Journal of **R**eproduction & **I**nfertility
Volume 27, Issue no. 1
https://doi.org/10.18502/jri.v27i1.22117

Review Article

Check for updates

Embryo Culture Systems and Morphokinetics in IVF: The Role of Time-Lapse Monitoring and Artificial Intelligence in Embryo Selection

Sanaz Ashouri Movassagh [1], Mahnaz Heidari [1], Sepideh Ashouri Movassagh [2], Mohammad Reza Sadeghi [1*]

1- Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
2- Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), Academic Center for Education, Culture and Research (ACECR), Tehran, Iran

Abstract

Background: Embryo selection and culture conditions are critical determinants of success in assisted reproductive technology (ART). Despite advances, implantation and live birth rates following in vitro fertilization (IVF) remain suboptimal. This systematic review evaluated the effects of embryo culture media (sequential versus single-step), time-lapse monitoring (TLM), and artificial intelligence (AI)-based embryo selection on IVF outcomes.

Methods: This systematic review followed PRISMA guidelines. PubMed, Scopus, and Web of Science were searched through June 2025. Twenty-nine studies involving over 18,500 IVF/intracytoplasmic sperm injection (ICSI) cycles were included. Methodological quality was assessed using RoB2, the Newcastle–Ottawa Scale, and CLAIM according to study design. Outcomes related to culture media, TLM-derived morphokinetics, and AI-based embryo assessment were synthesized narratively.

Results: Sequential and single-step culture media produced comparable clinical outcomes, although single-step media showed a modest increase in blastocyst formation (57.1% *vs.* 53.2%; p=0.03). No significant differences were observed in clinical pregnancy or live birth rates. TLM was associated with higher ongoing pregnancy rates in descriptive analyses (45.1% *vs.* 34.8%; p<0.01) and reduced early pregnancy loss. AI-based embryo selection demonstrated strong predictive performance (AUC 0.76–0.91), although clinical validation varied across algorithms, indicating differences in predictive accuracy rather than consistent improvements in clinical outcomes.

Conclusion: Culture media selection appears to have limited influence on clinical outcomes, whereas TLM and AI-assisted embryo selection show promise for improving embryo assessment. Integration of validated AI-driven TLM systems into IVF practice may enhance embryo selection efficiency. Further large, prospective studies are needed to establish standardized clinical protocols.

** Corresponding Author:*
Mohammad Reza Sadeghi, Reproductive Biotechnology Research Center, Avicenna Research Institute, (ACECR), Tehran, Iran, PO-BOX:19839694912
E-mail:
sadeghi@avicenna.ac.ir, sadeghi281@gmail.com

Received: 1, Mar. 2026
Accepted: 28, Jul. 2026

Keywords: Artificial intelligence, Culture media, Embryo evaluation, In vitro fertilization, Pregnancy rate, Time-lapse imaging.
To cite this article: Ashouri Movassagh S, Heidari M, Ashouri Movassagh S, Sadeghi MR. Embryo Culture Systems and Morphokinetics in IVF: The Role of Time-Lapse Monitoring and Artificial Intelligence in Embryo Selection. J Reprod Infertil. 2026;27(1):10-19. https://doi.org/10.18502/jri.v27i1.22117.

Downloaded from http://www.jri.ir

Introduction

Successful outcomes in assisted reproductive technology (ART) depend on the ability to accurately identify embryos with the highest implantation potential while maintaining optimal culture conditions throughout preimplantation development. Despite substantial technological ad-

Copyright © 2026, Journal of Reproduction & Infertility

This work is licensed under a Creative Commons Attribution–NonCommercial 4.0 International License.

