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Research Advances in Non-Invasive Preimplantation Evaluation of Embryonic Chromosomal Aneuploidy

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Abstract: Chromosomal aneuploidy is one of the leading causes of miscarriage. Preimplantation genetic testing for aneuploidy (PGT-A) can reduce this risk. However, all existing clinical approaches are based on invasive trophoblast biopsy, which constitutes a complex diagnosis and treatment procedure that carries the risk of embryonic damage and raises concerns regarding the long-term health of the offspring. Detection of cell-free DNA (cfDNA) isolated from blastocyst culture medium and blastocoel fluid enables non-invasive PGT-A (niPGT-A) of embryos. Multiple studies have shown its high accuracy, yet it still awaits clinical validation. Meanwhile, time-lapse imaging and artificial intelligence (AI) can predict embryo ploidy based on embryo morphology, and their performance is also quite remarkable. This review systematically compares these two non-invasive approaches, namely cfDNA-based niPGT-A and AI-based prediction. We analyze the technical bottlenecks of the former (such as cfDNA contamination, amplification bias, inconsistent results, etc.) and the interpretability-acceptability dilemma of the latter, and explore the value and strategies of combining the two methods, aiming to provide guidance for the precise development and clinical application of niPGT-A.

Keywords: Embryonic Chromosomal Aneuploidy; Blastocyst Biopsy; NiPGT-A; Time-lapse Embryo Imaging Technology; Artificial Intelligence

1. Introduction

Embryonic chromosomal aneuploidy abnormalities caused by errors in meiosis or mitosis are the primary causes of early pregnancy miscarriage, recurrent miscarriage, and fetal stillbirth [1]. A recent study found that among 467 samples of embryonic arrest in the early stages of pregnancy, 254 cases were caused by genetic factors, and the proportion of pregnancy loss caused by chromosomal aneuploidy was as high as 44.1% [2]. As the mother's age increases, the risk of meiotic errors in oocyte chromosomes rises significantly. The proportion of pregnant women over 35 years old carrying fetuses with chromosomal abnormalities is significantly higher than that of women under 35 (71.76% vs. 47.55%) [3]. Additionally, in a study related to recurrent pregnancy loss (RPL), it was found that 56.9% of the cases had chromosomal abnormalities, among which numerical chromosomal abnormalities accounted for 92.1% [4].

PGT-A technology significantly reduces fetal pregnancy loss caused by chromosomal abnormalities by screening and transplanting euploid embryos [5]. In 1996, Verlinsky et al. [6] first attempted preimplantation genetic screening for aneuploidy, detecting common aneuploidies in 135 older-age patients (there were 161 cycles in total) during in vitro fertilization (IVF). Eventually, 6 cases of healthy deliveries and 12 cases of ongoing pregnancies were achieved. In 2017, the technology was internationally unified and named PGT-A [7]. Blastocyst biopsy is currently the most widely used sampling method in the clinical detection of PGT-A. Blastocysts are more resilient than early-stage embryos and more tolerant of micromanipulation. There is no significant difference in the implantation rate between biopsied blastocysts and non-biopsied embryos (51% vs. 54%), while blastomere biopsy significantly reduces the implantation rate by 39% [8]. Moreover, when the trophectoderm (TE) score is grade A and the number of biopsied cells is less than 41, an

increase in the number of trophoblast cell samples used for biopsy does not significantly affect either the survival rate of blastocysts or the implantation rate [9]. Compared with the control group that selects blastocysts for transplantation based solely on morphological scores, PGT-A can significantly increase the delivery rate of the first transplantation and the viable birth rate of advanced-age women and reduce the miscarriage rate [10]. However, there are still potential risks associated with trophoblast biopsy. A study comparing the group that underwent PGT with the group that did not revealed that the incidence of pre-eclampsia in the PGT group was significantly higher than that in the non-PGT group. Moreover, the incidence of placenta previa was also higher in the PGT group [11]. However, due to the universality of cell migration, it is challenging to perform a biopsy on the TE to determine the actual differentiation status of the inner cell mass (ICM), and the positive diagnosis rate does not exceed 52.8% [5,12]. Due to problems such as insufficient accuracy, embryo damage, and operational complexity in PGT-A technology, scholars in reproductive medicine are exploring niPGT-A, a non-invasive detection method, aiming to obtain embryonic genetic information through non-invasive means and provide a basis for precise transplantation in assisted reproduction.

