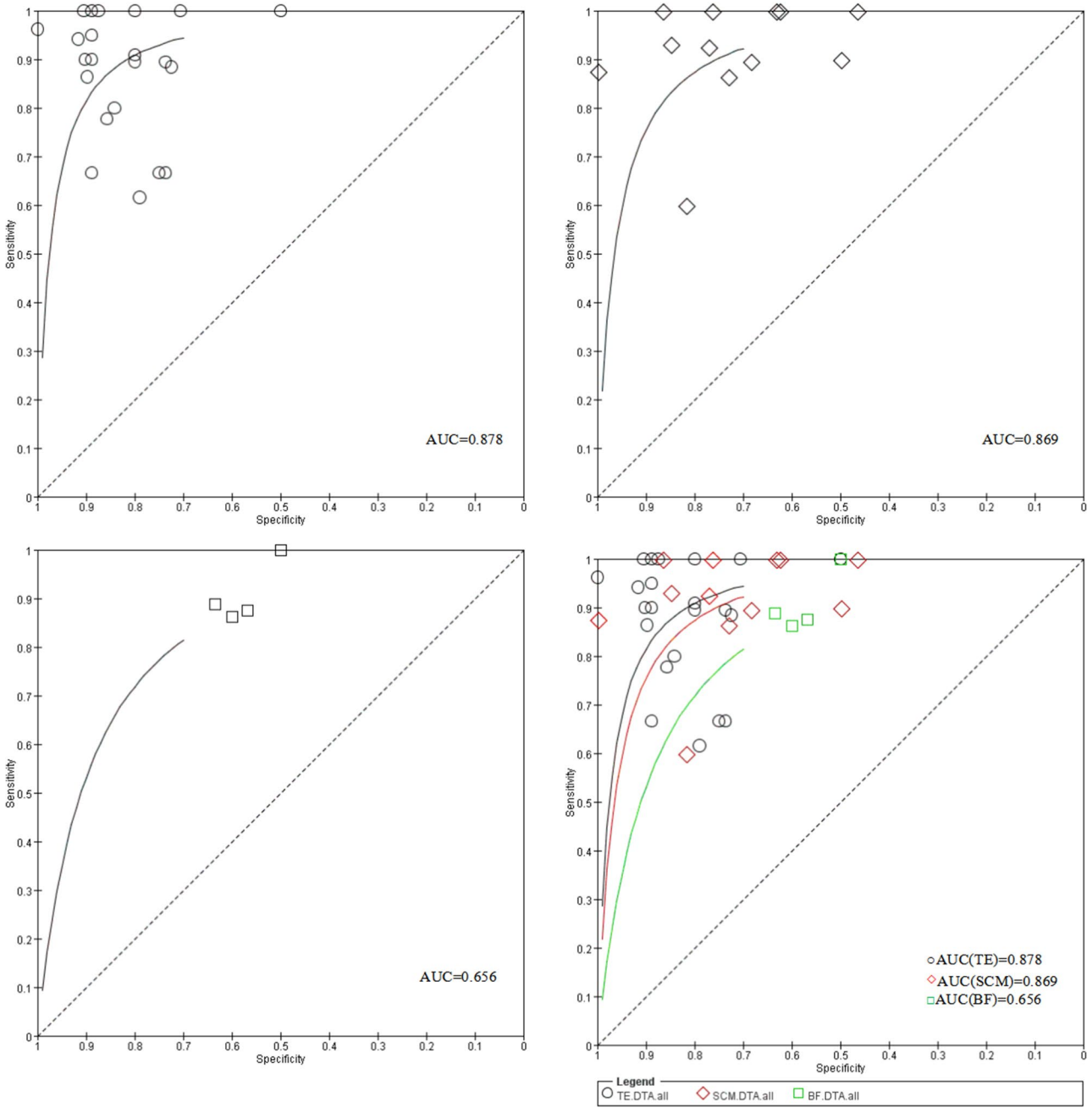

Fig. 5 Summary receiver-operating characteristic (SROC) curve for the PGT diagnosis: (**A**) trophectoderm biopsy (TE), (**B**) spent culture medium (SCM), (**C**) blastocyst fluid (BF), and (**D**) all markers. Each symbol represents a single study. The black dot represents the summary point and the dotted region represents the 95% confidence region. The diagonal dotted line represents AUC = 0.50 (random chance)

increases with age in women over 38 years of age (advanced age group). Given the high sensitivity observed in previous results, the impact of age on PGT-A diagnostics was considered. Samples were divided into two groups: PGT-A ≥ 38 years old and PGT-A < 38 years old (Table 3). The diagnostic sensitivity of PGT-A was higher in women of advanced age, though its specificity was slightly lower compared to those under 38. The AUC for PGT-A was 0.856 in the advanced age group, better than the 0.793 observed in younger women, indicating superior diagnostic performance in the advanced age group.

The diagnostic accuracy of PGT is also affected by the quality of the TE cell. Whole embryos comprise both ICM and TE, and the quality of trophoblast cells may impact the diagnostic validity of TE biopsies. To explore this, a stratified analysis was conducted based on different embryo qualities (Table 3). Embryos with a TE score of A/B were classified as high quality, while those with a

Table 3 Subgroup analysis of diagnostic accuracy of TE biopsy/ SCM(WB/ICM)

