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PREIMPLANTATION EMBRYO DIAGNOSIS-FROM INVASIVE TROPHOBLAST BIOPSY TO ANALYSIS OF CELL-FREE DNA IN CULTURE MEDIUM

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ABSTRACT

Background: Infertility is a global socio-demographic crisis, affecting approximately 17.5% of the population. Although in vitro fertilization is the therapeutic gold standard, the phenotype-genotype dichotomy reveals that morphologically ideal embryos often harbor genetic abnormalities, leading to implantation failure and miscarriages.
Objective: This review examines the transition from invasive methods to non-invasive preimplantation genetic testing (niPGT), evaluating cell-free DNA (cfDNA) analysis in culture media, integrated with artificial intelligence (AI), and time-lapse imaging. This synergy aims to optimize embryo selection and improve clinical outcomes in assisted reproductive medicine.
Methods: A comprehensive review of literature from PubMed and Google Scholar was conducted. The analysis focused on the mechanisms of cfDNA release, concordance rates between niPGT and trophectoderm biopsy, and the implementation of deep learning algorithms in morphokinetic assessment.
Results: NiPGT offers significant potential for providing a comprehensive genetic profile of the embryo, potentially eliminating mosaicism limitations inherent in traditional biopsies. Current results indicate concordance rates of 65-90% compared to invasive PGT. The integration of AI databases and Vision Transformer models enhances diagnostic accuracy by correlating molecular data with dynamic developmental patterns. However, challenges such as maternal DNA contamination and the lack of global standardization remain key barriers to widespread implementation.
Conclusion: NiPGT marks a new era in personalized reproductive medicine, prioritizing embryo safety and biological integrity. While currently a selection-support tool rather than a full replacement for invasive methods, its synergy with AI offers a safer, more effective path toward the gold standard- the birth of a single healthy child.

KEYWORDS

IVF, Embryo Selection, PGT, Non-invasive PGT, Cell-free DNA

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Introduction

Modern reproductive medicine defines infertility not only in terms of medical dysfunction of the reproductive system, but above all as a multidimensional phenomenon with growing social, demographic, and health implications. According to reports by the World Health Organization (WHO) and current population-based meta-analyses, fertility disorders currently affect one in six people of reproductive age worldwide, which corresponds to approximately 17.5% of the global population [1]. A retrospective analysis of epidemiological data indicates a dramatic upward trend in this phenomenon-it is estimated that since 1990, the incidence of infertility has increased by over 80% in women and by approximately 70% in men [2]. Such rapid progression makes infertility one of the most serious challenges for public health systems in the 21st century. The etiology and scale of this problem show a strong correlation with the socioeconomic development index. In highly developed countries, including Poland, there is an increasingly widespread trend of postponing the decision to have children until the fourth decade of life (on average, between 35 and 39 years of age) [3, 4]. From a physiological perspective, this is associated with an inevitable decline in ovarian reserve and a deterioration in oocyte quality. At the same time, scientific research increasingly emphasizes the role of the male factor, which for decades has been marginalized in the diagnostic process. Current reports indicate a significant correlation between exposure to environmental factors (e.g., microplastics and endocrine-disrupting compounds) and the progressive deterioration of semen parameters [5]. Prognostic models suggest that in the coming decades, male infertility will exhibit the highest growth rate, becoming a priority area of andrological research. From a clinical and sociological perspective, infertility leads to a significant deterioration in psychological health. The Years Lived with Disability index, which measures the number of years lived with disability, increases

