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Noninvasive methods for preimplantation blastocyst quality assessment in *in vitro* fertilization programs

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ABSTRACT

Since the first *in vitro* fertilization (IVF) procedure, assisted reproductive technologies have helped many patients overcome infertility. However, according to the 2022 National Registry of Assisted Reproductive Technologies of the Russian Association of Human Reproduction, the probability of achieving pregnancy through IVF remains below 50%. Morphological assessment of blastocyst quality remains the gold standard. Implantation rates have increased to some extent due to the selection of high-quality embryos. However, given the subjectivity of morphological evaluation, further research is needed to establish the correlation between embryo reproductive potential and morphology. Time-lapse imaging combined with artificial intelligence may enhance the objectivity of assessment and identify additional morphological features indicative of blastocyst quality. The detection of exosomes, proteins, and metabolites secreted into the culture medium during embryo development may provide insights into the physiological state of the embryo and its interactions with the surrounding environment, potentially serving as markers of implantation potential. This review provides an overview of the morphological and biochemical markers of blastocyst quality, their interrelationships, and the use of artificial intelligence in embryo selection for transfer. A literature search was conducted in the electronic databases PubMed and Google Scholar using the following keywords: IVF, blastocyst, human embryo, culture media, timelapse system, embryo string, embryo exosomes, morphology, artificial intelligence, proteome, and metabolome. The analysis included studies published in the past five years.

Keywords: blastocyst; implantation; culture medium; metabolome; proteome; exosomes; IVF.

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Неинвазивные методы преимплантационной оценки качества бластоцисты в программах экстракорпорального оплодотворения

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АННОТАЦИЯ

С момента первой процедуры экстракорпорального оплодотворения вспомогательные репродуктивные технологии помогли многим пациентам в лечении бесплодия. Однако, по данным национального регистра вспомогательных репродуктивных технологий Российской ассоциации репродукции человека (2022), вероятность беременности в результате экстракорпорального оплодотворения по-прежнему составляет менее 50%. Морфологическая оценка качества бластоцисты остаётся золотым стандартом. В определённой степени доля имплантации увеличилась благодаря отбору высококачественных эмбрионов. Однако в связи с субъективным характером морфологической оценки необходимы дальнейшие исследования для установления связи репродуктивного потенциала эмбрионов с их морфологией. Повысить объективность оценки и обнаружить новые морфологические признаки качества бластоцисты может система замедленной съёмки в комплексе с возможностями искусственного интеллекта. Детекция экзосом, белков и метаболитов, которые выделяются в процессе роста в культуральную среду, могут помочь определить способность бластоцисты к имплантации, так как они предоставляют информацию о физиологическом состоянии эмбриона и его взаимодействии с окружающей средой. В данном научном обзоре представлены сведения о морфологических, биохимических признаках качества бластоцисты, их взаимосвязи, а также применении искусственного интеллекта в отборе эмбриона для переноса. Поиск публикаций произведён в электронных базах данных PubMed и Google Scholar. Статьи искали по следующим ключевым словам: «IVF», «blastocyst», «human embryo», «culture media», «timelapse system», «embryo string», «embryo exosomes», «morphology», «artificial intilligence», «proteome», «metabolome». В работе проанализированы статьи, опубликованные в последние 5 лет.

Ключевые слова: бластоциста; имплантация; культуральная жидкость; метаболом; протеом; экзосомы; ЭКО.

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体外受精方案中胚泡质量的非侵入性植前评估方法

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摘要

自首次体外受精（IVF, in vitro fertilization）技术问世以来，辅助生殖技术已帮助众多患者克服不孕难题。然而，根据俄罗斯人类生殖协会（2022年）辅助生殖技术国家注册数据，IVF的妊娠成功率仍低于50%。目前，胚泡的形态学评估仍然是胚胎质量评估的“金标准”，在一定程度上，通过选择高质量胚胎，提高了胚胎植入率。然而，由于形态学评估具有一定主观性，因此需要进一步研究，以明确胚胎的生殖潜能与其形态之间的相关性。延时成像系统结合人工智能技术可提高评估的客观性，并识别新的胚泡质量形态学特征。此外，胚胎在培养过程中释放到培养液中的外泌体、蛋白质和代谢物的检测，可提供关于胚胎生理状态及其与环境相互作用的信息，从而有助于预测胚泡的植入潜能。本综述总结了胚泡质量的形态学及生化特征及其相互关系，并探讨了人工智能在胚胎筛选与移植决策中的应用。文献检索在PubMed和Google Scholar电子数据库中进行，使用的关键词包括：“IVF”（体外受精）、“blastocyst”（囊胚）、“human embryo”（人类胚胎）、“culture media”（培养基）、“timelapse system”（延时成像系统）、“embryo string”（胚胎细胞质串）、“embryo exosomes”（胚胎外泌体）、“morphology”（形态学）、“artificial intelligence”（人工智能）、“proteome”（蛋白质组）、“metabolome”（代谢组）。本研究分析了近5年发表的相关文献。