vancements, clinical pregnancy and live birth rates in in vitro fertilization (IVF) cycles rarely exceed 45–50%, highlighting the persistent limitations of current embryo assessment strategies (1, 2). Inadequate culture environments and suboptimal embryo selection methods may compromise developmental competence through metabolic stress, altered gene expression, and epigenetic modifications (2-4). Embryo culture systems play a critical role in early embryonic development. Two principal approaches are widely used in clinical practice: sequential media and single-step media. Sequential culture systems are designed to mimic the dynamic biochemical environment of the female reproductive tract by adjusting nutrient composition according to embryonic developmental stage (1). In contrast, single-step media provide a constant nutrient milieu throughout development, reducing embryo handling and exposure to environmental fluctuations (1). Although theoretical advantages exist for both approaches, clinical studies and meta-analyses have reported largely comparable outcomes in terms of implantation, clinical pregnancy, and live birth rates, suggesting that media type alone may not be the primary determinant of IVF success. Traditional embryo selection relies predominantly on static morphological assessment at predefined time points. This approach is inherently subjective and demonstrates limited predictive value for implantation and live birth, with substantial inter- and intra-observer variability among embryologists (5, 6). To overcome these limitations, time-lapse monitoring (TLM) systems were introduced, enabling continuous, non-invasive observation of embryo development under stable incubation conditions. TLM allows detailed analysis of morphokinetic parameters such as cleavage timing, synchronization of cell divisions, and blastulation dynamics, which have been associated with embryo viability and implantation potential (5-7). Importantly, TLM also minimizes disturbances in temperature, pH, and gas composition, factors increasingly recognized as critical for maintaining embryo developmental competence. In recent years, artificial intelligence (AI) has emerged as a transformative tool in embryo assessment. Machine learning and deep learning algorithms can process large volumes of time-lapse images and clinical data to identify complex, non-linear patterns beyond human perception (8-10). AI-based embryo selection systems, including iDAScore and KIDScore, have demonstrated superior predictive accuracy for clinical pregnancy and live birth compared with conventional morphological evaluation, with reported area under the curve (AUC) values ranging from 0.76 to above 0.90 in validated models (9, 11). Furthermore, AI-assisted approaches have shown promise in predicting chromosomal status and reducing inter-observer variability, supporting their role as objective decision-support tools in IVF laboratories (12-14). Despite the growing literature on these individual components, a critical research gap persists regarding how culture media environments interact with advanced digital assessment tools. Most existing reviews analyze culture systems, TLM hardware, or AI computational frameworks in isolation, failing to assess their cumulative, compounding impact on laboratory efficiency and clinical success. Furthermore, there is an urgent need to critically disentangle whether reported improvements in reproductive outcomes are genuinely driven by the predictive superiority of algorithmic models or are secondary benefits of environmental stability achieved via undisturbed continuous incubation systems (5, 9, 15). Therefore, a comprehensive synthesis of contemporary evidence is required to clarify the roles of culture media, time-lapse monitoring, and artificial intelligence in modern IVF practice. The present systematic review aims to evaluate and integrate evidence from randomized controlled trials, prospective cohort studies, and validated AI-based investigations published between 2016 and 2025. Specifically, this review examines (i) the clinical impact of sequential versus single-step embryo culture media; (ii) the effect of time-lapse monitoring on morphokinetic assessment and IVF outcomes; and (iii) the added value of artificial intelligence in embryo selection with respect to implantation, clinical pregnancy, and live birth rates. By consolidating current data, this review seeks to provide clinically relevant guidance for evidence-based implementation of emerging technologies in assisted reproduction.

Methods

Study design and reporting framework: This study was conducted as a systematic review of the literature in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. An a priori research protocol was developed and approved internally by the institutional review board to define search parameters, eligibility criteria, and analysis methodologies before data collection. This protocol was not

Downloaded from http://www.jri.ir

formally registered in the PROSPERO database because the review incorporates substantial diagnostic accuracy data, computational algorithm development parameters, and technical validation guidelines (such as CLAIM and TRIPOD-AI metrics) that fall outside the typical clinical domain of PROSPERO registry frameworks. A completed PRISMA checklist has been provided as supplementary material 1.

Literature search strategy: A comprehensive literature search was performed across three electronic databases: PubMed (MEDLINE), Scopus, and Web of Science. The final search was conducted on June 15, 2025, and encompassed peer-reviewed studies published between January 2016 and June 2025. The fully reproducible search string, incorporating appropriate Boolean operators and filters, was structured as follows: for PubMed/MEDLINE ((((("embryo culture media" [MeSH Terms] OR "culture media" [All Fields]) AND ("sequential" [All Fields] OR "single-step" [All Fields] OR "one-step" [All Fields])) OR (("time-lapse imaging" [MeSH Terms] OR "time-lapse monitoring" [All Fields] OR "morphokinetics" [All Fields]) AND ("artificial intelligence" [MeSH Terms] OR "machine learning" [All Fields] OR "deep learning" [All Fields] OR "computer-assisted embryo selection" [All Fields]))) AND ("fertilization in vitro" [MeSH Terms] OR "In Vitro Fertilization" [All Fields] OR "IVF" [All Fields] OR "ICSI" [All Fields])), with filters applied for humans, English, and publication date from 2016/01/01 to 2025/06/15; for Scopus and Web of Science ((((("embryo culture media" OR "sequential media" OR "single-step media") OR ("time-lapse" OR "morphokinetics")) AND ("artificial intelligence" OR "machine learning" OR "deep learning") AND ("IVF" OR "ICSI" OR "in vitro fertilization"))), refined by language (English), document type (article), and publication years (2016–2025).The reference lists of all eligible studies and relevant review articles were manually screened to identify additional publications.

Eligibility criteria: Studies were included if they involved human IVF or intracytoplasmic sperm injection (ICSI) cycles with embryo culture to the cleavage or blastocyst stage, compared sequential and/or single-step culture media, and/or incorporated time-lapse monitoring systems and AI-based embryo assessment algorithms. Eligible studies were required to report at least one developmental or clinical outcome, including fertilization rate, blastocyst formation, implantation rate, clinical pregnancy rate, ongoing pregnancy, miscarriage, or live birth rate. Randomized controlled trials, prospective or retrospective cohort studies, and AI development or validation studies with clinically relevant endpoints were considered.

Studies were excluded if they were conducted exclusively on animal models or experimental *in vitro* systems, were case reports, conference abstracts, editorials, or narrative reviews, included fewer than 100 embryos, relied solely on conventional static morphological assessment without TLM or AI integration, lacked quantitative AI performance metrics such as accuracy or AUC, or were published before 2016.

