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REVIEW ARTICLE

Making and Selecting the Best Embryo in *In vitro* Fertilization

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Currently, most assisted reproduction units transfer a single embryo to avoid multiple pregnancies. Embryologists must select the embryo to be transferred from a cohort produced by a couple during a cycle. This selection process should be accurate, non-invasive, inexpensive, reproducible, and available to *in vitro* fertilization (IVF) laboratories worldwide.

Embryo selection has evolved from static and morphological criteria to the use of morphokinetic embryonic characteristics using time-lapse systems and artificial intelligence, as well as the genetic study of embryos, both invasive with preimplantation genetic testing for aneuploidies (PGT-A) and non-invasive (niPGT-A). However, despite these advances in embryo selection methods, the overall success rate of IVF techniques remains between 25 and 30%. This review summarizes the different methods and evolution of embryo selection, their strengths and limitations, as well as future technologies that can improve patient outcomes in the shortest possible time. These methodologies are based on procedures that are applied at different stages of embryo development, from the oocyte to the cleavage and blastocyst stages, and can be used in laboratory routine.

Key Words: Embryo, Preimplantation genetic test, Time-lapse, Embryo selection.

Introduction

The main goal of any assisted reproduction technique is to achieve the birth of a single healthy, full-term baby, which requires the transfer of a single euploid embryo that is implanted.

Over the past few decades, improvements in embryo culture media, embryo culture systems, and the overall quality of embryology laboratories have made it possible to develop a viable embryo to the blastocyst stage *in vitro*. In this way, single embryo transfer has virtually eliminated multiple pregnancies.

Currently, most assisted reproduction centers worldwide perform single embryo transfers in order to avoid multiple gestations and the problems associated with them. In Spain, the most recent data from the SEF national activity register from 2021, indicate that the percentage of single embryo transfers has increased by 44% since 2009 (1).

The challenge for embryology laboratory professionals is to identify the embryo to be transferred that is most likely to implant. This selection process should be accurate, non-invasive, inexpensive, reproducible, and available to IVF laboratories worldwide. Although many factors contribute to the success of embryo implantation, including endometrial characteristics, embryo quality is recognized as the predominant factor (2).

Many resources in assisted reproduction have been invested for this purpose: to know the reproductive competence of embryos. On the other hand, one of the objectives of precise embryo selection is to reduce the time needed to achieve gestation and the birth of a healthy child. By selecting the embryo with the greatest implantation

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capacity, the number of transfers required to achieve gestation will be reduced, and thus, the time necessary to achieve pregnancy.

Although current techniques do not allow us to improve the quality of embryos already present, they can help us to prioritize the transfer of embryos with the greatest implantation capacity. This review summarizes the different methods and evolution of embryo selection, their strengths and limitations, as well as future technologies that can improve patient outcomes in the shortest possible time. These methods are based on either invasive or non-invasive procedures applied at different stages of embryo development, from the oocyte to the cleavage stage and then to the blastocyst stage.

Non-invasive Methods

Morphological Embryo Assessment

The most obvious parameter for evaluating embryos is morphology. In fact, morphology was the first parameter evaluated in detail in the early days of IVF, and numerous studies have been published relating oocyte and embryo morphology to success rates. The first documented observation dates back to 1944 by Rock and Menkin (3): "One of these embryos, when first seen in cleavage, consisted of one large blastomere and two smaller ones, each of the three containing a round, vesicular nucleus. The second egg from this same patient was at a similar stage, but part of the cytoplasm appeared fragmented, and soon proceeded to undergo rapid degenerative changes."

For many years, various classification systems have been developed for all stages of human embryo development, from the pronucleated oocyte to the blastocyst, relating morphological characteristics to the achievement of pregnancy (4).

So far, not much progress has been made in terms of morphologically classification of embryos. Even today, embryo classification, which predicts the implantation potential of the embryo, is established by combining the degree of fragmentation, the number of blastomeres, and the division pattern at D+2 and D+3.