关键词：胚泡；植入；培养液；代谢组；蛋白质组；外泌体；体外受精。

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INTRODUCTION

Infertility treatment is a medical, social and economic challenge. According to the Federal State Statistics Service, the infertility rate in 2022 was 203.29 newly diagnosed cases per 100,000 women aged 18–49 years, and this parameter does not tend to decrease. The World Health Organization (2023) estimates that 17.5% of the world's adult population suffers from infertility, or one in six people[1]. Since 2013, in vitro fertilization (IVF) has been included in the list of compulsory health insurance services. The pregnancy rate with IVF varies widely, from 8.6% to 46.2% [1]. There is a need to determine factors of IVF success and consider all these factors, risks, and treatment costs. For successful IVF, it is important to select an embryo to be transferred into the uterine cavity. This review discusses embryo quality assessment techniques that embryologists have been using in their practice for over 20 years, as well as more recent techniques and new parameters of blastocyst survival that require further investigation for full clinical implementation.

METHODS

PubMed, eLibrary, and Google Scholar databases were searched for publications. Relevant articles were found by keywords such as *IVF, blastocyst, human embryo, culture media, timelapse system, embryo string, embryo exosomes, morphology, artificial intelligence, proteome,* and *metabolome*. The analysis included articles published in the last five years.

RESULTS

Gardner Embryo Grading System

The embryo grading system proposed by Gardner et al. [2] is still generally accepted and most widely used in selecting embryos for IVF and intracytoplasmic sperm injection (ICSI). This grading system is based on maturation of the blastocyst, which is given a numerical score from 1 to 6 (1 for a blastocoel that is less than half the volume of the embryo, 6 for a hatched blastocyst), trophoblast and embryoblast morphology indicated by A, B, C. Recent studies have shown that blastocysts classified as good (3–6BB) and excellent (3–6AA, AB, BA) are more common to be implanted in the endometrium and are positively correlated with the percentage of resulting pregnancies and full-term births [3–6]. Some studies have also demonstrated the diagnostic value of trophoblast and embryoblast grades. One study used multivariate logistic analysis to show that trophoblast grade, as opposed to embryoblast grade, had a statistically significant effect on rates of resulting pregnancies and live births. The study compared blastocysts with grade A and C trophoblast cells. The transplantation of blastocysts with grade A trophoblast cells was 2.32 times more likely to result in pregnancy and 2.22 times more likely to result in a live birth ($p = 0.04$). However, embryoblast cell grade did not have a statistically significant effect on these parameters ($p = 0.47$) [7]. A similar study (2022) does not contradict the above study and shows that trophoblast grade ($p = 0.021$), as opposed to embryoblast grade ($p = 0.129$), also has statistically significant effect on the rate of biochemical pregnancy [8]. These findings may be due to the key role of the trophoblast in the implantation of the blastocyst, followed by the development of the amnion later in the embryonic process.

The Gardner embryo grading system is based solely on visual assessment of blastocyst morphology and does not consider morphometric parameters. A Japanese cross-sectional study proposed a morphometric system for evaluating blastocysts by measuring blastocyst diameter, embryoblast size, and estimated trophoblast cell counts to assess maturation of the blastocyst, trophoblast grade, and embryoblast grade, respectively. This study also examined the effects of these parameters on rates of pregnancy prolongation into the second trimester. In contrast to the embryoblast size and estimated trophoblast cell counts, the blastocyst diameter showed no effects on pregnancy course. The rates of pregnancy prolongation into the second trimester were 2.9% at an embryoblast size <1,500 mm^2 and 33.3% at an embryoblast size >4,500 mm^2. The estimated number of trophoblasts that survived into the second trimester was 2.9% with a cell count <60. A stable increase in pregnancy rates was reported with increasing cell counts, reaching 34.3% at a cell count >130. The combined effect on pregnancy outcome was calculated for the embryoblast size and estimated trophoblast cell counts, as these parameters were independently associated with the rates of progressive pregnancies. The rate of progressive pregnancies was 2.0% (1/49) at an embryoblast size <2,500 mm^2 and estimated trophoblast cell counts <70. These rates reached 47.8% (22/46) at an embryoblast size >3,500 mm^2 and estimated trophoblast cell count >110 [9].