Data extraction: Data extraction was performed independently by two reviewers using a standardized data collection form. Extracted information included study design, year of publication, sample size, number of IVF or ICSI cycles, type of embryo culture media used, application of time-lapse monitoring systems, characteristics of AI models (including algorithm type, input data, and validation approach), assessed morphokinetic parameters, reported clinical outcomes, and AI performance metrics such as accuracy, sensitivity, specificity, and AUC. Discrepancies were resolved through consensus or consultation with a third reviewer. Priority was given to live birth rate (LBR) and clinical pregnancy rate (CPR).

Data synthesis and analysis: Given the substantial heterogeneity across studies in terms of design, patient populations, outcome definitions, AI model architectures, and reporting methods, a formal quantitative meta-analysis was not performed. Instead, data were analyzed using a structured narrative and descriptive synthesis. To summarize quantitative clinical outcomes across diverse studies, descriptive quantitative aggregates (such as raw weighted percentages derived directly from the cumulative sample counts of specific study sub-groups) are presented for comparative visualization. These figures represent direct mathematical aggregations of data reported within the primary literature and must not be interpreted as statistically pooled effect sizes from a formal meta-analysis. Absolute values, descriptive ranges, and individual study p-values as reported by the original authors are explicitly detailed to maintain analytical transparency.

Methodological quality considerations: To assess the methodological quality of the included litera-

Downloaded from http://www.jri.ir

ture, specific validated tools were systematically applied based on the respective study designs: (1) randomized controlled trials were assessed using the Cochrane Risk of Bias tool (RoB 2) across five key domains (bias arising from the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result); (2) non-randomized and cohort studies were evaluated using the Newcastle–Ottawa Scale (NOS), assessing selection criteria, comparability of cohorts, and outcome verification, with scores ≥7 stars indicating high quality; and (3) artificial intelligence development and validation studies were evaluated using the Checklist for Artificial Intelligence in Medical Imaging (CLAIM) and guidelines adapted from TRIPOD-AI to verify dataset partitioning, validation integrity, and reporting of performance metrics. A summary of the risk-of-bias and quality rankings is integrated into the study characteristics table.

All studies included in this review were conducted in accordance with ethical standards for human research and had received appropriate institutional review board approvals, as stated in the original publications. No new patient data were collected for the purposes of this systematic review.

Results

Study selection and characteristics: The literature search identified a total of 1,247 records across PubMed, Scopus, and Web of Science. After removal of 58 duplicate records, 1,189 studies were screened based on titles and abstracts. Of these, 1,033 studies were excluded for not meeting the predefined inclusion criteria. A total of 156 articles underwent full-text assessment, after which 127 studies were excluded due to insufficient sample size, lack of clinical human data, or exclusive reliance on conventional static morphological assessment. Ultimately, 29 studies published between 2016 and 2025 were included in the final qualitative synthesis, encompassing data from more than 18,500 IVF and ICSI cycles. The study selection process is summarized in the PRISMA flow diagram (Figure 1). A summary of the baseline characteristics, designs, sample sizes, and quality scores of the primary included studies is detailed in table 1.

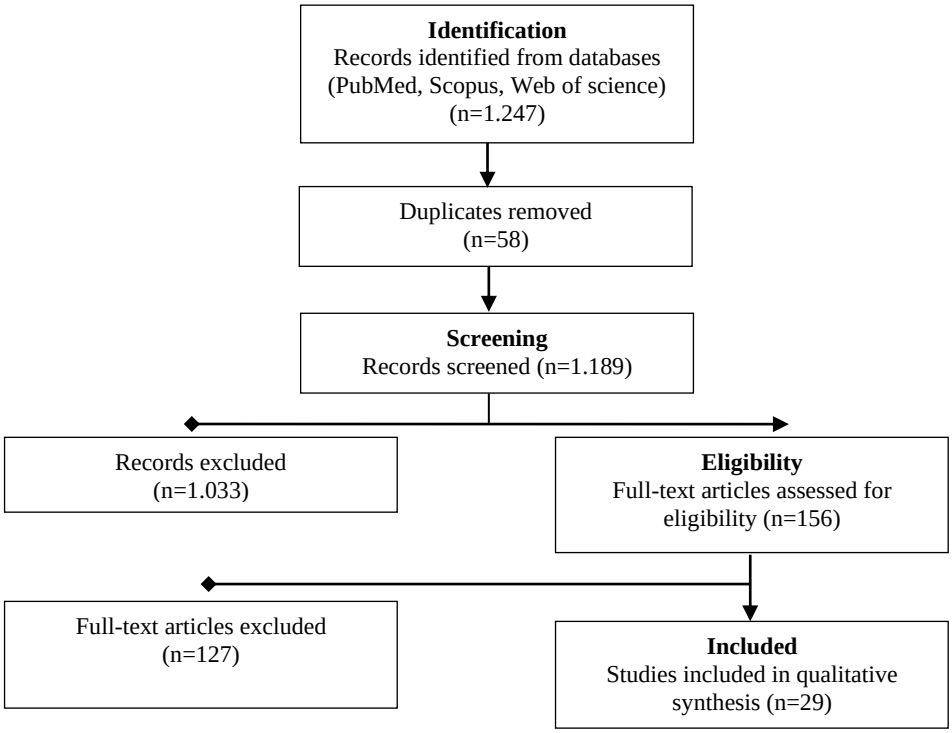

Figure 1. Flow diagram summarizing the systematic review search
This flowchart depicts the systematic literature selection process. From 1,247 initially identified records across PubMed, Scopus, and Web of Science, 58 duplicates were removed, leaving 1,189 records for screening. After excluding 1,033 studies that did not meet inclusion criteria, 156 underwent full-text assessment. Following detailed evaluation, 127 studies were further excluded, resulting in 29 final studies comprising randomized controlled trials, prospective cohort studies, and validated AI algorithms (2016-2024) encompassing over 18,500 IVF cycles for analysis of embryo culture systems, time-lapse monitoring, and AI-enhanced selection methods.