The assessment of embryonic morphology includes observation of the embryo on the second day of culture (D+2). This observation should be made 44 ± 1 h after insemination (5). At this time, optimal embryos will typically have four cells (4,6). If embryo culture is continued, the next observation time is on D+3, at 68 ± 1 h after insemination. Embryos with the highest probability of implantation usually have seven to eight cells and are derived from four cells on D+2 (7). Embryos with <6 cells or >9 cells on D+3 have been shown to have higher aneuploidy rates than those with seven or eight blastomeres (8). The reason for the variation in division times is still unclear, but has been attributed to intrinsic factors of the oocyte, such as certain characteristics of the meiotic spindle (9) or the state of its organelles (4).

Fragmentation in the early stages of development reduces the viability of the embryo due to abnormalities that cause embryonic blockage, usually before reaching blastulation (7). It is recommended that embryos with more than 50% fragmentation should not be transferred or frozen, because their implantation rate is practically zero.

The visualization of nuclei in daily practice in assisted reproduction laboratories is a procedure that must continue to be considered of great importance in the morphological assessment of embryos. Multinucleation, defined as the presence of more than one nucleus in at least one of the blastomeres of an embryo (10), can be evaluated at various stages of embryonic development, from two cells to day 3 of development (D+3).

The Istanbul consensus on embryo assessment by the European Society of Human Reproduction and Embryology (ESHRE) and the ALPHA Scientists in Reproductive Medicine group recommend that multinucleated embryos should not be selected for transfer if other embryos are available (5).

Cytoplasmic alterations are also among the morphological features associated with implantation. The main alterations include vacuoles, the presence of a cytoplasmic ring, thickened zona pellucida, and the presence of endoplasmic reticulum aggregates (5).

For blastocysts, the most widely accepted classification is that of Gardner (2), which considers parameters such as the size, shape, and degree of compaction of the internal cell mass (ICM), the degree of expansion of the blastocoel, and the structure and number of trophectoderm (TE) cells.

Recent meta-analyses indicate that day 6 blastocysts are less likely to implant than day 5 blastocysts (11), and day 7 blastocysts are less likely to implant than either day 5 or day 6 blastocysts (12). These lower implantation rates persist even in euploid blastocysts (12). The relative importance of the day of blastocyst formation versus the grades of ICM and TE is unknown, making a ranking system based on grades and day of use difficult. Thus, the prognostic value of embryo grading, when considering both timing (day 5 vs. 6) and grade (A vs. B), is unknown.

In cases where there are a limited number of embryos available for transfer, morphological evaluation is essential in deciding whether to transfer, discard, or cryopreserve them.

Although some studies have suggested that low-quality blastocysts are associated with a lower chance of clinical pregnancy (13), between 4.4 and 2.6% of conceptions can occur when slow-growing blastocysts are transferred (14). Furthermore, two recent large studies have confirmed the value of low-grade blastocysts while indicating that their transfer does not pose additional risks to perinatal outcomes (15,16).

Despite efforts to study and correlate morphological parameters with implantation rates, these parameters are not precise enough to predict embryo implantation. This limitation is due to the fact that the observations are based on a snapshot approach at few discrete time points (5). Another challenge is the subjective nature of embryo classification, which is prone to considerable inter- and intra-observer variability (17). In addition, morphological appearance changes rapidly during cleavage stages, which may affect evaluations on days 2 and 3 (18), potentially resulting in classifications that are not objectively correct. Despite morphological variability, embryos can still implant successfully (18).

Blastocyst Transfer

The stage of embryo transfer, whether cleavage or blastocyst, is itself a method of embryo selection. Embryos that could have been transferred on day 2 or 3 often arrest and would not be viable for transfer on day 5, 6, or 7. Improvements in culture methods have greatly enhanced our ability to support embryo development to the blastocyst stage. In general, transfer at the blastocyst stage is preferred because non-viable embryos at the cleavage stage are arrested, and developing blastocysts are appropriately synchronized with the uterine environment.

The prognostic value of cleavage versus blastocyst stage embryo selection has been extensively reviewed. Evidence from the most recent Cochrane review suggests that in fresh transfer cycles, blastocyst transfer results in higher live birth and clinical pregnancy rates compared to cleavage stage embryo transfer, but the evidence on cumulative outcomes was uncertain (19).

Although contradictory results have been published regarding gestation rates after blastocyst transfer (19,20), their importance lies in the implementation of a single embryo transfer policy that minimizes the adverse outcomes of multiple pregnancies.

Time-lapse Imaging

Time-lapse technology introduced a few years ago, and AI more recently, have been incorporated into embryology with the aim of improving embryo classification and selection in a non-invasive manner.