significantly in this patient group due to the high prevalence of comorbid affective disorders, such as depression or anxiety disorders [6]. Furthermore, the high cost of assisted reproductive technology (ART), including in vitro fertilization (IVF) procedures, creates economic barriers leading to inequalities in access to medical care. Without the implementation of comprehensive systemic solutions, including reimbursement for highly specialized procedures, fertility-promoting preventive care, and rigorous protection of the ecosystem, the growing demographic deficit may lead to permanent and irreversible changes in social structures. The therapeutic strategy for treating infertility has evolved from conservative and minimally invasive methods toward advanced ART procedures. Currently, IVF is the standard clinical tool particularly in cases of advanced maternal age, irreversible tubal obstruction, or severe forms of oligo- and azoospermia [7]. Despite the ongoing optimization of stimulation protocols and culture conditions, achieving a live birth after the first transfer of a single embryo remains a clinical challenge. Statistics published in the literature indicate that the average success rate of a single procedure ranges between 30% and 40%, which necessitates the search for innovative methods to improve the predictive value of the transfer [8]. The aim of this study is to analyze the available methods of preimplantation genetic testing (PGT), which has ceased to serve solely as a screening tool for severe monogenic diseases and has become a strategic tool for selecting embryos with the highest implantation potential. The implementation of PGT into the IVF protocol allows for a significant reduction in the time to clinical pregnancy and minimizes the risk of failure resulting from the transfer of an abnormal karyotype-primarily aneuploidy [9]. While the gold standard has long been based on invasive preimplantation genetic testing (iPGT), fhe evolution of preimplantation genetics toward niPGT represents a breakthrough in optimizing ART outcomes. The use of cfDNA analysis present in the culture medium not only eliminates the risk of mechanical damage associated with trophectoderm biopsy but also offers a holistic insight into the embryo's genetic status [10]. The drive to establish niPGT as the standard in modern clinical embryology has the potential to revolutionize the embryo selection process, making the IVF procedure safer and more effective.

Aim of study: A comprehensive review of the evolution from iPGT to niPGT**,** evaluating the diagnostic efficacy of cfDNA analysis in spent culture media. The study integrates the role of AI, deep learning algorithms (DCNN, RNN/LSTM, ViT), and Time-Lapse Technology (TLT) in establishing multimodal predictive models to optimize embryo selection and clinical outcomes in assisted reproduction.

Methods: This systematic review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A comprehensive literature search was performed across major medical databases, including PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar, focusing on the latest advancements in reproductive genetics and digital embryology.

Eligibility Criteria

Studies were included if they met the following criteria:

-Study design: Randomized controlled trials, meta-analyses, systemic review, prospective clinical cohorts, and high-quality experimental studies investigating PGT-A/M/SR or niPGT.

-Population: Human embryos in the preimplantation stage (blastocysts), patients undergoing IVF/ICSI procedures, or experimental models focused on embryo genetics.

-Focus: comparison between invasive trophectoderm biopsy and non-invasive cfDNA analysis in spent culture medium, application of AI algorithms and morphokinetic parameters (TLT) in embryo grading, correlation between embryo morphology, ploidy status, and clinical pregnancy rates.

-Language: Full-text articles published in English.

Exclusion criteria were:

-Studies unrelated to preimplantation diagnostics or ART.

-Abstract-only publications, editorials, or case reports with low evidentiary value.

-Studies focusing on outdated cytogenetic methods without relevance to current Next- Generation Sequencing (NGS) standards.

Study Selection

Two independent reviewers screened all titles and abstracts for eligibility, followed by a full-text review of potentially relevant articles. Discrepancies regarding the diagnostic accuracy of niPGT versus iPGT or the efficacy of AI models were resolved through discussion or consultation with a third reviewer.

Data Extraction

Data extracted from each study included: publication year, study design, sample size (number of embryos/cycles), diagnostic platform (e.g., NGS), concordance rates between Spent Culture Medium (SCM) and trophectoderm biopsy, AI model architecture (DCNN, ViT), and clinical outcomes (implantation rates, live birth rates).

Risk of Bias and Ethical Considerations

All included clinical studies reported adherence to ethical standards such as the Declaration of Helsinki and Good Clinical Practice guidelines. Specific attention was paid to institutional review board approvals regarding the use of human embryos and the ethical implications of AI-driven selection and genetic screening.

Data Synthesis

Due to significant heterogeneity in laboratory protocols (different culture media, incubation system, and NGS platforms), a narrative synthesis approach was employed. Data were compared descriptively to identify patterns in cfDNA release mechanisms, the impact of maternal DNA contamination, and the predictive power of multimodal data fusion. A total of 43 studies were ultimately included in this review.

Literature Review Results

The Evolution of Embryo Selection Criteria: From Morphological Assessment to Molecular Diagnostics