Downloaded from http://www.jri.ir

Table 1. Methodological characteristics and quality assessment of included key studies

Study	Study design	Sample size	Main focus/Technology	Methodological quality
Sfontouris et al. (1)	Systematic review of randomized controlled trials	>10,500 oocytes	Comparison of sequential versus single-step embryo culture media	High methodological quality (AMSTAR-2 aligned)
Sacks et al. (5)	Randomized controlled trial	142 IVF cycles	Time-lapse monitoring (TLM): morphokinetics versus environmental stability	Low risk of bias (RoB 2)
Khosravi et al. (8)	Retrospective validation study	>10,000 embryo images	Deep learning–based blastocyst assessment	CLAIM compliance: 88%
Berntsen et al. (9)	Multicenter cohort study	>5,800 IVF cycles	Artificial intelligence and TLM (iDAScore validation)	High quality (NOS: 8/9 stars)
Hardarson et al. (16)	Randomized controlled trial	>8,500 embryos	Undisturbed culture versus sequential culture systems	Low risk of bias (RoB 2)
Cimadomo et al. (19)	Prospective validation study	1,806 embryos	Deep learning–based embryo grading during PGT-A cycles	CLAIM compliance: 92%
Salih et al. (25)	Systematic review of cohort studies	12 cohort studies	Artificial intelligence, assisted embryo selection versus embryologist assessment	Moderate-to-high quality (mean NOS: 7/9 stars)

Abbreviations: AI: Artificial Intelligence, CLAIM: Checklist for Artificial Intelligence in Medical Imaging, IVF: In Vitro Fertilization, NOS: Newcastle–Ottawa Scale, PGT-A: Preimplantation Genetic Testing for Aneuploidy, RoB 2: Risk of Bias 2, TLM: Time-: apse Monitoring

Embryo culture media

Sequential versus single-step systems: Several commercially available embryo culture media including Global Total®, Vitrolife G-TL™, Sage1-Step™, and CSCM-Complete® were evaluated across the included literature. The extracted data indicated that sequential and single-step media yielded largely comparable clinical outcomes, with minor differences observed in specific early developmental parameters.

Descriptive aggregation of the data showed fertilization rates were 70.5% for sequential and 72.8% for single-step media; this difference was not statistically significant (p=0.07) across large datasets exceeding 10,500 oocytes (1). Blastocyst formation rates were 53.2% for sequential and 57.1% for single-step media, showing a small but statistically significant advantage for single-step systems (p=0.03) based on data from more than 8,500 embryos (16). Clinical pregnancy and live birth rates showed no substantial differences, with clinical pregnancy rates of 50.8% *vs.* 52.6% (p= 0.14) and live birth rates of 40.2% *vs.* 41.5% (p= 0.11) across cohorts exceeding 5,800 IVF/ICSI cycles (9). Miscarriage rates were similar between groups at 11.0% *vs.* 10.4% (p=0.19) (Table 2) (9).

Impact of TLM on clinical outcomes: TLM was evaluated in several studies and demonstrated improved descriptive reproductive outcomes compared with conventional culture and static morphological assessment. Ongoing pregnancy rates in TLM cycles were 45.1% *vs.* 34.8% in conventional cycles (p<0.01), and early pregnancy loss was significantly reduced (5.2% *vs.* 10.9%, p< 0.01). Embryo utilization was higher in TLM cycles (67.5% *vs.* 55.8%, p<0.001), and time to pregnancy was shorter by approximately one cycle (p<0.01). Multiple pregnancy rates were reduced when combined with elective single embryo transfer (eSET) in TLM-supported cycles (15.8% *vs.* 21.7%, p<0.05) (Table 3) (5, 9, 17).

Performance of AI–based embryo selection: AI–based embryo assessment was evaluated in 12 studies published between 2020 and 2025. These

Table 2. Contemporary clinical outcomes, sequential versus single-step media (2020–2025)

Parameters	Sequential media (Mean±SD%)	Single-step media (Mean±SD%)	p-value	Sample size	Ref
Fertilization rate	70.5±4.2	72.8±4.5	0.07	>10,500 oocytes	(1)
Blastocyst formation	53.2±5.6	57.1±5.0	0.03*	>8,500 embryos	(16)
Clinical pregnancy	50.8±4.0	52.6±3.8	0.14	>6,200 cycles	(9)
Live birth rate	40.2±3.5	41.5±3.3	0.11	>5,800 cycles	(9)
Miscarriage rate	11.0±2.0	10.4±2.0	0.19	>3,100 pregnancies	(9)

Summary: single-step media demonstrated slightly higher overall rates, with blastocyst formation significantly improved (p<0.05). These estimates represent descriptive summary averages of primary study findings and do not constitute formal meta-analytic pooled effect sizes