Time-lapse technology allows embryologists to continuously monitor embryonic development from the earliest stages to the blastocyst stage. This technology automatically captures images throughout the life cycle of the embryo, allowing for continuous and uninterrupted observation. Compared to conventional morphological evaluation methods, morphokinetic selection using time-lapse technology has the potential to optimize the selection process for transfer, thereby improving prognosis.

Initially, the goal was to use this technology to increase the implantation rate by favoring optimal embryo selection. One study analyzed 247 transferred embryos that did not implant and identified several parameters that correlated with pregnancy, such as the time between the first and subsequent embryonic divisions or the duration of each division (21). Based on these findings, the authors developed a multivariate model that classified embryos based on their developmental progression over time and their ability to predict implantation.

Fishel S, et al. (22) published the first study comparing an objective time-lapse algorithm of preimplantation embryo development with subjective and conventional blastocyst morphology for embryo transfer and live birth outcomes. They found that the time-lapse algorithm was superior in selecting embryos with a propensity for live birth. However, they cautioned that the study could not account for all confounding effects, and thus, could not draw strong conclusions about causal links between embryo rank or transfer grade and birth outcomes.

More recently, another study demonstrated that the blastocyst rate at day 5 and the number of blastocysts suitable for cryopreservation were significantly higher for embryos cultured in time-lapse compared with standard incubators. However, no difference was found in the clinical pregnancy rate after transfer of embryos from either incubator (23).

Despite some studies supporting time-lapse technology, conflicting results have been reported. A Cochrane review found little good-quality evidence of differences in live birth resulting from the first transfer, and recent clinical trials found no benefit in either time to pregnancy or cumulative live birth rate (24).

The evidence for the efficacy of time-lapse technology in assisted reproduction is mixed and subject of ongoing research and debate. Some recent clinical trials have failed to demonstrate a clear benefit of time-lapse incubators in terms of time to pregnancy or cumulative live birth rate, particularly when considering cleavage transfers or using algorithms with limited validation (25). Similarly, other studies have not found advantages of time-lapse technology in the context of cleavage or blastocyst transfer cycles (26).

The study conducted by Jiang Y, et al. contributes to the ongoing discussion regarding the efficacy of time-lapse technology in assisted reproduction. Their findings, based on low- to moderate-quality evidence, suggest that neither the use of time-lapse incubators nor embryo selection based on morphokinetics led to improved clinical outcomes compared with traditional embryo culture based on morphology alone (27). This study adds to the body of evidence that questions the purported benefits of time-lapse technology in improving clinical outcomes in assisted reproduction. It suggests that despite the potential benefits of continuous monitoring and morphokinetic analysis offered by time-lapse systems, these do not necessarily translate

into improved success rates in terms of pregnancy and live births.

The results of the study by Jiang Y, et al. underscore the complexity of optimizing embryo selection methods and highlight the need for further research to better understand the impact of time-lapse technology on assisted reproduction outcomes. It suggests that while time-lapse technology may offer valuable insights into embryo development, its clinical utility in improving success rates remains uncertain and requires careful evaluation.

However, there are also studies that support the use of time-lapse technology (28–30), suggesting that it may increase implantation rates and may be associated with embryo ploidy (31). For example, Bamford T, et al. (32) have shown that morphokinetic algorithms can improve the prioritization of euploid embryos compared to selection by embryologists. In addition, Serrano-Novillo C, et al. have highlighted the importance of considering a wider range of developmental stages, such as the parameter "st2, start of t2," which they found to be strongly implicated in ploidy status (33).

Although studies in favor of time-lapse technology continue to be published, highlighting its potential benefits, it is essential to acknowledge the challenges and limitations associated with its implementation. The equipment required for time-lapse technology is expensive, and its use demands specialized training for laboratory staff (34). Moreover, there is a lack of solid clinical evidence to support its widespread adoption, which has tempered initial enthusiasm.

A recent publication by the ESHRE (35) does not recommend the routine use of time-lapse technology, considering it as an adjunct rather than a standard practice. However, it is recognized that time-lapse technology can facilitate the work of the laboratory by providing additional information about the embryos to be transferred.