With the development of AI, its cross-application with different disciplines has become more and more mature. Radiomics, as a technical system for quantitative analysis of medical images, can convert images into high-dimensional data for mining and analysis [13]. When analyzing data obtained through radiomics, the application of machine learning or deep learning techniques represents a data-driven innovative approach, but AI still faces many challenges in predicting embryo ploidy [14]. Molecular non-invasive detection represented by niPGT-A and non-invasive imaging analysis driven by radiomics + AI together lead the development trend of non-invasive embryo evaluation in the territory of auxiliary reproduction, providing the possibility for low-damage and high-precision embryo screening.

2. Research Methods

In this study, a systematic retrieval was conducted in the PubMed and Web of Science databases, with the retrieval time range spanning from the establishment of each database to July 2026. The search strategy was formulated by combining the following core keywords and their synonyms/near-synonyms, including "embryonic chromosomal aneuploidy", "blastocyst biopsy", "non-invasive preimplantation genetic testing for aneuploidy", "time-lapse embryo imaging technology", "artificial intelligence" (and its extended terms such as "machine learning", "deep learning"). The above-mentioned keywords were combined using Boolean logical operators (AND/OR). For instance, terms related to niPGT-A were combined with aneuploidy, and time-lapse and AI technologies were used as supplementary search dimensions. The language of the retrieved literature was restricted to English.

Inclusion criteria: (1) The research content involves non-invasive detection methods for embryonic chromosomal aneuploidy, with particular attention to the niPGT-A technology based on cfDNA in the spent culture medium (SCM), as well as the auxiliary use of time-lapse imaging or AI in aneuploidy assessment; (2) The literature type is original research, clinical trial, or systematic review; (3) The publication language is English. Duplicate publications, conference abstracts, comments, and letters are excluded.

First, a preliminary screening is conducted based on the titles and abstracts. Subsequently, a secondary screening is carried out through a full-text review. Discrepancies are resolved through discussions or third-party arbitration. From the finally included literature, the following information is extracted: the first author, publication year, research design, sample size, detection technology platform, cfDNA source, consistency between niPGT-A and traditional biopsy, and key parameters related to the time-lapse/AI model (such as algorithm type, prediction accuracy, etc.).

This paper summarizes the literature according to the logical framework of "Technical principle and detection efficacy of niPGT-A-Emerging applications of time-lapse imaging and AI in aneuploidy assessment-Current technological bottlenecks and challenges (such as maternal contamination, low yield of cfDNA, and model generalization ability)-Future prospects".

3. Research Results

3.1 NiPGT-A Accuracy of Detection

To analyze the accuracy of niPGT-A test results, consistency analysis is usually conducted by comparing the niPGT-A test results with those of TE biopsy. Some research indicates that the consistency rates of ploidy and sex between niPGT-A and TE biopsy in 183 fresh embryos were 76.5% and 98.9%, respectively [15]. Rubio et al. [16] performed TE and SCM tests on 115 blastocysts, and the ploidy and sex results of the two tests also showed high consistency. Meanwhile, it was observed that when the euploidy results of TE and SCM tests were consistent, there was no miscarriage after embryo transfer. However, some studies have reported that the consistency between the two is relatively low. The complete chromosomal consistency between SCM and the whole embryo was 32.2%, significantly lower than the 69.33% between TE and the whole embryo [17]. The reason for the relatively low consistency may be related to the collection of SCM at different time points during embryo culture. Compared with Day 5 embryos, the ploidy consistency rate (72.7% vs. 60.0%) and sex-chromosome consistency rate (90.9% vs. 60.0%) of SCM in Day 6 embryos were significantly higher, which indicates that increasing the contact time between the culture medium and the embryo can improve these parameters [18]. The data on the consistency study between niPGT-A and TE biopsy or whole-embryo analysis results in recent years are detailed in Table 1. These data suggest that using SCM testing may be a reliable alternative to blastocyst biopsy.