	Index Test	*N*	Sensitivity % (95% CI)	Specificity % (95% CI)	AUC	DOR (97.5% CI)	Positive LR (97.5% CI)	Negative LR (97.5% CI)
TE biopsy	TE(WB/ICM)	16	0.839(0.790–0.879)	0.791(0.746–0.830)	0.878	20.300(12.700–30.800)	4.030(3.240–4.980)	0.205(0.151–0.270)
exclude mosaic	TE exclude mosaic (WB/ICM)	15	0.875(0.822–0.914)	0.864(0.802–0.909)	**0.926**	46.300(25.300–78.100)	6.560(4.420–9.550)	0.147(0.100-0.206)
PGT-A/SR	TE-PGT-A(WB/ICM)	13	0.821(0.758–0.871)	0.776(0.736–0.811)	0.806	16.300(10.000-25.200)	3.670(3.020–4.420)	0.233(0.165–0.315)
	TE-PGT-SR(WB/ICM)	2	0.928(0.752–0.982)	0.882(0.690–0.962)	**0.957**	153.000(14.600–636.000)	9.170(2.900–24.000)	0.102(0.021–0.292)
WB/ICM	TE(WB)	9	0.880(0.816–0.924)	0.786(0.696–0.856)	**0.907**	28.000(16.400–44.900)	4.200(2.950–5.920)	0.156(0.101–0.226)
	TE(ICM)	8	0.827(0.742–0.887)	0.793(0.726–0.848)	0.867	19.600(8.920–37.500)	4.050(2.860–5.590)	0.224(0.138–0.337)
prospective(A)	TE(WB/ICM)	12	0.820(0.756–0.871)	0.779(0.739–0.815)	0.807	16.600(10.100–25.800)	3.730(3.050–4.510)	0.233(0.164–0.317)
Age	PGT-A(age<38)	6	0.732(0.607–0.829)	0.766(0.699–0.822)	0.793	9.460(4.680–17.200)	3.150(2.310–4.190)	0.355(0.223–0.517)
	PGT-A(age ≥ 38)	4	0.817(0.711–0.890)	0.756(0.606–0.862)	0.856	15.400(5.510–34.600)	3.490(2.030–5.940)	0.252(0.144–0.402)
Embryo quality	TE-C	6	0.835(0.759–0.891)	0.772(0.708–0.826)	0.848	18.100(9.130–32.300)	3.700(2.760–4.880)	0.218(0.139–0.322)
	TE-A/B	8	0.778(0.681–0.852)	0.846(0.779–0.895)	0.885	20.600(9.310–39.700)	5.140(3.360–7.600)	0.267(0.172–0.386)
	Index Test	N	Sensitivity % (95% CI)	Specificity % (95% CI)	AUC	DOR (97.5% CI)	Positive LR (97.5% CI)	Negative LR (97.5% CI)
SCM	SCM(WB/ICM)	9	0.874(0.825–0.911)	0.719(0.650–0.780)	0.869	18.400(10.800–29.300)	3.140(2.480–3.970)	0.178(0.123–0.247)
exclude mosaic	SCM exclude mosaic (WB/ICM)	9	0.888(0.827–0.929)	0.782(0.705–0.843)	**0.910**	29.500(16.100–49.800)	4.120(3.030–5.570)	0.147(0.092–0.219)
frozen	SCM-frozen (WB/ICM)	8	0.885(0.820–0.929)	0.716(0.630–0.790)	0.886	20.500(10.200–37.000)	3.160(2.370–4.200)	0.165(0.099–0.255)
PGT-A	SCM-PGT-A(WB/ICM)	7	0.870(0.807–0.915)	0.668(0.562–0.759)	0.871	14.100(7.280–24.800)	2.660(1.980–3.590)	0.200(0.128–0.295)
WB/ICM	SCM(WB)	8	0.863(0.805–0.906)	0.739(0.679–0.773)	0.876	17.400(10.400–27.400)	3.190(2.650–3.820)	0.191(0.129–0.270)
	SCM(ICM)	3	0.918(0.809–0.967)	0.687(0.444–0.858)	**0.914**	32.500(5.710–106.000)	3.200(1.610–6.460)	0.137(0.045–0.318)
Embryo quality	TE-C	2	0.859(0.780–0.913)	0.674(0.540–0.784)	0.857	13.700(5.620–28.200)	2.700(1.830–4.010)	0.217(0.126–0.347)
	TE-A/B	2	0.835(0.563–0.951)	0.484(0.085–0.905)	0.825	12.000(0.322–69.300)	2.350(0.796–8.610)	0.587(0.0835-2.460)

WB: whole blastocyst; ICM: inner cell mass; TE: trophectoderm; SCM: spent culture media; BF: blastocyst fluid; TE-C: trophectoderm quantity is C; TE-A/B: trophectoderm quantity is A/B; DOR: diagnostic odds ratio; LR: likelihood ratio

score of C's were considered low quality. The diagnostic efficiency of PGT was superior in high-quality trophectoderm cells, with an AUC of 0.885, compared to 0.848 in low-quality trophectoderm cells.

The accuracy of PGT diagnosis is influenced by the type of gold standard used. The diagnostic performance of PGT was higher when WB was used as the gold standard (AUC: 0.907) compared to ICM (AUC: 0.867). These results indicate considerable heterogeneity between TE cells and ICM cells; thus, TE biopsy may not accurately represent the genetic status of ICM.

Mosaicism is prevalent in pre-implantation embryo diagnosis and TE biopsies face significant diagnostic challenges due to uncertainty in the location of mosaics. The impact of mosaic embryos on the diagnostic accuracy of PGT is currently unknown. To address this issue, we analyzed known euploid and aneuploid embryos while excluding all mosaic embryos. After exclusion, the curvilinear AUC for PGT increased to 0.926, which exceeded the 0.878 when mosaic embryos were not excluded, suggesting that mosaic embryos reduced the diagnostic accuracy of PGT (Table 3). Similarly, after exclusion of mosaic embryos, the AUC value for SCM

Table 4 Summary diagnostic accuracy measures of Multi-diagnosis (TE + SCM and TE + TE)

	Index Test	*N*	Sensitivity % (95% CI)	Specificity % (95% CI)	AUC	DOR (97.5% CI)	Positive LR (97.5% CI)	Negative LR (97.5% CI)
total	TE(WB/ICM)	16	0.839(0.790–0.879)	0.791(0.746–0.830)	0.878	20.300(12.700–30.800)	4.030(3.240–4.980)	0.205(0.151–0.270)
combined test	TE + SCM(WB/ICM)	7	0.921(0.848–0.961)	0.845(0.777–0.894)	**0.927**	71.300(25.400–160.000)	6.030(4.050–8.770)	0.098(0.046–0.183)
	TE + TE(WB/ICM)	2	0.924(0.741–0.981)	0.914(0.714–0.979)	**0.966**	224.000(16.700–1000.000)	13.600(3.130–42.300)	0.104(0.021–0.294)

WB: whole blastocyst; ICM: inner cell mass; TE: trophectoderm; SCM: spent culture media; TE + SCM: joint TE and SCM diagnostics; TE + TE: joint TE and TE re-biopsy diagnostics; DOR: diagnostic odds ratio; LR: likelihood ratio

analysis increased to 0.910, which was an improvement over the 0.869 when mosaic embryos were not excluded. The diagnostic sensitivity and specificity of both PGT and SCM increased after exclusion of mosaic embryos, suggesting that mosaic embryos have an impact on the accuracy of the different diagnostic methods.

SCM May supplement PGT methods

According to the subgroup analysis using WB/ICM as the gold standard, the diagnostic indices of ni-PGT are presented in Table 3. Contrary to the PGT results, SCM demonstrated higher sensitivity and diagnostic efficiency when ICM served as the gold standard, with an AUC exceeding 0.9, indicating strong diagnostic performance. This phenomenon may be attributed to the greater release of cfDNA from ICM into the culture medium during in vitro culture, making SCM a better representative of ICM's genetic information. The source of culture fluid also influenced the accuracy and reliability of ni-PGT, with higher diagnostic efficiency (AUC: 0.886) observed when the culture fluid was derived from frozen cycles. Since the cfDNA of SCM is derived from TE and ICM cells, ni-PGT demonstrated higher diagnostic efficiency in the PGT-A population (AUC: 0.871) compared to PGT (AUC: 0.806).