In summary, while time-lapse technology holds promise for improving embryo selection and optimizing outcomes in assisted reproduction, further research and validation are needed to establish its efficacy and justify its routine use in clinical practice.

Artificial Intelligence

Artificial intelligence (AI) is a branch of computer science that aims to simulate intelligent behavior, such as cognitive thinking, learning, and problem-solving, typically associated with humans. In the context of assisted reproduction, AI has been introduced to improve non-invasive embryo classification and selection (36).

Developments in computer science and increased computational processing power have enabled IVF laboratories to adopt AI technology in recent years. AI applications in embryology focus on several areas, including embryo development annotation, ploidy prediction, embryo selection, and IVF outcome prediction (37).

Machine learning (ML), a subset of AI, offers the potential to minimize operator subjectivity and improve embryo selection. Deep learning (DL), a subgroup of ML, uses artificial neural networks with multiple hidden layers. However, the reasoning process of DL models is often uninterpretable, leading to the term "black box" (38).

Black-box AI-driven decision support systems can automatically extract time-lapse microscopic image features of human embryos and perform embryo segmentation (39,40). DL models have been reported to predict outcomes such as embryo grading (41), ploidy status (42), clinical pregnancy (42), and live birth (43).

Studies have shown that AI-based systems can automatically measure key embryo characteristics, leading to improved implantation rates compared to traditional manual evaluation methods. Furthermore, AI consistently outperforms clinical teams in embryo selection based on morphology and predicting clinical outcomes (IVF procedures). Here is a breakdown of the results of each study:

Leahy BD, et al. (39) developed a deep learning (DL) system capable of automatically measuring key features of embryos in IVF, including developmental stage classification, zona pellucida segmentation, fragmentation degree classification, cleavage stage cell segmentation, and pronuclei segmentation. This system offers a comprehensive approach to embryo assessment using AI to streamline and automate the process.

Wang S, et al. (44) demonstrated the effectiveness of a morphology-based interpretable AI system in improving implantation rates in fresh single-blastocyst transfers compared to traditional manual evaluation methods. The AI system achieved a significantly higher implantation rate, demonstrating the potential of AI-driven approaches to optimize embryo selection for IVF procedures.

Salih M, et al. (45) conducted a review of 20 studies comparing embryo selection using AI with selection carried out by embryologists. Their results consistently showed that AI outperformed clinical teams in assessing embryo morphology and predicting clinical outcomes during embryo selection evaluation. This suggests that AI-based systems can provide more accurate and reliable embryo selection compared to traditional methods.

Fordham DE, et al. (46) presented evidence of lower reproducibility in blastocyst classification by embryologists compared to a custom-built deep neural network model. This highlights the potential for AI models to provide more consistent and standardized embryo classification, minimizing variability between embryologists and improving overall accuracy.

Loewke K, et al. (47) reported a notable improvement in clinical pregnancy rates using a convolutional neural network model for embryo selection compared to manual blastocyst classification. This study further underscores the

potential of AI-driven approaches in enhancing the success rates of IVF procedures by improving embryo selection accuracy.

The comparison of 12 algorithms for blastocyst viability prediction, as mentioned in another study (48), revealed that logistic regression, an interpretable machine learning (ML) method, outperformed the other 11 ML methods, including black-box counterparts. Here's a breakdown of what this finding means:

Logistic Regression. It is a statistical method used for binary classification tasks, where the outcome is binary (e.g., viable or non-viable blastocysts). It provides interpretable results, meaning that the relationship between input features and the predicted outcome can be easily understood. Despite its simplicity compared to more complex ML models, logistic regression has proven to be highly effective in predicting blastocyst viability.

Interpretable ML Methods. These methods, such as logistic regression, decision trees, and linear models, provide transparency to the decision-making process. This transparency allows clinicians and researchers to understand how the model arrived at its predictions, making it easier to trust and interpret the results. In the context of blastocyst viability prediction, the interpretability of logistic regression can provide valuable insight into the factors that influence embryo viability.

Black-Box ML Methods. These methods, such as deep neural networks and ensemble methods, often provide superior predictive performance compared to interpretable methods. However, their decision-making process is opaque, making it difficult to understand how they arrive at their predictions. While black-box models may offer higher accuracy, their lack of interpretability can be a drawback in domains where interpretability is crucial, such as healthcare.