Table 1. Summary Table of Relevant Studies on Nipgt-A in Recent Years

Author/ Year	Embryonic origin	Number of cultivation days	WGA	DNA sequencing	Consistency with PGT-A
Pan (2025) [15]	183 pairs of TE biopsy and blastocyst SCM samples from 60 patients.	D4~D7	TE: PicoPLEX Gold Single Cell DNA-Seq kit (Takara Bio, Mountain View, CA, USA); SCM: PicoPLEX Gold,PG-Seq Rapid Non-Invasive PGT kit (PerkinElmer,Waltham,MA, USA)or NICSlnst(Yikon Genomics,Shanghai,China)	NextSeq550 Illumina Sequencer (Illumina Inc., San Diego, CA, USA)	The ploidy concordance rate between niPGT-A and PGT-A was 76.5% (183 samples).
Wu (2025) [19]	212 TE biopsy samples and their corresponding SCM data from 98 couples.	D5~D6	SurePlex DNA amplification system (VeriSeq PGS Kit, Illumina)	Illumina MiSeq platform	The ploidy consistency of D5 blastocysts was 76.8% (63/82), that of D6 blastocysts was 73.4% (69/94), and the overall consistency was 75.9% (132/174).
Singh (2025) [19]	Forty-four blastocysts and SCMs from 14 patients undergoing IVF/ICSI cycles	D5~D6	Sureplex DNA amplification system (Illumina, CA, USA)	MiSeq (Illumina, California, USA).	The NGS results of 65.38% (17/26) of the embryo TE biopsy and SCM samples were consistent. The consistency rate for each chromosome was 85.13% (487/572), and the consistency rate for sex chromosomes was 73% (19/26).
Takeuchi (2024) [20]	Thirty-five blastocysts donated by 24 patients who underwent IVF	D5~D6	Ion ReproSeq PGS Kit (Thermo Fisher Scientific)	S5 sequencer (ThermoFisher Scientific)	The consistency rates between the whole embryo and SCM, the whole embryo and TE, and TE and SCM were 51.4% (18/35), 91.4% (32/35), and 51.4% (18/35), respectively.
Xu (2023) [21]	Thirty-five donated blastocysts	D5~D6	PicoPLEX WGA Kit (TAKARA, Japan)	DA8600 platform (Basecare)	Using the ICM as the gold standard, the chromosomal ploidy consistency rates of SCM, TE biopsy, and residual blastocysts were 58.33% (14/24), 68.75% (22/32), and 78.57% (22/28) respectively; the diagnostic consistency rates were 83.33% (20/24), 87.50% (28/32), and 92.86% (26/28) respectively; and the gender consistency rates were

					92.31% (24/26), 100% (32/32), and 100.00% (28/28) respectively.
Xie (2022) [22]	161 samples from 25 conventional IVF cycles and 122 samples from 37 ICSI cycles	D5~D6	-	NextSeq 550 sequencer (Cat No. SY-415-1002, Illumina, Inc., USA)	Using TE biopsy results as a control, there is no significant difference in the performance of niPGT between IVF and ICSI. After adjusting to a 50% mosaicism threshold, the concordance rates are 75.0% and 74.6% respectively, and the indicators such as sensitivity and specificity are also similar.
Sonehara (2022) [23]	Forty-six pairs of embryos and their corresponding SCMs were classified into two groups according to morphological grading.	D6~D7	PG-Seq Rapid Non-Invasive Kit (PerkinElmer)	iSeq 100 system (Illumina)	In low-grade embryos, whole-embryo analysis revealed that 63.6% (14/22) were aneuploid, and the concordance rate of niPGT-A was 54.5% (12/22). In high-grade embryos, whole-embryo analysis indicated that 41.7% (10/24) were aneuploid, and the concordance rate of niPGT-A was 62.5% (15/24).
Tsai (2022) [24]	Forty SCMs from seven couples, corresponding to 29 TE biopsy samples and 11 whole embryo control samples respectively.	D5~D6	ChromInst Library Preparation Kit or NICSInst Library Preparation Kit	Illumina MiSeq platform	The overall ploidy consistency between niPGT-A and PGT-A is 21/31 (67.7%), and the complete concordance rate is 8/31 (25.8%).
Sialakouma (2021) [25]	Forty embryos were collected from 13 infertile patients who underwent PGT-A.	D5~D6	SurePlex kit (BlueGnome Ltd., Mill Court, Great Shelford, Cambridge, UK)	Illumina VeriSeq PGS Kit analysis system	The overall concordance rate between niPGT-A and PGT-A was 27/33 (81.8%). The full concordance rate was 21/33 (63.6%). The aneuploidy concordance was 91.66%, and the euploidy concordance was 76.19%.