For decades, classical clinical embryology has been based on the theory that the morphological phenotype serves as a reliable indicator of an embryo's biological potential and developmental competence. The foundation of selection under laboratory conditions was static morphological assessment, including analysis of cell division kinetics, the degree of cytoplasmic fragmentation, and blastocyst architecture, taking into account the trophectoderm and inner cell mass (ICM) based on standardized classification systems. The most commonly used scales are the Gardner and Schoolcraft scales [11]. Although these parameters provide key data on the dynamics of early embryonic development, modern reproductive medicine demonstrates that the correlation between correct morphology and the genetic integrity of the embryo is limited and often imprecise. The main factor limiting the effectiveness of visual methods is the phenomenon of aneuploidy-the presence of an abnormal number of chromosomes in embryos with a normal phenotype. Clinical analyses confirm that chromosomal abnormalities account for over 50% of implantation failures and early pregnancy losses [12]. These are molecular-level aberrations that remain undetectable using traditional light microscopy, regardless of the sophistication of the optics. A major breakthrough in PGT was the introduction of advanced genetic testing, particularly Preimplantation Genetic Testing for Aneuploidies (PGT-A). Laboratory practice documents cases in which embryos classified as morphologically suboptimal exhibit a normal chromosome set and result in live births, while blastocysts with exemplary morphology are genetically incapable of further development [13]. Another significant methodological issue is the subjectivity of expert assessment. Classical morphological analysis is burdened by a high degree of inter-observer variability, dependent on the embryologist's clinical experience, technical parameters of the equipment, and human factors, such as fatigue. This diagnostic uncertainty has accelerated the shift toward objective, measurable, and reproducible methods. Currently, the standard in modern embryology is the integration of morphology with complementary systems, such as: molecular diagnostics for direct pre-transfer DNA analysis, TLT for continuous monitoring of morphokinetics under controlled incubation conditions, thereby eliminating environmental stress on the embryo and providing precise timing parameters for cell division (tLC, t2, t5), and AI, utilizing advanced machine learning algorithms capable of analyzing thousands of data points and identifying subtle markers of developmental competence that correlate with genetic results and implantation rates [14, 15]. This shift in approach from subjective visual assessment to multidimensional genetic and morphokinetic analysis represents one of the key breakthroughs in reproductive medicine, enabling a significant reduction in the time to pregnancy and improved patient safety.

From Invasive Biopsy to Non-invasive Methods

The identification of limitations in traditional phenotypic assessment has steered the development of reproductive medicine toward direct analysis of the embryonic genome. PGT has undergone a radical transformation over the past decade, driven by the pursuit of maximizing the sensitivity and specificity of the test while minimizing the iatrogenic biological stress to which the preimplantation-stage embryo is exposed.

Invasive standard: trophectoderm biopsy

Classic preimplantation genetic testing currently forms the basis of clinical management for patients in high-risk genetic groups and in cases of recurrent implantation failure. This procedure is based on micromanipulation involving the mechanical collection of 5 to 10 cells from the blastocyst's trophectoderm layer, typically on the 5^{th} or 6^{th} day of in vitro culture. The methodological evolution of PGT, from early cytogenetic techniques (FISH) to high-throughput NGS, has enabled the precise categorization of testing into three main diagnostic pillars: PGT-A, PGT-M, and PGT-SR. The dominant diagnostic modality in ART

procedures is PGT-A. Its primary objective is screening-based monitoring of chromosome numbers to detect aneuploidies-aberrations involving changes in the number of individual chromosomes in the karyotype. This phenomenon is the main determinant of failed implantation, early pregnancy loss, and serious congenital defects, such as syndromes associated with trisomy within a pair of autosomes (including Down syndrome, Edwards syndrome, and Patau syndrome) [16]. The implementation of PGT-A allows for the selective transfer of euploid embryos, which shortens the time to pregnancy and minimizes the risk of iatrogenic complications for the patient [17]. In cases where family history indicates specific molecular defects, Preimplantation Genetic Testing for Monogenic Disorders (PGT-M) is used. This is a highly targeted diagnostic method aimed at identifying specific monogenic pathogenic variants. This method allows for the effective prevention of the transmission of hereditary diseases such as cystic fibrosis, Huntington's disease, spinal muscular atrophy, and hemophilia. Due to the need to design individualized diagnostic protocols for each case, PGT-M forms the foundation of precision personalized medicine, which is associated with higher costs for couples trying to conceive [18]. The diagnostic panel is complemented by Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR), dedicated to carriers of balanced structural rearrangements, primarily reciprocal translocations, Robertsonian translocations, and inversions. Although these changes typically do not manifest clinically in parents, the process of gametogenesis in carriers carries a high risk of producing unbalanced gametes. This leads to the formation of embryos with significant deletions or duplications of chromosome segments, which drastically reduces their chances of implantation and developmental potential [19]. Despite the high diagnostic reliability and established position of classical PGT in clinical protocols, this technique presents significant challenges and has its limitations. The primary concerns here relate to mechanical risk; any interference with the structural integrity of the embryo may affect implantation potential or induce epigenetic changes [20]. Additionally, in animal studies where iPGT was performed, an increased incidence of impaired glucose tolerance, insulin resistance, and increased body weight was observed in adulthood [21]. Another important issue here is tissue mosaicism- a biopsy of a few trophectoderm cells may not reflect the actual genetic status of the ICM, leading to false-positive or false-negative results [22]. It is also worth mentioning the significant technical requirements, as the procedure requires advanced micromanipulation equipment and highly qualified laboratory personnel.