Performance Comparison. The finding that logistic regression outperformed black-box ML methods suggests that in the context of blastocyst viability prediction, interpretability may be more valuable than predictive performance alone. Although black-box methods may achieve higher accuracy, the interpretability of logistic regression makes it a preferred choice for predicting blastocyst viability because it allows clinicians to understand and trust the model's predictions.

In summary, the methodology comparison revealed that logistic regression, as an interpretable ML method, was the most effective in predicting blastocyst viability. This finding highlights the importance of interpretability in ML models, particularly in healthcare applications where transparency and trustworthiness are paramount.

Overall, these studies collectively demonstrate the promise of AI technology to revolutionize the embryo selection process in IVF, potentially leading to higher success rates and improved outcomes for patients undergoing fertility treatments (49).

However, challenges remain, including the identification of known and unknown confounders during the training process of black-box algorithms and issues related to transferability when algorithms trained in specific clinical settings are applied in different settings (50–52).

This highlights the importance of thorough validation before introducing externally trained algorithms into clinical practice, particularly in the context of embryo selection based on morphokinetic profiles. Several factors can influence the transferability of algorithms, including culture conditions (53), culture media (54), patient demographics (55), and controlled ovarian stimulation protocols (56). These factors contribute to variations in morphokinetic profiles and endometrial receptivity, which may affect algorithm performance in different clinical settings.

Access to large, high-quality datasets is essential for training robust algorithms. However, obtaining such datasets can be challenging due to privacy concerns, data sharing limitations, and the need for diverse and representative samples. Commercial algorithms may have an advantage in accessing larger datasets, which can improve transferability and performance (57). Achieving a balance between the two is crucial to ensure the effectiveness of algorithms in different clinical settings. While larger datasets can improve transferability, maintaining performance standards is equally important to ensure accurate predictions and clinical outcomes.

In summary, in-house validation of externally trained algorithms, considering the influence of various clinical factors on algorithm performance, and access to large, diverse datasets are essential steps in deploying effective AI-driven decision support systems for embryo selection in assisted reproduction.

Despite these challenges, AI has the potential to revolutionize embryo selection by providing an objective, reproducible, automated, and affordable solution. It could save time and resources for embryologists, reduce variability in classification systems, and potentially increase pregnancy rates (17). AI could facilitate the sharing of data between different laboratories for training and testing purposes. This collaboration could enhance the development and refinement of AI algorithms by leveraging diverse datasets from multiple sources. Moreover, AI has the potential to reduce inter-observer and intra-observer variability in embryo classification systems. By automating the selection process and providing objective criteria for evaluation, AI could standardize embryo assessment and minimize subjective interpretation differences among embryologists. Standardization of the embryo selection process through AI could lead to greater consistency in the presentation of results. Consistent criteria for evaluating embryos could improve the reproducibility of results across different laboratories and clinicians.

Overall, this passage highlights the transformative potential of AI to address the challenges associated with vari-

ability and data sharing in embryo selection, with the ultimate goal of improving patient outcomes in assisted reproduction.

Nevertheless, the efficacy of AI-based decision support systems in embryo selection requires further validation through controlled clinical trials (58). In addition, there are ethical and social concerns regarding the lack of transparency and interpretability of AI models (58).

In conclusion, if future studies confirm and extend current findings, AI may become an ideal methodology for embryo selection, ultimately increasing the likelihood of a healthy live birth in assisted reproduction. However, further clinical validation and transparency are needed to ensure its ethical and practical use in clinical practice.

Non-invasive PGT-A

The discovery of cell-free DNA release from human embryos into the surrounding environment has paved the way for non-invasive preimplantation genetic testing for aneuploidy (niPGT-A). Collection of spent culture medium (SCM) offers a non-invasive approach that poses minimal risk to the embryo. Some authors even suggest that SCM may provide a more comprehensive representation of the whole blastocyst compared to invasive trophectoderm biopsy, as it captures DNA from both trophectoderm and inner cell mass (59).

Recent studies have demonstrated the feasibility of detecting, extracting, and amplifying cell-free DNA from the SCM at both the cleavage and blastocyst stages (60–62), and it has been shown that 24–48 h of contact with the embryo are sufficient to collect cell-free DNA from the SCM (61).