The quality of TE cells may influence cfDNA release; therefore, a subgroup analysis was conducted based on TE cell quality (Table 3). Interestingly, low-quality TE exhibited higher sensitivity, specificity, and diagnostic efficiency than high-quality TE. Therefore, unlike TE biopsies, the diagnostic efficiency of ni-PGT was better in low-quality trophectoderm cells compared to high-quality cells.

SCM subgroup analysis with TE as the control

In the analysis using TE as the control group, a subgroup analysis of the PGT-A and PGT-SR populations was conducted to further assess the application value of ni-PGT across different groups (Supplementary table S1). The results indicated that ni-PGT demonstrated higher diagnostic sensitivity and efficiency in the PGT-SR population compared to the PGT-A population, aligning with the overall trend observed in PGT diagnostics. This finding reinforces the superior diagnostic performance of ni-PGT in the PGT-SR population when TE is used as the control group.

Additionally, subgroup analyses were performed based on the source of culture fluid (frozen versus fresh cycles). The results revealed that ni-PGT had higher specificity and diagnostic efficiency with frozen cycles compared to fresh cycles, consistent with the trend observed using WB/ICM as the gold standard. This suggests that ni-PGT performs better diagnostically with frozen samples. Further analysis of the effect of culture fluid sources on ni-PGT diagnostic efficiency in the PGT-A population confirmed that sensitivity, specificity, and diagnostic efficiency were all higher in frozen cycles compared to fresh cultures. This further substantiates the superior diagnostic performance of ni-PGT with frozen samples.

In assisted reproductive technology, assisted hatching (AH) aims to facilitate successful embryo hatching before implantation by thinning or perforating the zona pellucida using physical or chemical methods. During this process, blastocyst fluid may enter the spent culture medium (SCM), potentially impacting the diagnostic performance of ni-PGT. A subgroup analysis comparing embryos that underwent AH with those that did not was conducted (Supplementary table S1). The results revealed that the diagnostic sensitivity and efficiency of ni-PGT were higher in embryos that received AH compared to those that did not, suggesting that AH positively influences the diagnostic outcomes of ni-PGT.

Directions for PGT optimization

Based on previous results (Table 2), TE biopsy exhibited the highest diagnostic efficiency among the three methods, yet it did not reach the ideal level. To enhance the diagnostic performance of preimplantation genetic testing (PGT), optimization strategies were investigated. The diagnostic efficacy of combining TE with spent culture medium (SCM) and employing multiple biopsies using the WB/ICM gold standard was analyzed (Table 4). The analysis revealed that both TE + SCM diagnosis and multiple biopsies significantly improved sensitivity and specificity compared to TE biopsy alone.

Specifically, multiple biopsies achieved a higher AUC value of 0.966, slightly surpassing the 0.927 AUC of TE + SCM diagnosis. Both approaches outperformed TE biopsy, which had an AUC of 0.878. Overall, both multiple biopsies and TE + SCM diagnosis effectively enhanced PGT diagnostic efficiency, with multiple biopsies demonstrating the highest performance.

Discussion

Principal findings

The systematic review and diagnostic meta-analysis encompassed 36 studies on embryo ploidy diagnosis, involving a total of 4,230 embryos. The meta-analysis results indicate that TE-biopsy is currently the most accurate method for determining embryo ploidy, with SCM being only slightly less accurate. Both SCM and TE-biopsy are significantly more effective than the BF method. Despite TE-biopsy's superior accuracy, it is important to recognize that it still has a certain rate of missed detections and misdiagnoses, with an overall diagnostic AUC value of 0.878, falling short of the ideal diagnostic threshold of 0.9 or higher. At the same time, our subgroup analysis indicates that performing multiple biopsies can further reduce the rates of missed detections and misdiagnoses associated with TE-biopsy. However, while multiple biopsies may enhance the diagnostic efficiency of TE-biopsy, they introduce uncertainties regarding offspring safety and impose additional economic burdens. Addressing these issues will require further high-quality clinical studies. Additionally, our study reveals an intriguing finding: combining TE-biopsy with SCM diagnosis can improve diagnostic efficiency. The results from this combined approach are superior to those from either TE-biopsy or SCM alone and eliminate the need for additional biopsies, thus mitigating concerns about offspring safety. Therefore, when TE-biopsy alone does not yield accurate genetic information, SCM should be employed as a supplementary method. However, SCM is highly susceptible to contamination from maternal sources, which can lead to inaccurate test results. Future research should focus on identifying biomarkers present in SCM and integrating them into ploidy assessments for comprehensive modeling. This approach could help mitigate the impact of maternal contamination on the accuracy of SCM detection.

Our population subgroup analysis of TE-biopsy revealed that the AUC for the PGT-SR population was 0.957, significantly higher than the 0.806 observed in the PGT-A population. This disparity may account for why the diagnostic accuracy of TE-biopsy in our study is lower than what has been reported in the literature. Although the detection methods for PGT-SR and PGT-A are similar, PGT-SR detection incorporates the parental karyotype to enhance sequencing depth at breakpoint positions. This approach may help reduce missed detections and misdiagnoses. Additionally, our prospective subgroup analysis, which exclusively involved the PGT-A population, supports this explanation. Furthermore, our analysis of SCM indicated that embryo freezing is a crucial factor influencing SCM diagnostic accuracy.

Overall, these observational results underscore the variability in diagnostic accuracy among different methods for assessing embryonic genetic information. It is important to note, however, that population biases in current observational studies may affect these results. Specifically, the PGT-SR population is smaller than the PGT-A population, and eight of the nine studies involving SCM used frozen-thawed embryos. These factors may contribute to the comparable diagnostic efficiency of SCM and TE-biopsy. Therefore, a thorough and accurate evaluation of the diagnostic accuracy of TE, SCM, and BF methods is essential.

Strengths and limitations

This study is the first to comprehensively evaluate the accuracy of the three current PGT methods through an evidence-based approach and an extensive database search. To ensure the most accurate assessment, we used ICM or WB containing ICM as the gold standard. To minimize bias, we excluded studies with only abnormal embryos and included those that encompassed both euploids and aneuploids. Additionally, we employed recommended statistical methods for evaluating diagnostic accuracy and used a quality assessment tool designed specifically for diagnostic studies to assess bias risk. Our findings may inform future research or guide the optimization of existing PGT methods. A significant limitation of this study is the predominance of PGT-A populations in the TE-biopsy studies, with only two studies involving PGT-SR populations, potentially due to population differences. Furthermore, in the SCM analysis, eight of the included studies involved frozen-thawed culture media, while only one study used fresh cycles. Additionally, variations in culture systems and sample volumes across studies prevented us from conducting a subgroup analysis based on sample volume.