The above morphometric grading system for blastocyst is beneficial because it introduces numerical parameters and automates blastocyst morphologic evaluation. This reduces IVF/ICSI costs and optimizes speed and effectiveness of the fertility specialist's work.

None of the studies reviewed showed a statistically significant effect of maturation of the blastocyst (diameter) on pregnancy course, which may be related to its association with the other two parameters of the considered grading system [9].

Blastocyst Hatching

Blastocyst hatching (when the blastocyst sheds its clear outer membrane) is an important stage in embryo

[1] WHO: 1 in 6 people globally affected by infertility [cited 2024 Jul 20]. Available from: https://www.who.int/ru/news/item/04-04-2023-1-in-6-people-globally-affected-by-infertility

implantation [10]. Embryologists often use the Gardner embryo grading system to grade only more developed blastocysts (i.e., those that have fully hatched). In addition, the current grading system for blastocysts is subjective and controversial among embryologists. Therefore, it is necessary to identify potentially significant objective and quantitative criteria for blastocyst grading [11].

In a Korean study, transfer of a completely hatched blastocyst in the single embryo transfer group was associated with higher implantation and pregnancy rates compared to hatching blastocysts (15.7% and 15.6%, respectively; $p < 0.05$). In addition, in the double embryo transfer group, the implantation and pregnancy rates remained higher with the transfer of completely hatched blastocysts compared to hatching blastocysts (19.5% and 20.4%, respectively; $p < 0.05$). When completely hatched blastocysts were transferred, clinical pregnancy and implantation rates were higher, regardless of the age of the women and the quality of the embryos [12]. In a similar study, blastocysts were divided into three groups: group 1 for non-hatched blastocysts, group 2 for hatching blastocysts, and group 3 for completely hatched blastocysts. The blastocyst implantation rates were 28.6%, 43.6%, and 53.8% in groups 1, 2, and 3, respectively ($p < 0.001$); the pregnancy rates were 27.9%, 42.8%, and 53.2% ($p < 0.001$); the live birth rates were 23.1%, 32.0%, and 42.5% ($p < 0.001$). Group 3 had higher rates of these parameters. Group 2 had higher blastocyst implantation rates and higher pregnancy rates than group 1, but live birth rates were similar [11]. In contrast, Rodriguez-Purata et al. [13] found no correlation between spontaneous hatching and pregnancy course or outcomes. Another recent study shows that completely hatched blastocysts may have a lower chance of implantation than hatching blastocysts [14]. This may be caused by damage to the blastomeres during vitrification due to the lack of a protective zona pellucida surrounding the blastocyst. Therefore, a hatching effect on IVF and ICSI success rates remains poorly understood and further research is needed.