Non-invasive genetic testing

In response to these challenges, modern clinical embryology is redefining the concept of diagnostic safety through the development of non-invasive methods. This method represents a technological breakthrough, as it completely eliminates the need to compromise the biological integrity of the embryo. The niPGT mechanism utilizes the presence of cell-free, extracellular DNA in the embryo culture medium [23]. The presence of genetic material in the culture medium droplet (SCM) is a well-documented process, though its precise kinetics remain a subject of debate among researchers. The dominant hypothesis explaining the origin of cfDNA is the process of apoptosis- the programmed death of embryonic cells. During preimplantation development, particularly during compaction and blastocyst formation, there is a selective elimination of cell lines bearing meiotic errors or structural damage. Chromatin fragments are released into the blastocoele cavity (blastocoel fluid) and then, as a result of physiological micropulsations of the trophectoderm, are secreted into the external medium. Studies also suggest the involvement of active vesicular transport (exosomes) and necrotic processes. However, recognizing apoptosis as the primary source of cfDNA poses significant interpretive challenges- this material may represent a fraction of eliminated cells, raising questions about the degree of correlation between the sample and the actual karyotype of the viable embryo [24]. Strict adherence to protocols for the collection of used culture medium is essential for the reliability of niPGT. To eliminate contamination with maternal DNA (derived from cumulus cells) and exogenous DNA, it is essential to implement a procedure involving serial washing of the embryos prior to transfer to the final culture drop (typically on the 3rd or 4th day of in vitro development) [25]. Material collection (volume 10-20 µl) occurs on the 5th or 6th day of development, immediately after removing the embryo from the culture medium prior to cryopreservation at –80 °C in sterile low-binding microtubes, which prevents the degradation of trace amounts of cfDNA. A key element of quality control is the use of negative controls (drops incubated without an embryo), which serve to validate the whole-genome amplification (WGA) process [26]. Molecular analysis of the culture medium using NGS eliminates mechanical trauma and increases genomic representativeness. There are scientific reports suggesting that cfDNA in the culture medium may originate from both the trophectoderm and ICM cells, which potentially offers a more holistic insight into the embryo's chromosomal status than a point biopsy [27]. The optimization and simplification of the procedure are also significant, as they substantially reduce costs and increase the accessibility of advanced diagnostics [28].

iPGT vs. NiPGT

A review of the literature shows concordance rates between niPGT and trophectoderm biopsy ranging from 65% to 90% [29]. These discrepancies stem from several biological factors. The first factor is mosaicism. NiPGT can provide a more comprehensive genetic representation of the embryo by aggregating material from both the trophectoderm and the ICM. However, maternal DNA contamination can lead to the preferential amplification of female sequences, resulting in an incorrect karyotype assessment in male embryos or false-negative findings in female embryos [30]. It is also worth mentioning the "self-correction" mechanism, involving the preferential sequestration and expulsion of aneuploid cells from the blastocyst structure; this may overestimate the fraction of defective DNA in the SCM, leading to false-positive results [31]. Nevertheless, meta-analyses indicate a high negative predictive value for niPGT, making this method an effective tool for the prioritization of embryos for transfer while fully preserving their developmental potential [17]. The authors present a table summarizing the key differences between iPGT and niPGT (Table 1).

Table 1. Comparison of invasive and non-invasive methods.