Comparison with other studies

Our study is the first to use ICM or WB as a reference standard to evaluate the accuracy of TE-biopsy for embryo ploidy diagnosis. The analysis reveals that the AUC for TE-biopsy is 0.878. Additionally, the overall sensitivity and specificity of TE-biopsy are 0.839 (95% confidence interval [CI]: 0.790–0.879) and 0.791 (95% CI: 0.746–0.830), respectively. Although TE-biopsy is currently the most accurate method for diagnosing embryonic ploidy, it is important to note that its AUC does not exceed 0.9, indicating that some misdiagnosis and missed

diagnoses may still occur. This limitation might be influenced by the population studied. Our subgroup analysis of PGT-A and PGT-SR shows that the AUC for PGT-A is 0.806, which is adequate for screening purposes but may have limited application. Additionally, clinical outcomes for PGT-A in non-aged patients do not show significant improvement compared to conventional IVF methods [59]. This finding is supported by the study conducted by Yan et al., which also found no significant difference in clinical outcomes between PGT-A and IVF in non-aged patients [60]. In contrast, the AUC for the PGT-SR population is 0.957, indicating a high diagnostic accuracy. For individuals with balanced translocations, TE-biopsy can effectively identify and exclude nearly all unbalanced translocation embryos, which significantly reduces the miscarriage rate and enhances clinical outcomes [3]. Unlike the PGT-A population, PGT-SR patients have a lower probability of producing normal gametes [61]. For embryos with unbalanced translocations, there are typically two abnormal chromosomes corresponding to the karyotype of the translocation carrier [62]. Consequently, TE-biopsy in the PGT-SR population is less likely to result in misdiagnosis or missed diagnoses.

In addition to differences in population, the type of embryo may also contribute to suboptimal diagnostic results from TE-biopsy. Embryonic mosaicism is prevalent in preimplantation embryos [63]. Due to the variable degree and location of mosaicism, a single TE-biopsy may not capture the complete genetic status of the entire embryo [45]. Our research results indicate that removing the influence of mosaic embryos significantly enhances the diagnostic accuracy of TE-biopsy. The AUC for TE-biopsy improved to 0.926, surpassing the 0.9 threshold, with sensitivity and specificity values reaching 0.875 (95% CI: 0.822–0.914) and 0.864 (95% CI: 0.802–0.909), respectively. Despite these improvements, effective diagnostic methods for determining the comprehensive genetic status of mosaic embryos remain lacking. Additionally, the impact of the sequencing platform must be considered, as current PGT methods cannot differentiate between low-proportion mosaic embryos that are artifacts of background noise and true mosaicism [64]. A multicenter, double-blind, non-selective study found no significant difference in clinical outcomes between the transplantation of low-proportion mosaic embryos (20–50%) and euploid embryos [65]. Thus, there is a need for more sensitive bioinformatics algorithms to better differentiate between mosaicism and background noise in the future. Nonetheless, PGT-A retains significant clinical value, particularly for aged patients. As women age, the likelihood of producing abnormal gametes increases, resulting in a higher proportion of aneuploid embryos among aged patients [66, 67]. Numerous studies have reported that PGT-A can substantially improve clinical outcomes for these patients [68, 69]. Our results also indicate that the diagnostic efficiency of TE-biopsy is higher in aged patients undergoing PGT-A compared to younger patients. This finding may elucidate the effectiveness of PGT-A in aged populations.

Since TE-biopsy can cause trauma to the embryo, raising concerns about long-term offspring safety, and given that the diagnostic accuracy of TE-biopsy does not achieve an AUC value above 0.9, it is essential to explore less invasive and more accurate diagnostic methods. Research has identified embryonic cfDNA in the blastocyst cavity and culture medium as potential sources for ploidy diagnosis [70]. Magli et al. reported that the accuracy of BF-based MiPGT, using TE-biopsy as a control, was 97% [20]. However, it is important to note that this study employed array comparative genomic hybridization (aCGH) rather than next-generation sequencing (NGS), which may not provide the same diagnostic accuracy as TE-biopsy used as a control. To further assess the effectiveness of BF-based MiPGT, this study used WB or ICM as the gold standard for evaluating BF diagnostic accuracy. The results revealed that the AUC for BF was 0.656, with a specificity of only 0.593, both significantly lower than those of TE-biopsy. Additionally, research indicates that BF's diagnostic efficiency for embryo ploidy is markedly inferior to that of SCM [21]. This lower accuracy may be attributed to the influence of surrounding cells within the blastocoel [35]. Studies have demonstrated that biopsies of cells surrounding the blastocoel exhibit a very low consistency rate with ICM results, which may account for the reduced diagnostic accuracy of BF [35].

The results of the first study on ni-PGT in 2016 indicated that ni-PGT achieved an accuracy of approximately 87% when TE-biopsy was used as the control [27]. In 2019, the same research team employed WB as the gold standard and reported that ni-PGT accuracy improved to 93.8%, surpassing the 82% accuracy of TE-biopsy [49]. This high consistency underscores the potential of current SCM-based ni-PGT for diagnosing embryo ploidy. However, a multicenter prospective study using TE-biopsy for 1,301 embryos as the control found that SCM's diagnostic consistency was only 78.2% [25]. Overall, accuracy results for SCM vary widely, ranging from 43.5 to 100%, depending on the control used, including TE-biopsy, WB, and ICM [3, 23, 52]. This variability may be attributed to differences in control standards. Using TE-biopsy, which has limited diagnostic accuracy, as a control may not accurately reflect SCM's effectiveness. This study, employing WB/ICM as the gold standard, found that SCM's diagnostic efficiency is comparable to that of TE-biopsy, with SCM achieving an AUC of 0.869, slightly lower than TE-biopsy's 0.878. These findings highlight SCM's significant potential for clinical diagnosis of embryonic karyotype.

It is important to note that eight of the nine studies included in the SCM analysis originated from cryopreservation cycles. This may be due to the fact that freezing processes destroy granulosa cells attached to the embryo's surface and reduce maternal DNA contamination after multiple washes, thereby enhancing SCM's diagnostic efficiency in these cycles. Additionally, freezing can also destroy some embryonic cells, resulting in the release of more embryo-derived DNA, which further improves SCM's diagnostic capability. This observation aligns with current research indicating a 95% success rate for embryo cryopreservation, with the remaining 5% of embryos failing to be implanted after freezing, highlighting that cryopreservation does indeed cause some cellular destruction. Furthermore, a rapid SCM diagnostic method has been reported that can provide an embryo's karyotype test report within 9 h, demonstrating SCM's significant potential for diagnosing embryo karyotype during freezing cycles [71]. Although numerous studies indicate no significant difference in clinical outcomes between frozen-cycle and fresh-cycle embryo transfers, the number of SCM-related studies using gold standards as controls is limited [72–74]. Consequently, further subgroup analysis across different populations is not feasible. In the supplementary results, TE-biopsy was used as a control to explore SCM's effectiveness in different populations. The diagnostic efficiency of SCM was found to be better in the PGT-SR population compared to the PGT-A population. Although the specificity of SCM in the PGT-SR group was lower than in the PGT-A group, this difference may be attributed to the fact that 2 of the 3 studies in the PGT-SR subgroup were from fresh cycles and had a larger sample size. Moreover, results using TE as a control mirrored those obtained with the gold standard, with frozen cycles showing superior results compared to fresh cycles. Despite the supplementary analysis not employing the gold standard, TE-biopsy, as a preferred clinical method for diagnosing embryonic karyotype, was used to reduce bias and ensure that overall result trends remained consistent.