Morphology of Vitrified Embryos after Thawing

Cryopreservation protocols are currently widely used in IVF. Such protocols reduce the risk of multiple pregnancies, avoid ovarian hyperstimulation syndrome, have economic benefits, and reduce partner stress before IVF [15]. Freezing and thawing can have a negative effect on a blastocyst. Vitrification minimizes the formation of ice crystals in the blastocoel, although some crystals still develop [16]. During vitrification, the volume of the embryo decreases and its ability to return to its original shape upon thawing may affect IVF success. The effect of blastocyst morphology on live birth rates was evaluated in a cohort study [17]**.** Blastocoel re-expansion was graded immediately before embryo transfer as follows: A, fully re-expanded; B, partially re-expanded (≥50%); C, partially re-expanded (<50%), and D, collapsed. Live birth rates ranged from 11.4% in group D to 38.9% in group A ($p < 0.001$). Park et al. [18] evaluated morphology and morphokinetics of thawed blastocysts and their correlation with the Gardner blastocyst grade and pregnancy course. Vitrified and thawed embryos were divided into four groups based on their diameter: group 1, <135 μm; group 2, 135–145 μm; group 3, 146–155 μm; and group 4, ≥156 μm. The diameter of blastocysts in groups 2, 3 and 4 correlated positively with their grade. However, some blastocysts in group 1 were also classified as good, but their number was significantly lower. The study also measured blastocyst re-expansion rates and divided embryos into three groups: group 1, ≤50 nm/min; group 2, ≥50.1 nm/min to ≤100 nm/min; and group 3 ≥100.1 nm/min to ≤150 nm/min. A strong correlation was found between the blastocyst re-expansion speed and carrying of a pregnancy, as well as a correlation between the blastocyst re-expansion speed and blastocyst grade, with higher re-expansion rate resulting in higher blastocyst grade ($p < 0.001$). Therefore, higher-grade blastocysts are less susceptible to damage when vitrified and thawed. Hershko-Klement et al. [19] evaluated the predictive value of changes in the size of the blastocyst after a certain period of incubation. Measurements were taken immediately after thawing and after 120 ± 15 minutes. Minimum and maximum cross-sectional axes were measured. Three groups were defined: group 1 for embryos that continued to shrink by ≥10 μm; group 2 for embryos that ranged from –9 μm to +9 μm; and group 3 for re-expansion of ≥10 μm. Pregnancy rates were 18.9% in group 1, 27.0% in group 2, and 51.2% in group 3 ($p = 0.007$). Therefore, morphology and morphokinetics of thawed vitrified embryos may significantly affect IVF and ICSI success rates. Therefore, embryologists should consider blastocyst parameters both before and after vitrification.

Embryonic (Cytoplasmic) Strands

The development and clinical implementation of time-lapse imaging has opened new possibilities for detailed assessment of embryo morphology. This system captures the development of an embryo at regular intervals of 5–15 minutes [20]. This technology discovered embryonic strands. The function of embryonic strands in early embryonic development remains poorly understood. These are typically long, thin filaments (0.1–0.3 mm) that connect the embryoblast and trophoblast during blastocyst formation [21]. In the early 2000s, the detection of embryonic strands in a blastocyst was considered an unfavorable sign of embryo viability [22]. In recent years, however, it has been considered favorable [23–25]. A recent study evaluated effects of embryonic strands on blastocyst grade and implantation. Significantly more embryos with embryonic strands developed into high grade blastocysts (AA, AB, BB, and BA) compared with embryos without strands. The implantation rate was 39.7% in the group with embryonic strands versus 14.3% in the group without them, although this difference was not statistically

significant (p = 0.16). This study also discovered antero- and retrograde transport across embryonic strands between the embryoblast and trophoblast [26]. A similar study found that high-grade embryos had one or more strands predominantly in the early stages of blastocyst development, which disappeared as the embryo grew. The shape and width of the strands depended on the blastocyst maturation. The transfer of blastocysts with embryonic strands resulted in a 1.416-fold higher rate of full-term pregnancies compared with blastocysts without strands [27]. Although the function of embryonic strands in the early stages of embryo development remains poorly understood, studies describe their beneficial effects on blastocyst development and IVF success. Therefore, this morphological feature requires further investigation (methods for morphological evaluation of blastocyst grade are presented in Table 1).

Role of Time-Lapse Imaging in Assessing Blastocyst Implantation Potential

As mentioned above, the development of time-lapse imaging has helped to identify a new structure, embryonic strands. However, the application of this technology goes beyond that. This technique makes the process of assessing blastocyst morphology more objective, but manual mapping and evaluation of all images is a long and labor-intensive process, so data analysis can be optimized using artificial intelligence. A 2023 study [28] developed a BELA algorithm to predict embryo ploidy without the subjective involvement of embryologists. BELA uses multitask learning to predict blastocyst grades, which further predict ploidy status, and matches the performance of models trained on data provided by embryologists. In a similar study, a deep learning algorithm was developed to predict the development of an embryo from morula to blastocyst stage with 66.42% to 77.74% accuracy, detecting abnormalities at the earliest stages of development [29]. A 2024 study [30] used time-lapse imaging of mouse embryos to detect features predictive of normal embryonic development to the blastocyst stage, such as the absence of multinucleation (presence of more than one nucleus in a blastomere) and homogeneous cytoplasm. In addition, software was developed to construct 3D models of blastocysts based on 2D images. Students of the Digital Department of Sechenov First Moscow State Medical University (Moscow, Russia) have developed a system for automated analysis of the morphokinetcs of human embryos from fertilization to the blastocyst stage.[2] Time-lapse imaging combined with machine learning will optimize and facilitate the work of embryologists.