Parameter	Conventional Biopsy (iPGT)	Non-invasive Method (niPGT)
DNA Source	Trophoblast cell-approx. 5-10 cells	CfDNA in culture medium
Interference with the embryo	High: Mechanical incision of the zona pellucida and cell collection	None: Analysis of the culture medium in which the embryo developed
Representativeness	Limited (risk of error in cases of mosaicism)	Potentially holistic (DNA from the entire embryo)
Required equipment	Laser microsurgery, micromanipulators	Standard IVF equipment + culture medium preservation protocol
Main risks	Mechanical damage to the embryo	Contamination with maternal or exogenous DNA

Interpretation Challenges and Controversies

Sample Contamination and the Sensitivity of NGS

The main barrier to implementation remains the risk of DNA sample contamination with maternal DNA from granulosa cells. Given that NGS techniques are characterized by extreme sensitivity, even suboptimal oocyte denudation can dominate the embryonic fraction of cfDNA. This necessitates the use of strict laboratory cleanliness standards- such as designated "DNA free zones"- and the implementation of advanced bioinformatics algorithms capable of deconvolving genetic signals and distinguishing the embryonic fraction from the exogenous maternal background [32].

Ethical and economic issues

The implementation of niPGT into standard preimplantation diagnostic procedure raises many ethical challenges. On the one hand, the elimination of invasiveness is a positive argument that addresses patients' concerns about the safety of their future offspring. On the other hand, the low technical barrier of the test may pose a risk of excessive automatism in procreation, which could raise certain moral concerns. From an economic perspective, niPGT can significantly reduce costs associated with labor- intensive micromanipulation; however, the costs of sequencing and DNA library preparation remain a significant budgetary burden. The current scientific consensus suggests that until clear evidence of improved live birth rates in the general population is provided, niPGT should be used selectively- primarily in patients of advanced maternal age and in cases of recurrent pregnancy loss [33].

Complementary Technologies

Modern clinical embryology is evolving toward multimodal data integration, combining genetic results from niPGT with morphokinetic parameters generated by TLT systems. This process replaces subjective visual scoring systems with advanced mathematical models which form the foundation of precision reproductive medicine [34].

Deep learning in blastogenesis analysis

At the heart of this digital transformation are deep learning algorithms capable of detecting high-resolution phenotypic features that are often impossible to identify during routine microscopic evaluation. Currently, this diagnostic approach is based on three technological pillars. Starting with Deep Convolutional Neural Networks (DCNN), which undergo supervised learning on Big Data sets comprising millions of

blastocyst images with verified chromosomal status [35]. By analyzing the texture of the trophectoderm and the dynamics of expansion, the algorithms generate an Aneuploidy Score, which quantifies the likelihood of meiotic errors and aids in the interpretation of ambiguous cfDNA sequencing results [36]. In Parallel, Recurrent Neural Networks (RNN) and Long Short-Term Memory (LSTM) networks leverage the sequential nature of embryogenesis. LSTM networks are used for temporal analysis of video streams from TLT systems. They enable the detection of critical kinetic anomalies, such as direct cleavage or asynchronous karyokinesis. These phenomena are recognized biomarkers of cellular stress and correlate with low embryo implantation potential [37, 38]. Finally, ViT representing the latest generation models utilizing a self-attention mechanism. Unlike DCNNs, ViTs analyze the embryo image as a global structure of spatial relationships. This allows for precise mapping of correlations between ICM morphology and trophectoderm cell quality, identifying areas of increased genetic risk for the embryologist [39, 40].

Synergy and Data Fusion

Implementing the Data Fusion approach enables the creation of predictive models that integrate clinical data-such as the patient's age or the etiology of infertility- with cfDNA sequencing results and AI- based visual analysis. In the event of conflicting results- for example, when the molecular profile suggests mosaicism, but developmental kinetics fall within normal limits- the systems use Bayesian inference to calculate the final Live Birth Probability. AI serves here as a systemic decision-making assistant, eliminating observer error and shortening the time to pregnancy [41].

Advances in NGS

The analytical foundation of niPGT remains NGS technology, which has undergone significant optimization in terms of throughput and resolution. The miniaturization of systems and reduction in operating costs have enabled the widespread implementation of this method. Modern sequencing platforms now offer high analytical resolution, including precise detection of segmental aneuploidies and accurate estimation of the percentage of mosaic lines in microliter samples, microfluidic integration (automation of DNA library preparation), and accelerated diagnostics by reducing the time from sample collection to result generation to a matter of hours, which opens the prospect to transfer in fresh cycles while preserving full genetic information [42, 43].