Our subgroup analysis utilized WB or ICM as the gold standard. The results indicated that TE biopsy exhibited higher diagnostic accuracy when WB was employed as the gold standard. WB encompasses both trophoblast cells and the inner cell mass, making TE biopsy results more aligned with WB. Since TE biopsy samples are exclusively derived from trophoblast cells, and given that both trophoblast cells and the ICM exhibit inherent heterogeneity, TE biopsy may not accurately reflect the genetic status of the ICM. Interestingly, SCM showed better diagnostic accuracy with ICM than with WB, possibly because ICM releases more cfDNA into SCM. However, it is important to note that the current study demonstrated that, even in fresh cycles, regardless of the number of embryo washings performed, cfDNA from SCM may contain DNA from maternal polar bodies, granulosa cells, and paternal sperm. This contamination complicates genetic status assessment and reduces the accuracy of SCM [71]. While SCM's accuracy improves significantly after cryopreservation, secondary cryopreservation of embryos may affect clinical outcomes, including pregnancy and miscarriage rates [75]. The goal for SCM application should be to accurately determine the genetic status of embryos, whether from fresh or frozen cycles. Future advancements in whole-genome amplification technology may allow for more precise amplification of cfDNA from ICM in SCM, potentially enhancing the accuracy of embryo genetic diagnosis.

Future prospects

The results from existing studies and our own research indicate that both TE-biopsy and SCM have certain limitations and neither method can provide complete diagnostic accuracy for the entire embryo. For patients undergoing PGT, additional TE biopsies are performed to mitigate risk; however, some missed diagnoses can still occur, which, although rare, is unacceptable for PGT patients. Our findings suggest that performing multiple biopsies may further reduce the likelihood of missed diagnoses and misdiagnoses. Specifically, the diagnostic AUC value for multiple biopsies was 0.966, with both sensitivity and specificity exceeding 0.9, which significantly enhances the diagnostic effectiveness of TE-biopsy. It is important to note that this subgroup analysis included only two studies, and additional biopsies may raise concerns about long-term offspring safety. Research indicates that for embryos with a TE grade of A, biopsy more than 16 cells does not significantly affect implantation rates. However, for embryos with TE grades of B/C, biopsy more than 16 cells can markedly reduce implantation rates. A recent study also found that performing two biopsies was associated with significantly lower clinical pregnancy rates and increased miscarriage rates. Thus, while multiple biopsies can enhance diagnostic accuracy, their impact on clinical outcomes and long-term offspring safety remains unclear. Future high-quality clinical research is needed to address these issues. To mitigate concerns about the safety and accuracy of individual diagnostic methods, we combined TE-biopsy with SCM in our subgroup analysis. This combined approach improved upon the limitations of each separate method, achieving an AUC value of 0.927 and sensitivity of 0.921—nearly equivalent to the 0.924 achieved with multiple biopsies. However, inconsistent diagnostic results between embryos can pose challenges for clinical decision-making. Based on the current evidence, no effective measures have been identified to resolve discrepancies in test results between the two methods. This

limitation represents a significant constraint in our study. We anticipate that future high-quality research will contribute to the optimization of PGT's clinical application, offering clearer and more effective guidance for clinical decision-making.

Therefore, combining TE-biopsy with SCM represents a promising approach for enhancing diagnostic accuracy while addressing the limitations and safety concerns associated with each method individually.

Conclusion and implications

This systematic review and diagnostic meta-analysis confirm that TE-biopsy remains the most accurate method for diagnosing embryo ploidy, yet its diagnostic AUC value is only 0.878, which falls short of clinical diagnostic standards. This discrepancy can be attributed to differences between PGT-A and PGT-SR populations: the AUC for the PGT-A population is 0.806, while the PGT-SR population shows a higher AUC of 0.957. Our study indicates that the current diagnostic accuracy of TE-biopsy, particularly in the PGT-A population, is limited to a screening level. Additionally, our subgroup analyses based on age and population type effectively outline the current applications of PGT. We also assessed the potential of ni-PGT through SCM across different cycle sources and gold standards. Our findings suggest that combining TE-biopsy with SCM may enhance diagnostic accuracy, although more data are required to validate this combined approach. Ultimately, our results clarify the selection of embryo ploidy diagnostic methods and offer insights for improving future diagnostic accuracy. These advancements are essential for more precise genetic assessment of embryos.

Abbreviations

PGT	preimplantation genetic testing
mi-PGT	minimally invasive preimplantation genetic testing
ni-PGT	non-invasive preimplantation genetic testing
TE	trophectoderm
WB	whole blastocyst
ICM	inner cell mass
cfDNA	cell-free DNA
BF	blastocoel fluid
SCM	spent culture medium
AH	assisted hatching
NGS	next-generation sequencing
aCGH	array comparative genomic hybridization
AUC	area under the curve
DOR	diagnostic odds ratio
LR	likelihood ratio
LR+	Positive likelihood ratio
LR-	negative likelihood ratio
TP	true positive
FP	false positive
FN	false negative
TN	true negative
RE	random effect
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-025-01376-1 .

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Acknowledgements

None.

Author contributions

Y.Jia. and H.Z. conceptualized and designed the research,W.Z., L.Yun. and Y.Jia. supervised the study. C.K. and H.Z. performed the literature search. C.K., H.Z. and Y.Jia. selected articles and C.K. and H.Z. extracted the data. C.K., H.Z. L.Yu., H.Y. and Y.Jia. interpreted the data and wrote the manuscript. Z.X., L.Yo, X.L, J.W., L.M., Z.P., Z.M., L.X., X.Y., Z.H., T.M., W.M., M.R., Y.Ji., B.Y., D.R. and D.B. revised the manuscript. All authors approved the final submission of the manuscript.