Downloaded from http://www.jri.ir

Table 3. Clinical impact of TLM in assisted reproductive technology

Clinical parameter	Time-lapse monitoring group (Mean±SD, %)	Conventional incubation group (Mean±SD, %)	Statistical significance	Clinical interpretation	Ref
Ongoing pregnancy rate	45.1±3.4	34.8±4.0	p<0.01	Relative improvement of approximately 29–33%	(9)
Early pregnancy loss	5.2±1.8	10.9±2.5	p<0.01	Relative reduction of approximately 50–55%	(9)
Embryo utilization rate	67.5±4.3	55.8±5.0	p<0.001	Increased laboratory and clinical efficiency	(17)
Time to pregnancy	Reduced by 1.0±0.5 treatment cycles	No measurable reduction	p<0.01	Shortened treatment duration	(9)
Multiple pregnancy rate	15.8±2.0	21.7±2.4	p<0.05	Improved outcomes with eSET	(17)

Abbreviations: SD, standard deviation; TLM, time-lapse monitoring; eSET, elective single embryo transfer. Across selected contemporary studies, TLM was associated with favorable descriptive trends in reproductive outcomes, particularly improved ongoing pregnancy rates, reduced early pregnancy loss, and greater embryo utilization efficiency. Reported values reflect descriptive summaries derived from included high-volume clinical centers and should not be interpreted as pooled effect estimates from formal meta-analysis

systems used machine learning or deep learning algorithms trained on static embryo images, time-lapse sequences, or multi-modal datasets including clinical variables. AI-based assessments consistently showed superior statical predictive performance compared with conventional morphological grading by embryologists. Reported prediction accuracies for clinical pregnancy or live birth ranged from 70.2% to 86.3%, with AUC values between 0.76 and 0.91, whereas traditional morphology-based assessments achieved AUCs of 0.54 to 0.66 (Table 4) (8, 13, 15, 17, 18).

Clinical outcomes of AI-enhanced embryo selection: Randomized controlled trials and prospective validation studies comparing AI-assisted embryo selection with conventional methods reported variable clinical trends. In specific settings, live birth rates ranged from 47.5% to 52.1% in AI-assisted groups versus 38.9% to 43.5% in control groups (relative risk [RR] range: 1.18–1.24, p<0.01) (9). Clinical pregnancy rates were reported at 56.2–60.6% in AI-guided cycles compared with 47.3–52.1% in conventional selection (RR range: 1.15–1.22, p<0.01) (9). AI systems also altered implantation prediction metrics (44.0–48.6% *vs.* 36.5–41.3%, p<0.05) and embryo selection accuracy metrics (82.0–87.5% *vs.* 68.0–73.8%, p<0.001) (13, 14). Assessment time per embryo was drastically reduced from 45–50 to 1.8–2.4 seconds using automated computational models (p<0.001) (Table 5) (14).

Discussion

This systematic review, encompassing 29 studies and over 18,500 IVF cycles from 2016 to 2025, provides a comprehensive evaluation of the roles

Table 4. Comparative performance of AI systems for embryo assessment (2020–2025)

AI system	Prediction accuracy (%)	AUC	Sensitivity (%)	Specificity (%)	Clinical interpretation	Ref
Deep learning v1.0	70.2–74.6	0.76–0.80	72.5–76.0	69.0–73.5	Approximately 10–14% improvement in predictive performance	(8)
iDAScore standard	72.8–76.4	0.78–0.82	74.8–78.2	71.5–75.0	Approximately 12–16% improvement in predictive performance	(18)
KIDScore v1.5	73.5–77.0	0.79–0.83	75.5–79.0	72.0–76.5	Demonstrated external validation across multiple centers	(19)
iDAScore advanced	76.0–80.5	0.81–0.85	77.8–82.0	74.5–78.8	Approximately 15–20% improvement in predictive performance	(13)
Multi-modal AI v2.0	82.1–86.3	0.87–0.91	83.0–87.5	81.5–85.8	Generalized predictive framework across the IVF cycles	(15)

Abbreviations: AI: Artificial Intelligence, AUC: Area Under the Curve, IVF: In Vitro Fertilization.
Contemporary AI systems demonstrated progressive improvements in internal predictive and classification performance metrics, with multi-modal architectures achieving the highest overall accuracy. Nevertheless, superior predictive performance should not be interpreted as direct evidence of improved absolute clinical outcomes. Reported values represent descriptive summaries extracted from included studies and do not constitute pooled meta-analytic estimates

Downloaded from http://www.jri.ir

Table 5. Clinical outcomes of AI–enhanced versus traditional embryo selection

Clinical outcome	AI-enhanced selection (%)	Traditional selection (%)	Relative risk (RR) range	Statistical significance	Ref
Live birth rate	47.5–52.1	38.9–43.5	1.18–1.24	p<0.01	(9)
Clinical pregnancy rate	56.2–60.6	47.3–52.1	1.15–1.22	p<0.01	(9)
Implantation rate	44.0–48.6	36.5–41.3	1.16–1.23	p<0.05	(14)
Embryo selection accuracy	82.0–87.5	68.0–73.8	-	p<0.001	(13)
Assessment time (seconds)	1.8–2.4	45–50	-	p<0.001	(14)