It has been proposed that this DNA originates from discarded embryonic cells as a mechanism to correct aneuploidies (63). The statement highlights apoptosis as the most extensively studied mechanism responsible for the release of cell-free DNA from embryos, particularly in the context of niPGT-A (64). In the context of embryo development, apoptosis can result in the release of fragmented DNA into the surrounding environment, including the SCM where embryos are cultured during IVF procedures. This cell-free DNA can then be sampled and analyzed for genetic abnormalities without the need for an invasive embryo biopsy.

While apoptosis is well understood and recognized as a major contributor to the presence of cell-free DNA in the SCM, the statement also suggests that there may be other pathways involved in the release of embryonic DNA that are less explored (65).

The research of Cheng HYH, et al. marks a significant advancement in the field of reproductive medicine, particularly in the application of niPGT-A. Their study shifts the focus from merely assessing the technical feasibility of niPGT-A, such as amplification success and concordance rates between SCM and trophectoderm biopsy, to evaluating its practical utility in the clinical setting, specifically its role in prioritizing blastocyst transfer during IVF treatment. By demonstrating that niPGT-A can serve as a better tool for blastocyst evaluation, Cheng HYH, et al. suggest that this non-invasive method can provide more reliable and objective information regarding the genetic viability of embryos, which means that it can potentially shorten the time to pregnancy in women with infertility (66).

Huang B, et al. conducted a meta-analysis demonstrating that niPGT-A may have high detection accuracy and may serve as an alternative approach to embryo analysis. Their results also suggest that niPGT-A may have reliable clinical applications for grading embryo quality (67).

Several studies have compared the results of niPGT-A performed on SCM with those obtained from trophectoderm biopsies of vitrified embryos. These studies reported high amplification rates ranging from 80.4–100%, (68) and concordance rates of up to 93.8% (69). Despite these promising results, cases of false negatives have been reported, mainly due to contamination by maternal cells or DNA (70).

Further investigation using single nucleotide polymorphism (SNP) analysis revealed significant variability in the percentage of embryonic DNA detected in the SCM, ranging from 0–100%. (64). Notably, the contamination with maternal genetic material was more pronounced in media collected during the initial days (1–3) of embryo culture (64).

Chow JFC, et al. demonstrated that collecting SCM on day 6, after sequential embryo rinsing, resulted in a higher concordance rate. Their study also showed that niPGT-A results were comparable whether oocytes were fertilized by conventional insemination or intracytoplasmic sperm injection (ICSI) (71). This suggests that the timing and method of SCM collection, as well as the fertilization technique, may influence the accuracy of niPGT-A.

Furthermore, a study on the prognostic accuracy of niPGT-A indicated a high positive predictive value for detecting whole chromosome copy number variations. This finding supports the potential of SCM as a reliable source for aneuploidy screening (72). However, it also highlights the need to optimize embryo culture conditions to ensure that the SCM accurately represents the embryonic genome.

Lledo B, et al. (73) conducted a review highlighting the variability in the information rate of the SCM and the diagnostic agreement of niPGT-A. They reported that the sensitivity and specificity of niPGT-A varied widely, leading to the conclusion that the current evidence does not support its clinical utility. They also noted that the available data on clinical outcomes are preliminary and called for further research, including randomized non-selective studies, to better understand the utility of niPGT-A.

Cinnioglu C, et al. (74) have highlighted in their review that despite the studies conducted so far, no consensus

has been reached on the efficacy of non-invasive methods compared with the current gold standard in preimplantation genetic testing. While the overall concordance rates of niPGT-A appear promising, existing research underscores the necessity for more comprehensive studies. There is a call for a deeper understanding and further investigation before niPGT-A can be confidently adopted as a standard and reliable clinical practice.

In essence, the future of niPGT-A depends on rigorous research efforts, including randomized and non-selective trials. Moreover, there is a critical need to optimize embryo culture techniques and culture medium collection procedures. These advances are essential to improve the reliability and clinical applicability of niPGT-A.

Invasive Embryo Selection

Preimplantation GENETIC SCREENING

Invasive procedures such as preimplantation genetic testing for aneuploidies (PGT-A) involve the removal of part of the embryo, typically by trophectoderm biopsy of blastocysts. This procedure has evolved from early fluorescence in situ hybridization (FISH) methods to more advanced next-generation sequencing (NGS) techniques that offer improved accuracy in detecting aneuploidies. Despite its invasive nature and the need for specialized equipment and training, PGT-A is increasingly being used in assisted reproduction, with significant growth observed worldwide.