Funding

This study was funded by grants: Social development projects of Yunnan Province: 202302AA310044;Natural Science Foundation of China: 32270981;High level Project of Yunnan Province: YNQR-QNRC-2018-126; XDYC-MY-2022-0055; The Joint Special Funds for the Department of Science and Technology of Yunnan Province Kunming Medical University: 202301AY070001-003; 202401AY070001-301; 202301AY070001-048; 202301AY070001-298;Yunnan Provincial Reproductive and Obstetrics and Gynecology Clinical Medicine: 2022LCZXKF-SZ12; 2022LCZXKF-SZ11; 2022LCZXKF-SZ17; 2022LCZXKF-SZ05; 2022LCZXKF-SZ15; 2022LCZXKF-SZ18;Joint Medical Project of Kunming University of Science and Technology: KUST-KH2022025Y; KUST-KH2023004Z; KUST-KH2022003Z; Kunming University of Science and Technology Medical School research development fund project: SRDP-2023-003;Yunnan Provincial Young and Middle-aged Academic and Technical Leaders Reserve Talents Program: 202305AC160022.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Reproductive Medicine, NHC Key Laboratory of Healthy Birth and Birth Defect Prevention in Western China, First People's Hospital of Yunnan Province, Kunming 650500, China
[2]KUST-YPFPH Reproductive Medicine Joint Research Center, Kunming, Yunnan, China

Received: 10 December 2024 / Accepted: 3 March 2025
Published online: 11 March 2025

References

1. Esfandiari N, Bunnell ME, Casper RF. Human embryo mosaicism: did we drop the ball on chromosomal testing? J Assist Reprod Genet. 2016;33:1439–44.
2. Victor AR, Griffin DK, Brake AJ, Tyndall JC, Murphy AE, Lepkowsky LT, Lal A, Zouves CG, Barnes FL, McCoy RC, Viotti M. Assessment of aneuploidy

concordance between clinical trophectoderm biopsy and blastocyst. Hum Reprod. 2019;34:181–92.
3. Jiao J, Shi B, Sagnelli M, Yang D, Yao Y, Li W, Shao L, Lu S, Li D, Wang X. Minimally invasive preimplantation genetic testing using blastocyst culture medium. Hum Reprod. 2019;34:1369–79.
4. Sachdev NM, McCulloh DH, Kramer Y, Keefe D, Grifo JA. The reproducibility of trophectoderm biopsies in euploid, aneuploid, and mosaic embryos using independently verified next-generation sequencing (NGS): a pilot study. J Assist Reprod Genet. 2020;37:559–71.
5. Chavli E, van den Born M, Eleveld C, Boter M, van Marion R, Hoefsloot L, Laven J, Baart E, Van Opstal D. Chromosomal mosaicism in human blastocysts: a cytogenetic comparison of trophectoderm and inner cell mass after next-generation sequencing. Reprod Biomed Online. 2022;45:867–77.
6. Tšuiko O, Zhigalina DI, Jatsenko T, Skryabin NA, Kanbekova OR, Artyukhova VG, Svetlakov AV, Teearu K, Trošin A, Salumets A, et al. Karyotype of the Blastocoel fluid demonstrates low concordance with both trophectoderm and inner cell mass. Fertil Steril. 2018;109:1127–e11341121.
7. Klimczak AM, Seli E, Scott RT. Noninvasive prenatal testing in women undergoing in vitro fertilization with preimplantation genetic testing. Curr Opin Obst Gynecol. 2021;33:184–7.
8. Bunnell ME, Dobson LJ, Lanes A, Ginsburg ES. Use of preimplantation genetic testing for Monogenic disorders and subsequent prenatal care and diagnostic testing. Prenat Diagn. 2022;42:1022–30.
9. Munné S, Grifo J, Wells D. Mosaicism: survival of the fittest versus no embryo left behind. Fertil Steril. 2016;105:1146–9.
10. Fragouli E, Munne S, Wells D. The cytogenetic constitution of human blastocysts: insights from comprehensive chromosome screening strategies. Hum Reprod Update. 2019;25:15–33.
11. Rodrigo L, Clemente-Císcar M, Campos-Galindo I, Peinado V, Simón C, Rubio C. Characteristics of the IVF cycle that contribute to the incidence of mosaicism. Genes 2020, 11.
12. Greco E, Minasi MG, Fiorentino F. Healthy babies after intrauterine transfer of mosaic aneuploid blastocysts. N Engl J Med. 2015;373:2089–90.
13. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, Cheng E-H, Lee M-S, Horcajadas JA, Corti L, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. Fertil Steril. 2021;115:1212–24.
14. Navratil R, Horak J, Hornak M, Kubicek D, Balcova M, Tauwinklova G, Travnik P, Vesela K. Concordance of various chromosomal errors among different parts of the embryo and the value of re-biopsy in embryos with segmental aneuploidies. Mol Hum Reprod. 2020;26:269–76.
15. Shi X, Tang Y, Liu C, Li W, Lin H, Mao W, Huang M, Chu Q, Wang L, Quan S, et al. Effects of NGS-based PGT-a for idiopathic recurrent pregnancy loss and implantation failure: a retrospective cohort study. Syst Biology Reproductive Med. 2023;69:354–65.
16. Sanders KD, Silvestri G, Gordon T, Griffin DK. Analysis of IVF live birth outcomes with and without preimplantation genetic testing for aneuploidy (PGT-A): UK human fertilisation and embryology authority data collection 2016–2018. J Assist Reprod Genet. 2021;38:3277–85.
17. Ma S, Liao J, Zhang S, Yang X, Hocher B, Tan J, Tan Y, Hu L, Gong F, Xie P, Lin G. Exploring the efficacy and beneficial population of preimplantation genetic testing for aneuploidy start from the oocyte retrieval cycle: a real-world study. J Translational Med 2023, 21.
18. Kasaven LS, Marcus D, Theodorou E, Jones BP, Saso S, Naja R, Serhal P, Ben-Nagi J. Systematic review and meta-analysis: does pre-implantation genetic testing for aneuploidy at the blastocyst stage improve live birth rate? J Assist Reprod Genet. 2023;40:2297–316.