Cell Culture Fluid Tests

Currently, IVF clinics use morphological criteria to select embryos for transfer [31]. Morphologic assessment is still widely used, because it is a fast, technically simple, non-invasive and cost-effective technique. However, it has some limitations, such as a certain subjectivity of an embryologist in selecting the embryos for transfer and a low ability to predict pregnancy (the rates of implantation, pregnancy onset and full-term delivery did not exceed 50%) [32]. The prevalence of aneuploidy in preimplantation embryos still seems to be the cause of implantation failure and miscarriage. Although still controversial, there is increasing evidence that identification of euploid embryos for transfer significantly improves implantation, clinical pregnancy, and live birth rates in patients with various conditions, including advanced maternal age and recurrent miscarriage, as well as in patients with a favorable prognosis after single embryo transfer [33]. All previous embryo grading technologies were extremely dangerous because they required the collection of embryonic material (e.g., karyotyping) [34]. In addition, a biopsy at the blastocyst stage requires a qualified embryologist with specialized equipment, which is a major factor affecting the cost and availability of the procedure. Active development of noninvasive techniques is promising and highly compatible with morphologic assessment without embryo biopsy [35]. Such non-invasive techniques may include detection of embryonic exosomes, as well as analysis of the proteome and metabolome in the cell culture fluid.

The attachment of an embryo to the endometrium plays a critical role in the success of embryo implantation and the onset of pregnancy [36]. The role of exosomes and microRNA in embryo implantation and mother-embryo communication has been demonstrated. Exosomes are cellular membrane vesicles ranging in size from 30 nm to 150 nm that deliver biologically active substances from cell to cell [37]. The contents of exosomes provide intercellular communication by delivering multiple factors such as DNA, microRNA, proteins, and lipids [38]. Exosomal biomarkers (tetraspanins CD9, CD63 and CD81) do not have sufficient diagnostic value because they are specific not only for exosomes but also for other types of extracellular vesicles, and due to the widespread presence of tetraspanins in the cell membrane, other classes of extracellular vesicles may carry these markers [32]. MicroRNAs (POU5F1, NANOG, Oct4, Sox2, Klf4, c-Myc) are also used as exosome biomarkers to indicate their embryonic rather than maternal origin [39, 40]. As mentioned above, the contents of exosomes include various factors such as DNA, RNA, etc. However, studies prefer microRNAs as exosome biomarkers because of their higher stability and rapid detectability [41]. Several studies have found a correlation between IVF success and changes in the levels of certain microRNAs. A 2019 study identified a microRNA-191-3p sequence with significantly higher levels in implanted embryos compared with those that were miscarried [42]. The expression of microRNA-142-3p in the cell culture fluid was

[2] A new embryo grading system is developed at Sechenov University. Available at: https://www.sechenov.ru/pressroom/news/v-sechenovskom-universitete-sozdali-novuyu-sistemu-dlya-analiza-kachestva-embrionov-/ Accessed on 29 July 2024

Table 1. Morphological methods for assessing blastocyst implantation potential

Blastocyst implantation potential assessment method	Research methods	Evaluated parameters	Reference
Embryo quality classification	Light microscopy	Embryo development stage (1–6) Embryoblast quality (A, B, C) Trophoblast quality (A, B, C)	[2–6]
Morphometric method for blastocyst quality assessment	Light microscopy and image analysis using image J software (ver.1.52)	Embryoblast area Estimated number of trophoblast cells	[9]
Assessment of blastocyst hatching	Light microscopy	Degree of blastocyst escape from the zona pellucida (unhatched, hatching, hatched)	[11, 12]
Morphological evaluation of vitrified embryos after thawing	Light microscopy Time-lapse image analysis	Degree of blastocoel expansion Blastocyst expansion rate Changes in blastocyst size after incubation	[17–19]
Detection of embryonic strings	Light microscopy and time-lapse image analysis	Presence of embryonic strings in the blastocoel	[26, 27]

significantly higher in non-implanted blastocysts compared with implanted blastocysts [43]. The culture medium of implanted high-grade embryos was found to have lower miRNA diversity compared with failed low-grade embryos [44, 45]. The concentration of exosomes in the culture medium may also be a marker of potential IVF success. A 2017 study detected DNA-containing exosomes using flow cytometry with propidium iodide staining and found that implanted blastocysts released fewer exosomes into the culture medium [46]. Similar data were obtained in a study conducted the same year; the extracellular vesicle concentration in culture media of embryos, whose transfer resulted in pregnancy, was approximately 50% of the extracellular vesicle concentration in negative outcomes [44].