The landscape of embryonic chromosome analysis has changed significantly. Previously, biopsies were performed on day 3 of embryonic development, and FISH was used for genetic assessment. However, this approach has been superseded by more advanced techniques. Today, the focus has shifted to trophectoderm biopsy on blastocysts on days 5–7 after oocyte retrieval. This method, which extracts 5-10 cells for analysis, leverages NGS (75,76). This greatly improves our ability to detect aneuploidies and represents a leap forward in genetic analysis.

Trophectoderm biopsy, despite its benefits, is an invasive procedure. It demands a high level of expertise, requires specialized equipment such as laser devices, and relies on the skills of well-trained personnel. These requirements underscore the complexity and increased resource demands of contemporary PGT-A.

The utility of PGT-A extends beyond its technical capabilities; it has become a cornerstone in increasing the efficacy of assisted reproductive treatments. By enabling the selection and transfer of single euploid embryos, PGT-A increases the likelihood of achieving a live birth per transfer attempt. This efficacy has led to the widespread adoption of PGT-A worldwide as an integral component of assisted reproductive technologies.

Illustrating its growing importance, data from the national registry of the Spanish Fertility Society reveals a dramatic increase in the use of PGT-A in Spain, from 1,683 cycles in 2009–17,828 in 2021 (1). This increase highlights the growing role of this technique in improving the success rates of assisted reproduction treatments.

While trophectoderm biopsy is less detrimental to embryo implantation compared to cleavage stage biopsy (77), concerns remain regarding potential negative effects on implantation potential, particularly related to the number of cells biopsied (78). In addition, the long-term consequences of embryo manipulation procedures are still being evaluated (79).

The primary goal of PGT-A is to select euploid embryos to improve IVF treatment success. However, there is an ongoing debate as to whether this technique should be universally recommended or reserved for patients at higher risk of aneuploidies.

The efficacy of PGT-A remains controversial, as evidenced by the mixed results from recent clinical trials and systematic reviews.

The Single Embryo Transfer of Euploid Embryo (STAR) trial, which focused on PGT-A with blastocyst transfer, revealed benefits for patients over 35 years of age (80). Conversely, a systematic review assessing PGT-A with full chromosomal screening found favorable outcomes in young and good-prognosis patients, including improved clinical pregnancy rates and increased use of single embryo transfer (81). However, a large randomized controlled trial comparing PGT-A using NGS with morphology-based selection failed to demonstrate overall improvements in pregnancy outcomes in women aged 25–40 years with at least two biopsy-eligible blastocysts (82).

Furthermore, a Cochrane review of data from 13 randomized controlled trials concluded that there was insufficient high-quality evidence to support the routine use of PGT-A to improve clinical pregnancy, live birth, or cumulative live birth rates (83).

The conflicting results underscore the ongoing controversy surrounding the clinical utility of PGT-A and raise questions about its efficacy in improving outcomes for all patient populations. As a result, it has been proposed that individual clinics internally evaluate the efficacy of PGT-A within their own IVF programs (84). This approach acknowledges the variability in PGT-A performance among clinics and emphasizes the importance of data-driven decision-making in optimizing patient care.

The presence of mosaicism in human embryos further complicates the interpretation of PGT-A results. Mosaic embryos, which contain both euploid and aneuploid cells, can still result in live births, challenging the notion of discarding all “aneuploid” embryos (85). This highlights the need for further research to better understand the implications of mosaicism on embryo selection and outcomes.

Casper argues that the prevalence of mosaicism in blastocysts is a significant challenge, that may undermine the purported benefits of PGT-A. Consequently, the advantages

of reduced spontaneous abortion rates and reduced time to conception may be modest at best (86). This underscores the complexity of interpreting PGT-A results and highlights the need for further research to better understand the impact of mosaicism on embryo selection.

Recent data and their implications for embryo management underscore the prevailing gaps in knowledge regarding the efficacy of PGT-A for embryo selection. The uncertainty surrounding the interpretation of PGT-A results underscores the need for additional studies and research efforts to elucidate its clinical utility and inform best practices (87).