3.2 NiPGT-A Effectiveness of Detection

Currently, the effectiveness of niPGT-A remains in the clinical research stage, and some studies have shown positive results. Rubio et al. [16] divided the patients undergoing testing into two groups. In one group, both the TE biopsy and SCM results were euploid, while in the other group, the results were aneuploid. By comparing the embryo transfer success rates of the two groups, it was found that the transfer success rate of the "euploid" group (52.9%) was approximately three times that of the "aneuploid" group (16.7%), and no miscarriages occurred after the transfer of the 9 embryos in this group. Another study reported the cumulative live birth rates with NICS. In the NICS group, cumulative live births were achieved in 44 out of 98 oocyte retrieval cycles (44.9%). In the TE-PGT group, cumulative live births were achieved in 53 out of 104 oocyte retrieval cycles (51.0%). In the ICSI group, cumulative live births were achieved in 64 out of 229 oocyte retrieval cycles (27.9%). This study indicated that NICS is as effective as embryo biopsy, and compared with the ICSI group, both detection techniques significantly increased the cumulative live-birth rate [26]. However, some viewpoints suggest that there is a self-correction mechanism during the blastocyst stage, and some aneuploid cells in the early stage may be corrected to euploid cells during later development. This mechanism may lead to false-positive results in cfDNA detection [15]. Additionally, due to the existence of chimeras, the consistency of SCM and TE biopsy in ploidy and gender determination varies in different reports, ranging from 51.40% to 85.13% and from 73.0% to 98.9% respectively [15,18,20]. The cfDNA test cannot fully confirm the embryo's actual genome, which may lead to incorrect assessments and inappropriate treatment of some embryos with good developmental potential. An embryo allocation system that employs a random forest machine learning algorithm effectively prevents embryo

wastage. According to the NICS test results, blastocysts are classified into three grades (A, B, and C) based on the probability of euploidy. The clinical outcomes of A- and B-grade embryos are comparable to those of euploid embryos, with A- and B-grade embryos accounting for 78.8% of the total number of embryos. Among them, the probability of euploidy in group A embryos is over 90%, and they should be given priority for transplantation. Moreover, analyzing embryos with cfDNA amplification failure within the classification system can largely prevent embryo wastage [27].

These studies and clinical trials indicate that niPGT-A can effectively reduce the number of abortions, decrease embryo wastage, and increase the pregnancy rate. However, the above studies were limited to small-scale clinical trials, and larger randomized controlled clinical trials are needed to further confirm these findings.

3.3 Detection Effectiveness of AI Models

As the application of AI in assisted reproductive technology (ART) gradually progresses from theoretical exploration to clinical practice, it has brought revolutionary possibilities to key aspects of IVF, such as embryo screening and implantation prediction. A series of breakthrough achievements have been made in related research and application exploration. A non-invasive Life Whisperer AI model integrates computer vision image processing and deep learning techniques. It uses the AI model to predict embryo viability based on standard optical microscopic images of 8,886 embryos and their corresponding clinical outcomes. The overall accuracy of this model is 64.3%, which is 24.7% higher than that of embryologists' judgments [28]. A study investigated the relationship between clinical image AI algorithms (ERICA) and early spontaneous abortion, showing that the risk of miscarriage was significantly lower for high-quality/high-grade embryos compared to average/low-grade embryos, with an overall prediction accuracy rate of 67.4%. Although the difference is not significant among chromosomally normal embryos, it preliminarily indicates that ERICA can provide patients with reference information on abortion risk to assist clinical decision-making [29].