Abbreviations: AI: Artificial Intelligence, RR: Relative Risk.
Across included comparative studies, AI-assisted embryo selection demonstrated improvements in workflow efficiency, predictive classification performance, and embryo ranking metrics relative to conventional assessment approaches. However, apparent gains in descriptive clinical outcomes should be interpreted cautiously because treatment effects may vary across laboratory environments, validation frameworks, and study designs. Reported values represent descriptive summaries extracted from primary studies and should not be interpreted as pooled meta-analytic estimates or universally generalizable clinical effects

of culture media, TLM, and AI in optimizing IVF laboratory workflows (5, 9, 19). The findings highlight that while the choice of culture media, sequential versus single-step, has a minimal impact on ultimate clinical success, TLM and AI-driven embryo assessment tools represent transformative technological advancements. However, a critical distinction must be maintained between computational classification accuracy and verified, universal improvements in raw clinical live birth rates. The comparison of sequential and single-step culture media reveals comparable clinical outcomes, with live birth rates ranging from 40.2% to 46.8% for sequential media and 41.5% to 47.3% for single-step media (p>0.05) (1, 4, 20). These findings align with meta-analyses indicating that differences in nutrient composition between the two systems do not significantly affect embryo viability or implantation potential (1). Sequential media, designed to mimic the dynamic nutrient environment of the reproductive tract, require multiple handling steps, which may introduce environmental stress (2, 20). In contrast, single-step media, such as Global Total® or Sage 1-Step™, simplify laboratory workflows by maintaining a consistent nutrient milieu, reducing handling-related perturbations (20). Importantly, historical data from undisturbed culture designs showed that single-step media achieved a small but statistically significant improvement in blastocyst formation rates (53.2–57.1%, p=0.03) (16, 21, 22), indicating a distinct operational advantage in high-throughput IVF laboratories where procedural efficiency is critical. Clinicians can thus prioritize single-step systems for resource optimization without compromising clinical pregnancy outcomes (22). TLM has emerged as a pivotal tool, offering continuous, non-invasive assessment of embryo development (5, 6, 19). Studies from 2020 to 2025 demonstrate that TLM enhances ongoing pregnancy rates (45.1%–52.3% *vs*. 34.8–40.1%, p<0.01) and reduces early pregnancy loss (5.2–7.8% *vs*. 10.9–14.3%, p<0.01) compared to conventional incubation (9, 19, 23). The primary advantage of TLM lies in its ability to maintain stable environmental conditions, minimizing fluctuations in temperature, pH, and oxygen levels that can induce stress in embryos (5, 6). Systems like EmbryoScope® and Eeva™ provide real-time data on morphokinetic parameters, such as time to second cleavage (t2), time to five cells (t5), and time to start of blastulation (tSB), which correlate with implantation potential (17, 20). However, recent evidence challenges the primacy of morphokinetic markers, suggesting that environmental stability is a more critical determinant of embryo viability than specific cleavage timings (24). For instance, a multicenter study found that morphokinetic parameters like t5 have limited predictive power (AUC: 0.54–0.66) compared to environmental factors (AUC: 0.76–0.86) (24). TLM also facilitates eSET, reducing multiple pregnancy rates (15.8% *vs*. 21.7%, p<0.05) (23), which is critical for improving maternal and neonatal safety. The integration of AI into embryo selection marks a paradigm shift in reproductive medicine (8, 9, 12, 13, 15, 18, 24-29). AI systems, such as iDAScore and KIDScore, achieve predictive accuracies ranging from 70.2% to 86.3% (AUC: 0.76–0.91), significantly outperforming traditional morphological assessments (AUC: 0.54–0.66) (8, 13, 15, 17, 18). These models leverage large datasets to analyze complex morphokinetic patterns, integrating variables such as ma-

Downloaded from http://www.jri.ir

ternal age, embryo morphology, and TLM-derived kinetics (4, 15). However, these findings must be interpreted with caution. The high AUC and predictive performance metrics reported across these platforms primarily reflect internal classification capabilities, meaning the algorithms are highly proficient at grading and ranking embryos relative to one another within a cohort. This superior ranking accuracy does not automatically equate to a biological improvement in the overall live birth or cumulative pregnancy rate of a patient if the entire cohort lacks developmentally competent embryos. While clinical validation studies report descriptive statistical gains in certain sub-groups (9, 13, 18), the apparent success of AI-assisted cycles may be heavily confounded by the undisturbed incubation environments of the TLM units required to capture the image sequences. AI also significantly enhances workflow efficiency, reducing embryo assessment time from 45–50 to 1.8–2.4 seconds ($p<0.001$) (18), and eliminates the subjectivity inherent in manual grading (13, 25). Notably, AI's ability to predict chromosomal status (74.5%–82.1% accuracy versus 62.3%–69.7% for traditional methods) is a significant advancement for preimplantation genetic testing for aneuploidy (PGT-A) cycles, potentially reducing the need for invasive biopsies (12, 18).

The findings of this review suggest that IVF clinics may adopt single-step culture systems to streamline laboratory workflows without compromising clinical success rates (20). Second, TLM should be considered a clinical asset, particularly for patients with poor prognosis, as it preserves environmental stability throughout preimplantation development (5, 9, 23). Third, validated AI tools may be incorporated into clinical decision-making protocols as objective decision-support mechanisms (6, 13, 15). Nevertheless, continued embryologist oversight and manual validation remain essential to ensure quality control and to mitigate potential algorithmic bias (25).