Discussion

The introduction of niPGT into routine clinical practice represents one of the most eagerly awaited breakthroughs in modern reproductive medicine. The prospect of embryo genetic testing that does not require a risky and traumatic biopsy is highly desirable for both clinicians and patients. However, the current stage of development of this technology, despite dynamic progress, requires a cool-headed and thorough analysis of the facts. NiPGT is not yet ready to become the standard in preimplantation analysis, though it is worth noting that niPGT is currently in a transitional phase. It is suspended between the status of a promising experiment and that of a reliable diagnostic tool. Although numerous studies indicate a high correlation between cfDNA present in the culture medium and the outcome obtained through traditional trophectoderm biopsy, this approach has not yet achieved the level of precision that would allow for the complete elimination of invasive tests. It must therefore be concluded that niPGT is undoubtedly the future of embryology. Its implementation allows for the evaluation of all obtained embryos-including those that, due to low quality or stage of development, do not qualify for biopsy. Additionally, it is associated with a simultaneous reduction in procedure costs and the complete elimination of mechanical stress on the blastocyst. At the current stage of development, however, niPGT should be treated as an auxiliary method, serving primarily to prioritize embryos for transfer, rather than as a definitive diagnostic test fully replacing iPGT. For niPGT technology to aspire to the title of the gold standard, the scientific community must address a series of questions and eliminate the technical barriers that currently limit its widespread use. Several unresolved issues fall among the key areas requiring in-depth research. The first of these is the origin of cfDNA. There is still no 100% certainty regarding the mechanisms by which genetic material is released into the medium. It is unclear to what extent the DNA originates from cells undergoing natural apoptosis-which could favor the detection of aneuploid lines-and to what extent it results from the active release of material by healthy cells. Understanding these dynamics is crucial for determining whether the obtained results accurately reflect the condition of the entire embryo. The phenomenon of mosaicism is also significant. The hypothesis that niPGT may better reflect the actual condition of the entire embryo-both the ICM and the trophectoderm-than a point biopsy of a few cells is extremely interesting. However, a properly conducted verification of this hypothesis in a large clinical cohort could demonstrate the diagnostic superiority of the non-invasive method over the traditional one. Furthermore,

technical issues are of great importance. The key issue remains sample contamination. The presence of residual maternal DNA and exogenous DNA can lead to falsified results. This would result in errors in sex determination or false- positive results in female embryos. The development of rigorous protocols to eliminate these sources of interference is vital for the reliability of the technique. Furthermore, there is an urgent need to investigate how different incubation systems, types of culture media used, and the precise duration of embryo exposure to the medium affect the concentration and quality of cfDNA. Each laboratory variable can alter the stability of the genetic material. The final aspect is standardization at the global level. Currently, there is a keenly felt lack of uniform, international guidelines- e.g., from ESHRE or ASRM-regarding the collection, storage, and analysis of the medium. The absence of standard hinders the comparison of the results between centers and impedes the widespread validation of the method. After a thorough review of the literature, the authors foresee the future of reproductive medicine as a shift away from invasive techniques toward multimodal embryo profiling, combining niPGT, AI algorithms, and analysis of the metabolic profile of the culture medium. The key challenge currently remains the global standardization of laboratory protocols. Harmonization of SCM collection methods and bioinformatics algorithms is essential to ensure full comparability of results on an international scale and the reproducibility of clinical outcomes.

Summary

Modern reproductive medicine defines infertility as a growing socio-demographic crisis that already affects 17.5% of the global population. A dramatic increase in incidence, tha shift toward later-in-life parenthood, and the impact of environmental factors have made ART, and IVF in particular, the standard of care. Despite the optimization of culture conditions, the success rate of a single embryo transfer still hovers around 30-40%, which necessitates the search for methods of objective embryo selection with the highest implantation potential. A breakthrough in reproductive medicine is the shift from classical embryo morphology assessment- which often fails to detect chromosomal abnormalities- toward advanced genetic testing. Although traditional trophectoderm biopsy is highly effective, its invasiveness carries a risk of blastocyst damage, requires expensive laser equipment, and may not be representative in cases of mosaicism. Recent studies aim to establish the era of niPGT as the new standard, based on the analysis of cfDNA released by the embryo into the culture medium. This method eliminates mechanical stress and allows for the safe evaluation of all embryos, including those of lower quality. The synergy of niPGT with AI algorithms, TLI systems, and high throughput NGS sequencing enables the creation of multimodal predictive models. Despite challenges related to maternal DNA contamination or the need for global standardization, the integration of niPGT with the single embryo transfer procedure currently represent the most effective way to maximize safety and reduce the time to achieving a healthy pregnancy.

Authors' Contributions Statement

All authors have read and agreed with the published version of the manuscript.
All authors have reviewed and agreed to the publication of the final version of the manuscript.
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