19. Gleicher N, Metzger J, Croft G, Kushnir VA, Albertini DF, Barad DH. A single trophectoderm biopsy at blastocyst stage is mathematically unable to determine embryo ploidy accurately enough for clinical use. Reproductive Biology Endocrinol 2017, 15.
20. Magli MC, Pomante A, Cafueri G, Valerio M, Crippa A, Ferraretti AP, Gianaroli L. Preimplantation genetic testing: Polar bodies, blastomeres, trophectoderm cells, or blastocoelic fluid? Fertil Steril. 2016;105:676–e683675.
21. Shi W, Zhao Z, Xue X, Li Q, Yao Y, Wang D, Wang J, Lu S, Shi J. Ploidy testing of Blastocoel fluid for screening May be technically challenging and more invasive than that of spent cell culture media. Front Physiol 2022, 13.
22. Tobler KJ, Zhao Y, Ross R, Benner AT, Xu X, Du L, Broman K, Thrift K, Brezina PR, Kearns WG. Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis. Fertil Steril. 2015;104:418–25.
23. Kang X, Wen M, Zheng J, Peng F, Zeng N, Chen Z, Wu Y, Sun H. Influence of the number of washings for embryos on non-invasive preimplantation chromosome screening results. Front Endocrinol (Lausanne). 2024;15:1363851.
24. Hu Z, Li Y, Chen K, Li M, Tian M, Xiang L, Wu X, Zeng P, Li M, Shao J, et al. The comparison of two Whole-Genome amplification approaches for noninvasive preimplantation genetic testing (ni-PGT) and the application scenario of ni-PGT during the fresh cycle. J Mol Diagn. 2023;25:945–56.
25. Rubio C, Navarro-Sánchez L, García-Pascual CM, Ocali O, Cimadomo D, Venier W, Barroso G, Kopcow L, Bahçeci M, Kulmann MIR, et al. Multicenter prospective study of concordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts. Am J Obstet Gynecol. 2020;223:e751751–751713.
26. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature. 1990;344:768–70.
27. Xu J, Fang R, Chen L, Chen D, Xiao J-P, Yang W, Wang H, Song X, Ma T, Bo S, et al. Noninvasive chromosome screening of human embryos by genome sequencing of embryo culture medium for in vitro fertilization. Proc Natl Acad Sci. 2016;113:11907–12.
28. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JPA, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009;339:b2700–2700.
29. Liu W, Liu J, Du H, Ling J, Sun X, Chen D. Non-invasive pre-implantation aneuploidy screening and diagnosis of beta thalassemia IVSII654 mutation using spent embryo culture medium. Ann Med. 2016;49:319–28.
30. Hanson BM, Tao X, Hong KH, Comito CE, Pangasnan R, Seli E, Jalas C, Scott RT. Noninvasive preimplantation genetic testing for aneuploidy exhibits high rates of deoxyribonucleic acid amplification failure and poor correlation with results obtained using trophectoderm biopsy. Fertil Steril. 2021;115:1461–70.
31. Ho JR, Arrach N, Rhodes-Long K, Ahmady A, Ingles S, Chung K, Bendikson KA, Paulson RJ, McGinnis LK. Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos. Fertil Steril. 2018;110:467–e475462.
32. Chen L, Sun Q, Xu J, Fu H, Liu Y, Yao Y, Lu S, Yao B. A Non-invasive chromosome screening strategy for prioritizing in vitro fertilization embryos for implantation. Front Cell Dev Biology 2021, 9.
33. Sun B-l, Wang Y, Sixi W, Zhou L, Zhang C-h, Wu Z-X, Qiao J, Sun Q-y, Yao Y-x, Wang J et al. Effectiveness of non-invasive chromosomal screening for normal karyotype and chromosomal rearrangements. *Frontiers in Genetics* 2023, 14.
34. Zhao H, Yuan P, Chen X, Lin H, Zhao J, Huang J, Qiu Q, Ji X, Zhang Q, Wang W. The aneuploidy testing of blastocysts developing from 0PN and 1PN zygotes in conventional IVF through TE-biopsy PGT-A and minimally invasive PGT-A. Front Reproductive Health 2022, 4.
35. Griffin DK, Brezina PR, Tobler K, Zhao Y, Silvestri G, McCoy RC, Anchan R, Benner A, Cutting GR, Kearns WG. The human embryonic genome is karyotypically complex, with chromosomally abnormal cells preferentially located away from the developing fetus. Hum Reprod. 2023;38:180–8.
36. Li X, Hao Y, Chen D, Ji D, Zhu W, Zhu X, Wei Z, Cao Y, Zhang Z, Zhou P. Non-invasive preimplantation genetic testing for putative mosaic blastocysts: a pilot study. Hum Reprod. 2021;36:2020–34.
37. Popovic M, Dheedene A, Christodoulou C, Taelman J, Dhaenens L, Van Nieuwerburgh F, Deforce D, Van den Abbeel E, De Sutter P, Menten B, Heindryckx B. Chromosomal mosaicism in human blastocysts: the ultimate challenge of preimplantation genetic testing? Hum Reprod. 2018;33:1342–54.
38. Vera-Rodriguez M, Diez-Juan A, Jimenez-Almazan J, Martinez S, Navarro R, Peinado V, Mercader A, Meseguer M, Blesa D, Moreno I, et al. Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development. Hum Reprod. 2018;33:745–56.
39. Wu L, Jin L, Chen W, Liu JM, Hu J, Yu Q, Ren XL, Huang B, He H. The true incidence of chromosomal mosaicism after preimplantation genetic testing is much lower than that indicated by trophectoderm biopsy. Hum Reprod. 2021;36:1691–701.
40. Sialakouma A, Karakasiliotis I, Ntala V, Nikolettos N, Asimakopoulos B. Embryonic Cell-free DNA in spent culture medium: A Non-invasive tool for aneuploidy screening of the corresponding embryos. Vivo. 2021;35:3449–57.
41. Lei C, Fu J, Li X, Zhou J, Xiao M, Zhang S, Sun Y, Sun X. Re-denudation of residual cumulus cells on day 3 increases the accuracy of cell-free DNA detection in spent embryo culture medium. J Assist Reprod Genet. 2022;39:1653–60.
42. Yeung QSY, Zhang YX, Chung JPW, Lui WT, Kwok YKY, Gui B, Kong GWS, Cao Y, Li TC, Choy KW. A prospective study of non-invasive preimplantation genetic