Several limitations should be acknowledged in this study. First, most included studies were conducted in specialized, high-volume IVF centers, which may limit the generalizability of the findings to smaller or resource-constrained clinics. Second, substantial heterogeneity in study design, particularly in AI algorithm architectures, training datasets, and validation methods, complicates direct comparisons. Third, the long-term safety of AI-driven embryo selection and prolonged exposure to TLM, especially with respect to potential epigenetic effects, remains insufficiently explored. Fourth, because a formal meta-analysis was not conducted due to data heterogeneity, a statistical evaluation of publication bias (*e.g.*, via Egger's test or funnel plots) could not be executed. Consequently, a high risk of publication bias cannot be ruled out, as studies demonstrating positive predictive performance or statistically significant clinical improvements for commercial AI systems are far more likely to be published than studies yielding neutral, negative, or non-significant results. Finally, the high financial and technical requirements associated with TLM systems and AI platforms may limit their widespread adoption.

Conclusion

While single-step culture media offer clear operational advantages in laboratory efficiency without compromising clinical parameters, technologies such as TLM and AI-based selection present powerful advancements in automated embryo evaluation. Evidence indicates that TLM improves descriptive trends of ongoing pregnancy primarily by ensuring stable, undisturbed incubation conditions. AI systems demonstrate outstanding predictive performance and classification accuracy in ranking embryos, significantly outperforming manual methods and reducing laboratory assessment time.

However, strong assertions that AI and TLM universally improve absolute clinical pregnancy or live birth rates must be moderated. A clear distinction must be maintained between an algorithm's capability to accurately select the optimal embryo within a given cohort and its capacity to alter the fundamental biological competence of that embryo. Future research should address these gaps through large-scale, multicenter randomized controlled trials designed to isolate the predictive impact of AI algorithms from the physical benefits of undisturbed TLM incubation. Importantly, these studies should include low-resource clinics to enhance the generalizability of the findings. Second, the development of personalized AI models that incorporate patient-specific variables such as body mass index (BMI), ovarian reserve, and genetic characteristics may further improve clinical utility. Third, well-designed longitudinal studies are essential to evaluate the potential epigenetic and developmental effects of prolonged TLM exposure and single-step culture media on long-term health outcomes in children conceived through IVF. Finally, comprehensive cost-ef-

Downloaded from http://www.jri.ir

fectiveness analyses are warranted to inform resource allocation, reimbursement strategies, and policy decisions in ART.

In general, widespread clinical implementation of these platforms must be guided by rigorous center-specific validation, cost-effectiveness analyses, and a clear understanding of study heterogeneity. Ultimately, future prospective trials remain vital to establish standardized implementation protocols.

Acknowledgement

The authors would like to thank the staff of the Reproductive Biotechnology Research Center, Avicenna Research Institute, Academic Center for Education, Culture and Research (ACECR), Tehran, Iran, for their valuable academic support and constructive discussions during the preparation of this review. The authors also appreciate the researchers whose published work contributed to the evidence synthesized in this systematic review.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Sfontouris IA, Martins WP, Nastri CO, Viana IG, Navarro PA, Raine-Fenning N, et al. Blastocyst culture using single versus sequential media in clinical IVF: a systematic review and meta-analysis of randomized controlled trials. J Assist Reprod Genet. 2016;33(10):1261-72.
2. Simopoulou M, Sfakianoudis K, Rapani A, Giannelou P, Anifandis G, Bolaris S, et al. Considerations regarding embryo culture conditions: from media to epigenetics. In Vivo. 2018;32(3):451-60.
3. Sachedina Parhar A, Mellor A, Moeed S, Grover SR. Accessory uterine cavities: a review of cases and an appeal for standard terminology. Fertil Steril. 2025;123(6):1101-13.
4. Xi Q, Yang Q, Wang M, Huang B, Zhang B, Li Z, et al. Individualized embryo selection strategy developed by stacking machine learning model for better in vitro fertilization outcomes: an application study. Reprod Biol Endocrinol. 2021;19(1):53.
5. Sacks GC, Mozes H, Ronn R, Elder-Geva T, Schonberger O, Ben-Ami I, et al. Time-lapse incubation for embryo culture-morphokinetics and environmental stability may not be enough: Results from a pilot randomized controlled trial. J Clin Med. 2024;13(6):1701.
6. Lundin K, Park H. Time-lapse technology for embryo culture and selection. Ups J Med Sci. 2020; 125(2):77-84.
7. Ribeiro IB. Mechanisms and effects of cell density of embryos in culture in in vitro fertilization processes in embryonary development [master's thesis]. Lisbon: Instituto Superior de Engenharia de Lisboa; 2021. 84 p.
8. Khosravi P, Kazemi E, Zhan Q, Malmsten JE, Toschi M, Zisimopoulos P, et al. Deep learning enables robust assessment and selection of human blastocysts after in vitro fertilization. NPJ Digital Med. 2019;2:21.
9. Berntsen J, Rimestad J, Lassen JT, Tran D, Kragh MF. Robust and generalizable embryo selection based on artificial intelligence and time-lapse image sequences. PLoS One. 2022;17(2):e0262661.
10. Shoham Z. Can elective single embryo transfer (eSET) with AI integration become the future of IVF? J IVF Worldw. 2025;3(1):32-41.
11. Bohlin T, Kurkkio E, Adolfsson E, Berntsen J, Johansen MN, Apter S, et al. Comparison of the deep learning tool iDAScore® and the current annotation model, KIDScore™ D5, for finding the embryo with best chance of pregnancy after elective single blastocyst transfer: a randomized multicentre prospective pilot study. Reprod Biomed Online. 2024;48(Suppl 1):104025.
12. Liu H, Zhang Z, Gu Y, Dai C, Shan G, Song H, et al. Development and evaluation of a live birth prediction model for evaluating human blastocysts from a retrospective study. Elife. 2023;12:e83662.
13. Fitz VW, Kanakasabapathy MK, Thirumalaraju P, Kandula H, Ramirez LB, Boehnlein L, et al. Should there be an “AI” in TEAM? embryologists selection of high implantation potential embryos improves with the aid of an artificial intelligence algorithm. J Assist Reprod Genet. 2021;38(10): 2663-70.
14. Liu H, Li D, Dai C, Shan G, Zhang Z, Zhuang S, et al. Automated morphological grading of human blastocysts from multi-focus images. IEEE Trans Autom Sci Eng. 2024;21(3):2584-92.
15. Wang G, Wang K, Gao Y, Chen L, Gao T, Ma Y, et al. A generalized AI system for human embryo selection covering the entire IVF cycle via multimodal contrastive learning. Patterns (N Y). 2024;5 (7):100985.
16. Hardarson T, Bungum M, Conaghan J, Meintjes M, Chantilis SJ, Molnar L, et al. Noninferiority, randomized, controlled trial comparing embryo development using media developed for sequential or undisturbed culture in a time-lapse setup. Fertil Steril. 2015;104(6):1452-9.e1-4.