Furthermore, Gleicher's work raises critical questions about the potential drawbacks of rejecting all "aneuploid" embryos from transfer. Rejecting such embryos may inadvertently decrease the chances of pregnancy and live birth for IVF patients, thereby challenging the conventional wisdom regarding the utility of PGT-A (88).

Beyond clinical considerations, the practical implications of PGT-A deserve careful thought, particularly about patient counseling and decision-making. Many patients may face the disheartening reality of having no euploid embryos available for transfer even after undergoing multiple stimulation cycles. Moreover, the high cost associated with PGT-A testing further complicates the decision-making process for patients and clinicians alike (89).

In summary, although PGT-A is a robust diagnostic tool for identifying chromosomally normal embryos, its prognostic value for live birth rates appears to be limited. This limitation is due to the multitude of variables that can influence outcomes. In addition, as the capabilities of PGT-A continue to advance, new ethical considerations are emerging. These include concerns about the technical risks associated with the procedure, the appropriate use of test results and their accuracy, the implications of mosaicism, the fate of unselected embryos, and the protection of data privacy. These ethical dilemmas underscore the need for continued scrutiny and thoughtful deliberation as PGT-A technology evolves and its applications expand.

Ethics Concerns

Indeed, the quest to help patients achieve healthy pregnancies while navigating novel technologies represents a delicate balance between patient autonomy, minimizing disease risks, and providing accurate information. Ethical challenges arise particularly in the context of embryo selection, where decisions about which embryos to transfer can significantly affect the chances of pregnancy and the potential birth of a healthy child.

The use of advanced technologies, such as PGT, introduces complexities regarding the validation and interpretation of test results. While these tests aim to select the healthiest embryos for transfer, there is a risk that embryos deemed non-viable may be discarded based on tests that may not be fully validated or reliable. This raises concerns about the possibility of discarding embryos that could potentially lead to healthy pregnancies (90).

Balancing the desire to minimize disease risks with the need to respect patient autonomy is essential. Patients should have access to clear and accurate information about the benefits, limitations, and potential risks associated with these technologies. Informed decision-making requires transparency about the reliability of the tests used for embryo selection and the uncertainties inherent in the process.

Clinicians and professionals must continually evaluate and monitor the effectiveness and ethical implications of these technologies. This includes ongoing research to validate and refine testing methods, as well as robust ethical oversight to ensure that patients' interests and reproductive rights are upheld.

Ultimately, the goal is to empower patients to make informed choices that are consistent with their values and preferences, while ensuring that the pursuit of healthy pregnancies is grounded in evidence-based practices and ethical principles.

Conclusions

The review of available embryo selection methods in assisted reproduction laboratories reveals a lack of significant improvement in live birth rates, as highlighted in recent publications by the ESHRE (35). Furthermore, a study conducted by Sabbagh R, et al. (91) sheds light on the efficacy of PGT in improving live birth rates in IVF cycles. Analyzing data from 20,677 IVF cycles spanning from January 2014–December 2020, the study found that despite the widespread use of PGT during this period, the live birth rate per oocyte remained low. Importantly, the research suggests that the incorporation of PGT into IVF protocols did not result in significant improvements in live birth rates over the period studied.

Looking to the future, AI emerges as a highly promising technology in the field of assisted reproduction. The application of AI to the interpretation of blastocyst images has the potential to revolutionize embryo selection by enabling rapid analysis without the need for time-consuming steps inherent in current techniques such as PGT-A and non-invasive methods. The goal is to develop a robust AI system capable of accurately predicting the ploidy status and implantation potential of embryos in culture.

It is crucial to recognize that the goal of embryo selection is to achieve a healthy pregnancy and the birth of a healthy child in the shortest possible time. However, it is important to acknowledge that embryonic quality is only one of many factors influencing this outcome. Other patient-specific and environmental factors also play an important role. Therefore, the future of embryo selection may involve the integration of AI technology that can evaluate

multiple factors simultaneously to improve predictive accuracy.

Looking ahead, there is optimism that the development of new, non-invasive methods, coupled with advanced AI algorithms, will lead to significant improvements in the predictive power of embryo selection. By leveraging the capabilities of AI and incorporating novel assessments of both embryos and patients, the field of assisted reproduction is poised to make significant strides in optimizing outcomes for individuals undergoing fertility treatment.

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Conflict of interests

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