Analysis of time-lapse videos of 418 embryos detected by PGT-A was carried out. The Trans U-Net and Vision Transformer models were used to extract and quantify expansion kinetic features. By analyzing the embryo expansion patterns in time-lapse imaging through deep learning, the differences between euploid and aneuploid embryos were identified. The results showed that aneuploid embryos had a slower expansion rate and more frequent collapse events [30], whereas morphodynamic variables were significantly associated with euploid embryos. Yuan et al. [31] employed a highly robust machine learning model that integrates blastocyst morphological features with patients' clinical parameters to predict the ploidy of blastocysts. The model achieved an area under the curve (AUC) of 0.879, demonstrating high predictive accuracy. In 2024, Xin et al. [32] reported that the pooled sensitivity of AI in predicting embryo euploidy was 0.71, the specificity was 0.75, and the AUC was 0.80. In addition, they observed that the AUC for diagnostic accuracy of studies published after 2023 was 0.71 [95% CI (0.66, 0.74)], while that of studies published before 2023 was 0.62 [95% CI (0.58, 0.66)], indicating that AI has good potential in embryo ploidy prediction. Although it cannot completely replace invasive detection at present, AI is developing rapidly. Over time, it is reasonable to anticipate that the diagnostic accuracy of these models will improve further.

4. Analyze and Discuss

4.1. NiPGT-A Based on High-Throughput Sequencing of Cell-Free DNA

4.1.1 Comparison of two sampling approaches and applications of cell-free DNA

The cfDNA available for detection in pre-implantation embryos mainly originates from blastocoel fluid and SCM of embryos. In 2013, Palini et al. [33] first confirmed that amplifiable cfDNA exists in approximately 90% of blastocoel fluid samples. Through WGA combined with array comparative genomic hybridization (aCGH) technology, gender and aneuploidy detection can be achieved. A new method for minimally invasive genetic testing of embryos using this DNA was proposed, which can avoid the risks of embryo biopsy. However, the volume of blastocoel fluid is extremely small, with only about 0.01 μl of extractable sample, and the concentration of available cfDNA in it is even lower [34]. When the cfDNA in blastocoel fluid is used for genetic analysis after WGA, the consistency with the results of TE biopsy

reaches 97%, but the amplification success rate ranges from 40% to 98%, which is greatly affected by DNA yield and integrity [35]. Although it avoids the risks of traditional PGT-A embryo biopsy, this operation is not completely non-invasive.

In 2016, Xu et al. [36] proposed and verified a non-invasive chromosome screening (NICS) method based on SCM genome sequencing, achieving whole-genome chromosome screening based on SCM-DNA for the first time without embryo biopsy. Through multiple annealing and looping-based amplification cycles (MALBAC) technology, efficient amplification and accurate sequencing of low-starting DNA were realized. The cfDNA content in SCM is significantly higher than that in blastocoel fluid, and the sequencing success rate (100.0%) is higher than 69.8% of blastocoel fluid. Taking the ICM/whole embryo as the gold standard, the overall ploidy consistency (83.8%) between SCM sequencing results and the gold standard is higher than that of blastocoel fluid (69.6%), and the consistency of SCM sequencing results in embryos obtained by IVF and ICSI is better than that of blastocoel fluid. Therefore, in the development of niPGT-A technology, more exploration is carried out on the cfDNA in SCM [37].

To determine the source of cfDNA in SCM, single-cell genome DNA methylation sequencing (scBS-seq) was employed. The results indicated that this DNA originated from blastocysts (inner cell mass/trophectoderm), cumulus cells, and polar bodies [38]. The release mechanism of cfDNA in blastocoel fluid and SCM may be embryo cell apoptosis, lysis, or shedding, but the specific mechanism is unknown. The niPGT-A detection results of 52 blastocyst SCMs show that, compared with PGT-A of TE biopsy, niPGT-A can simultaneously reflect the ploidy of TE and ICM, with a false-negative rate of 0%. The positive predictive value and specificity are significantly higher than those of TE biopsy, and it is less affected by embryo chimerism [12]. The niPGT-A detection based on SCM has a higher positive predictive value and specificity. Compared with blastocoel fluid-based niPGT, the sampling method of SCM has less interference with embryos and a higher sequencing success rate, thus providing important theoretical and practical support for the non-invasive and accurate development of PGT-A.