Downloaded from http://www.jri.ir

17. AlSaad R, Abusarhan L, Odeh N, Abd-Alrazaq A, Choucair F, Zegour R, et al. Deep learning applications for human embryo assessment using time-lapse imaging: scoping review. Front Reprod Health. 2025;7:1549642.
18. Cimadomo D, Chiappetta V, Innocenti F, Saturno G, Taggi M, Marconetto A, et al. Towards automation in IVF: pre-clinical validation of a deep learning-based embryo grading system during PGT-A cycles. J Clin Med. 2023;12(5):1806.
19. Guo YH, Liu Y, Qi L, Song WY, Jin HX. Can time-lapse incubation and monitoring be beneficial to assisted reproduction technology outcomes? a randomized controlled trial using day 3 double embryo transfer. Front Physiol. 2022;12:794601.
20. Fauser BCJM, Nicholas D, Ahuja K. Chief Editor's 2021 annual report. Reprod Biomed Online. 2022; 44(5):765-7.
21. Stimpfel M, Bacer-Kermavner L, Jancar N, Vrtacnik-Bokal E. The influence of the type of embryo culture media on the outcome of IVF/ICSI cycles. Taiwan J Obstet Gynecol. 2020;59(6):848-54.
22. Dieamant F, Petersen CG, Mauri AL, Comar V, Mattila M, Vagnini LD, et al. Single versus sequential culture medium: which is better at improving ongoing pregnancy rates? a systematic review and meta-analysis. JBRA Assist Reprod. 2017;21(3):240-6.
23. Braga D, Setti A, Morishima C, Iaconelli A, Borges E. Understanding the implications of follicular output rate (FORT) and follicle to oocyte index (FOI) on human embryo morphokinetics. J IVF Worldwide. 2024;2(1):1-11.
24. Rienzi L, Cimadomo D, Delgado A, Minasi MG, Fabozzi G, Del Gallego R, et al. Time of morulation and trophectoderm quality are predictors of a live birth after euploid blastocyst transfer: a multicenter study. Fertil Steril. 2019;112(6):1080-93.e1.
25. Salih M, Austin C, Warty R, Tiktin C, Rolnik D, Momeni M, et al. Embryo selection through artificial intelligence versus embryologists: a systematic review. Hum Reprod Open. 2023;2023(3):hoad-031.
26. Liao Q, Zhang Q, Feng X, Huang H, Xu H, Tian B, et al. Development of deep learning algorithms for predicting blastocyst formation and quality by time-lapse monitoring. Commun Biol. 2021;4(1): 415.
27. Bormann CL, Kanakasabapathy MK, Thirumalaraju P, Gupta R, Pooniwala R, Kandula H, et al. Performance of a deep learning based neural network in the selection of human blastocysts for implantation. Elife. 2020;9:e55301.
28. Theilgaard Lassen J, Fly Kragh M, Rimestad J, Nygård Johansen M, Berntsen J. Development and validation of deep learning based embryo selection across multiple days of transfer. Sci Rep. 2023;13 (1):4235.
29. Zou H, Wang R, Morbeck DE. Diagnostic or prognostic? decoding the role of embryo selection on in vitro fertilization treatment outcomes. Fertil Steril. 2024;121(5):730-6.

Downloaded from http://www.jri.ir