testing for aneuploidies (NiPGT-A) using next-generation sequencing (NGS) on spent culture media (SCM). J Assist Reprod Genet. 2019;36:1609–21.
43. Zhang J, Xia H, Chen H, Yao C, Feng L, Song X, Bai X. Less-invasive chromosome screening of embryos and embryo assessment by genetic studies of DNA in embryo culture medium. J Assist Reprod Genet. 2019;36:2505–13.
44. Xie P, Zhang S, Gu Y, Jiang B, Hu L, Tan Y-q, Yao Y, Tang Y, Wan A, Cai S et al. Non-invasive preimplantation genetic testing for conventional IVF blastocysts. J Translational Med 2022, 20.
45. Chuang T-H, Hsieh J-Y, Lee M-J, Lai H-H, Hsieh C-L, Wang H-L, Chang Y-J, Chen S-U. Concordance between different trophectoderm biopsy sites and the inner cell mass of chromosomal composition measured with a next-generation sequencing platform. MHR: Basic Sci Reproductive Med. 2018;24:593–601.
46. Kelly GM, Kuznyetsov V, Madjunkova S, Antes R, Abramov R, Motamedi G, Ibarrientos Z, Librach C. Evaluation of a novel non-invasive preimplantation genetic screening approach. PLoS ONE 2018, 13.
47. Shitara A, Takahashi K, Goto M, Takahashi H, Iwasawa T, Onodera Y, Makino K, Miura H, Shirasawa H, Sato W, et al. Cell-free DNA in spent culture medium effectively reflects the chromosomal status of embryos following culturing beyond implantation compared to trophectoderm biopsy. PLoS ONE. 2021;16:e0246438.
48. Wrenzycki C, Shitara A, Takahashi K, Goto M, Takahashi H, Iwasawa T, Onodera Y, Makino K, Miura H, Shirasawa H et al. Cell-free DNA in spent culture medium effectively reflects the chromosomal status of embryos following culturing beyond implantation compared to trophectoderm biopsy. PLoS ONE 2021, 16.
49. Huang L, Bogale B, Tang Y, Lu S, Xie XS, Racowsky C. Noninvasive preimplantation genetic testing for aneuploidy in spent medium May be more reliable than trophectoderm biopsy. Proc Natl Acad Sci. 2019;116:14105–12.
50. Gui B, Yao Z, Li Y, Liu D, Liu N, Xia Y, Huang Y, Mei L, Ma R, Lu S, et al. Chromosomal analysis of blastocysts from balanced chromosomal rearrangement carriers. Reproduction. 2016;151:455–64.
51. Lledo B, Morales R, Ortiz JA, Rodriguez-Arnedo A, Ten J, Castillo JC, Bernabeu A, Llacer J, Bernabeu R. Consistent results of non-invasive PGT-A of human embryos using two different techniques for chromosomal analysis. Reprod Biomed Online. 2021;42:555–63.
52. Xu CL, Wei YQ, Tan QY, Huang Y, Wu JJ, Li CY, Ma YF, Zhou L, Liang B, Kong LY, et al. Concordance of PGT for aneuploidies between blastocyst biopsies and spent blastocyst culture medium. Reprod Biomed Online. 2023;46:483–90.
53. Takahashi H, Takahashi K, Goto M, Hirakawa T, Hasegawa H, Shitara A, Iwasawa T, Togashi K, Makino K, Shirasawa H, et al. Consistency between chromosomal status analysis of biopsied human blastocyst trophectoderm cells and whole blastocyst cells. Reproductive Med Biology. 2021;20:444–50.
54. Kulmann MIR, Riboldi M, Martello C, Bos-Mikich A, Frantz G, Dutra C, Donatti LM, Oliveira N, Frantz N. First baby born in Brazil after simultaneous diagnosis through Non-Invasive and conventional PGT-A. Revista Brasileira De Ginecol E Obstetrícia / RBGO Gynecol Obstet. 2021;43:878–82.
55. Kuznyetsov V, Madjunkova S, Abramov R, Antes R, Ibarrientos Z, Motamedi G, Zaman A, Kuznyetsova I, Librach CL. 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.
56. Li P, Song Z, Yao Y, Huang T, Mao R, Huang J, Ma Y, Dong X, Huang W, Huang J et al. Preimplantation genetic screening with spent culture medium/blastocoel fluid for in vitro fertilization. Sci Rep 2018, 8.
57. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155:529–36.
58. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol. 2005;58:882–93.
59. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, Silverberg K, Kalista T, Handyside AH, Katz-Jaffe M, 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;112:1071–e10791077.
60. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, Sun X, Ling X, Li H, Hao C, et al. Live birth with or without preimplantation genetic testing for aneuploidy. N Engl J Med. 2021;385:2047–58.
61. Scriven PN. PGT-SR (reciprocal translocation) using trophectoderm sampling and next-generation sequencing: insights from a virtual trial. J Assist Reprod Genet. 2021;38:1971–8.
62. Pei Z, Deng K, Lei C, Du D, Yu G, Sun X, Xu C, Zhang S. Identifying balanced chromosomal translocations in human embryos by Oxford nanopore sequencing and breakpoints region analysis. Front Genet. 2021;12:810900.
63. van Echten-Arends J, Mastenbroek S, Sikkema-Raddatz B, Korevaar JC, Heineman MJ, van der Veen F, Repping S. Chromosomal mosaicism in human preimplantation embryos: a systematic review. Hum Reprod Update. 2011;17:620–7.
64. Fragouli E, Alfarawati S, Spath K, Babariya D, Tarozzi N, Borini A, Wells D. Analysis of implantation and ongoing pregnancy rates following the transfer of mosaic diploid–aneuploid blastocysts. Hum Genet. 2017;136:805–19.
65. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, Cimadomo D, Benini F, Farcomeni A, Cuzzi J, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet. 2021;108:2238–47.
66. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, Scott RT. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril. 2014;101:656–e663651.
67. Wei L, Zhang J, Shi N, Luo C, Bo L, Lu X, Gao S, Mao C. Association of maternal risk factors with fetal aneuploidy and the accuracy of prenatal aneuploidy screening: a correlation analysis based on 12,186 karyotype reports. BMC Pregnancy Childbirth 2023, 23.
68. Vitagliano A, Paffoni A, Viganò P. Does maternal age affect assisted reproduction technology success rates after euploid embryo transfer? A systematic review and meta-analysis. Fertil Steril. 2023;120:251–65.
69. Barad DH, Albertini DF, Molinari E, Gleicher N. IVF outcomes of embryos with abnormal PGT-A biopsy previously refused transfer: a prospective cohort study. Hum Reprod. 2022;37:1194–206.
70. Yang L, Shi W, Li Y, Tong J, Xue X, Zhao Z, Zhang N, Wang D, Fatim I, Liao M, Shi J. SCM is potential resource for non-invasive preimplantation genetic testing based on human embryos single-cell sequencing. Gene 2023, 882.
71. Chen Y, Gao Y, Jia J, Chang L, Liu P, Qiao J, Tang F, Wen L, Huang J. DNA methylome reveals cellular origin of cell-free DNA in spent medium of human preimplantation embryos. J Clin Invest 2021, 131.
72. Maheshwari A, Bari V, Bell JL, Bhattacharya S, Bhide P, Bowler U, Brison D, Child T, Chong HY, Cheong Y, et al. Transfer of thawed frozen embryo versus fresh embryo to improve the healthy baby rate in women undergoing IVF: the E-Freeze RCT. Health Technol Assess. 2022;26:1–142.
73. Maheshwari A, Bell JL, Bhide P, Brison D, Child T, Chong HY, Cheong Y, Cole C, Coomarasamy A, Cutting R, et al. Elective freezing of embryos versus fresh embryo transfer in IVF: a multicentre randomized controlled trial in the UK (E-Freeze). Hum Reprod. 2022;37:476–87.
74. Guerif F, Lemseffer M, Bidault R, Gasnier O, Saussereau MH, Cadoret V, Jamet C, Royere D. Single day 2 embryo versus blastocyst-stage transfer: a prospective study integrating fresh and frozen embryo transfers. Hum Reprod. 2009;24:1051–8.
75. Chapple V, KB, FQ, KP RW, Natalwala J. YL: Impacts of double biopsy and double vitrification on the clinical outcomes following euploid blastocyst transfer: a systematic review and meta-analysis. Hum Reprod 2024:39.

Publisher's note