4.1.2 Paternal/Maternal contamination of cell-free DNA

niPGT-A achieves non-invasive detection by analyzing cfDNA in SCM. However, its accuracy is affected by parental contamination. After conventional in vitro fertilization, unfertilized sperm may adhere to the embryo's zona pellucida, allowing their DNA to be released into the SCM and become a primary source of contamination originating from the father [39]. It is widely recognized that the injection of a single sperm into an egg cell through the ICSI technique can reduce DNA contamination caused by sperm cells in biopsy samples. However, with the advancement of detection technologies, it has now been confirmed that the impact of sperm cell contamination is negligible [40]. In fact, the amplification of sperm DNA requires relatively strong lysis conditions. The lysis conditions of WGA technology, such as MALBAC are relatively mild. These techniques improve the uniformity and accuracy of amplification through special primer design and amplification methods and have extremely low requirements for the initial template amount [41,42]. These characteristics make these techniques unable to effectively amplify highly condensed sperm DNA but perfectly suitable for the amplification of embryo biopsy cells. Data show that no paternal contamination was detected in 161 IVF blastocyst SCM samples, while the maternal contamination rate was as high as 29.8% [22].

Quantitative parental contamination testing (qPCT) can effectively detect paternal/maternal contamination. In the artificial contamination model, the proportion of maternal contamination is related to the number of cumulus cells [39]. Approximately half of the 191 high-quality SCM samples had cumulus cell contamination, and the proportion of polar body contamination was approximately 27%. Together with cumulus cells, they constitute maternal contamination and bring about a rise in the false-negative rate of aneuploidy detection in SCM and a decrease in the overall concordance rate with TE biopsy [38]. By comparing different SCM collection times and two embryo washing protocols (one-step method and sequential method), it was found that collecting SCM on the 6th day and using the sequential washing method can effectively reduce the contamination originating from maternal DNA and improve the accuracy of

detection [43]. A computational algorithm based on DNA methylation developed by Chen et al. [44] can effectively reduce the false-negative rate caused by non-embryonic contaminating DNA in SCM, especially significantly improving the sensitivity of aneuploidy detection when there is 50% cumulus cell contamination. Therefore, the accuracy of niPGT-A detection is mainly affected by maternal contamination. Reducing maternal contamination through scientific methods can avoid misdiagnosis caused by maternal DNA contamination.

4.2. AI-based Prediction of Embryo Chromosomal Euploidy Based on Embryo Morphology

4.2.1 Theoretical basis

AI is a broad term that encompasses machine learning and its sub-field, deep learning. Artificial neural networks (ANNs) are the most commonly used approach in AI, with common types including convolutional neural networks (CNN) and recurrent neural networks (RNN) [45]. Deep learning based on ANNs is a method that employs multi-layer neural networks for feature extraction and prediction. Machine learning, on the other hand, enables computers to autonomously learn the patterns in data through data training and make predictions on new data, learning the mapping relationship from input to output.

During the development process, embryos exhibit specific morphological features and dynamic developmental parameters, and there are statistical differences in these features between euploid and aneuploid embryos [30]. Deep learning models can automatically extract subtle features from time-lapse photography images or static images that are difficult to identify with the human eye. By training on a large amount of annotated embryo image data with known ploidy results, a complex mapping relationship between features and ploidy status can be established [46]. Some models combine clinical data and image features and use machine learning algorithms to construct a joint prediction model. This integration can compensate for the limitations of single image features and improve the robustness of the prediction [47].

4.2.2 Detection methods

Morphodynamics studies the continuous and time-defined processes of cell interactions and morphological changes during early embryogenesis, particularly in the stages of embryo development. Continuous observation of these processes can provide valuable insights into embryo quality, developmental potential, and the likelihood of successful pregnancy [48]. A time-lapse system is used to regularly capture detailed multi-planar images of the embryo development process; integrating this technology into IVF can provide particular explanation of embryo morphokinetics [49]. A large number of quantitative features are extracted from these medical images and then analyzed using AI. This minimizes the subjective factors of operators and enhances the accuracy of embryo ploidy prediction [14].

AI Support System (DSS) can be classified into the following types [14]: (1) Black-box models: These directly establish the mapping relationship between input and output through deep learning based on the original images. (2) Grey-box models: An additional feature annotation step (manual or automatic) is added between the images and the prediction, and the structured data are input into the black-box model. (3) Glass-box models: These combine image features with interpretable machine learning models (such as linear/logistic regression and decision trees). In the ploidy testing of IVF embryos, three different technical approaches of the AI decision support system based on morphokinetics and time-lapse imaging monitoring technology jointly transform the morphokinetic characteristics of embryos into quantitative evaluation criteria through AI technology, laying a methodological foundation for the validation of detection effectiveness and the realization of clinical value.

AI shows good potential in the field of assisted reproduction, but it also has its limitations. The clinical application of AI requires a large amount of data. To standardize the prediction results, the training of AI must overcome the limitations in data [50]. Moreover, the "black-box" nature of deep learning poses many challenges in embryo ploidy prediction, which restricts the universality of AI models [14]. In addition, the use of AI in the area of reproductive medicine will inevitably lead to some debates on ethical issues and challenges in patient information and data supervision.

5. Conclusion

Embryonic chromosomal aneuploidy is the core factor leading to early pregnancy loss and

recurrent miscarriage, particularly affecting high-risk groups such as advanced-age women and those with repeated implantation failure. Traditional PGT-A biopsy methods carry the risk of embryo damage. Meanwhile, affected by embryonic mosaicism, biopsy methods may not accurately reflect the chromosomal ploidy of the ICM. In recent years, the discovery of trace amounts of cfDNA in blastocoel fluid and SCM has greatly stimulated the exploration of the application potential of niPGT-A. Since using cfDNA from SCM for niPGT-A has a higher sequencing success rate and less interference with the embryo, it is considered a better option for niPGT-A. However, regarding the reliability of the niPGT-A strategy, in the published studies, there are still significant differences in the proportion of samples with obtained data and the consistency of genetic results with those of whole embryos or biopsy specimens. The mechanism of cfDNA release is not yet fully understood. Although there is good data supporting the use of cfDNA for niPGT-A, the reliability of niPGT-A in detecting the chromosomal status of embryos will continue to be tested until the cellular mechanisms involved in DNA release are fully understood. Moreover, problems such as maternal contamination and false positives still pose challenges. AI combined with time-lapse imaging monitoring of embryos can extract morphological and dynamic features to predict chromosomal ploidy, but it is limited by problems such as small data scale and the "black-box" characteristic. Currently, AI models cannot replace embryo ploidy testing. However, they can avoid unnecessary biopsy operations through decision-making assistance. To promote the clinical application of AI in this field, it is necessary to further develop and integrate comprehensive databases and conduct large-scale, multi-center prospective studies.

Currently, two main technical routes have emerged in the field of niPGT-A: cfDNA detection and AI morphological prediction. The former directly analyzes the chromosomal ploidy of embryos, and its accuracy is closer to that of traditional TE biopsy. It is suitable for clinical scenarios requiring clear genetic diagnosis but is restricted by bottlenecks such as low cfDNA yield, unclear release mechanism, and lack of standardized procedures. The latter is based on time-lapse imaging data modeling, with better non-invasiveness and simple operation. It is more suitable for embryo developmental potential assessment and rapid screening, but its model interpretability is insufficient, and its clinical acceptance needs to be improved. The two routes complement each other in terms of applicable scenarios. Future research can focus on the following aspects: (1) Elucidate the mechanisms of cfDNA release and degradation, optimize sample collection and enrichment procedures, and promote the standardization of niPGT-A testing; (2) Improve the interpretability of AI models and construct more clinically reliable prediction models by combining multi-omics data; (3) Explore the combined application of cfDNA detection and AI morphological prediction to achieve integrated non-invasive assessment of "genetic diagnosis + developmental potential assessment" and provide a more comprehensive solution for the clinical translation of niPGT-A.

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