The analysis of culture media identifies biomarkers associated with embryo implantation rates and provides a non-invasive technique for molecular grading of embryos, because unlike genes, proteins and metabolites yield more accurate information about the physiological state of the embryo and its interaction with the environment [47]. A culture media multiomics strategy has been developed to identify proteins and metabolites associated with embryo grade and implantation rates [48]. The study found that, unlike low-grade embryos, high-grade embryos had higher levels of arginase-1 (an enzyme involved in the urea cycle, that catalyzes the hydrolysis of arginine to ornithine and urea and neutralizes cytotoxic products of nitrogen metabolism) and lower levels of ubiquitin carboxy-terminal hydrolase L1 (an enzyme involved in the processing of ubiquitin precursors and ubiquitinated proteins); ferritin light chains; triose phosphate isomerase, fructose-bisphosphate aldolase A, phosphoglycerate kinase 1 (enzymes involved in glucose metabolism); cytoplasmic actin 2. Further comprehensive analysis of the proteome and metabolome in the culture medium revealed that high-grade embryos had faster rates of lipid metabolism and slower rates of glucose and amino acid metabolism (for more detailed information on enzymes and metabolites, see Table 2.) Therefore, low-grade embryos showed abnormally high energy metabolism activity and low lipid metabolism activity. These data support the "quiet embryo hypothesis", which states that abnormally high metabolic activity is associated with low embryo grade. High-grade embryos were then transferred into the uterine cavity during IVF; on day 28, an ultrasound was performed to divide the embryos into implanted (with a detectable amniotic sac in the uterine cavity) and non-implanted (with no detectable amniotic sac). Compared to group 2, group 1 embryos had higher culture medium levels of acute phase proteins such as attractin, ceruloplasmin, haptoglobin, haptoglobin-associated protein, apolipoprotein A2, plasma kallikrein, macrophage-colony stimulating factor 1 receptor, and monocyte differentiation antigen CD14. This suggests that implanted embryos have a better immune response and a higher survival rate under adverse conditions [49]. The study also found that implanted high-grade embryos had higher culture medium levels of glycine (an intermediate metabolite of lipid metabolism) and lower levels of n-butylamine, so these two substances can be used as biomarkers of higher reproductive potential [50]. A 2019 study found a peptide derived from blastocyst culture medium (PDBCM) in the culture medium of high-grade blastocysts. This is a degradation product of the HERC2 protein (E3 ubiquitin ligase). PDBCM was almost completely absent from the culture medium of growth-arrested blastocysts. The authors suggest that this peptide is both an intermediate in protein degradation and an endogenous peptide with biological activity that may affect the speed of development and viability of blastocysts [51]. Freis et al. [52] found several protein biomarkers of future implantation success, including activated leukocyte cell adhesion molecule, ephrin type-B receptor 4, junctional adhesion molecule A, selectin E, C-C motif chemokine ligand 24, Fas receptor, platelet-derived growth factor subunit A, PECAM-1, tissue inhibitor of metalloproteinase-4, paraoxonase 3, cystatin B, bleomycin hydrolase, and von Willebrand factor. Most of these proteins

Table 2. Biomarkers of high blastocyst implantation potential and high quality

Biomarkers	Concentration change (↑/↓)	Role in blastocyst function	Reference
Arginase-1	↑	Urea cycle enzyme	[50]
Triosephosphate isomerase, fructose-bisphosphate aldolase A, phosphoglycerate kinase 1, pyruvic acid	↓	Glucose metabolism	
Glycine, apolipoprotein A2	↑	Lipid metabolism	
N-butylamine, cyclohexanol	↓	Intermediate metabolites	
Oleamide (a metabolite of oleic acid), linoleamide (a metabolite of linoleic acid), arachidonic acid	↑	Fatty acid metabolism	
Octadecanamide	↓	Fatty acid metabolism metabolite	
Attractin, ceruloplasmin, haptoglobin, haptoglobin-related protein, plasma kallikrein, macrophage colony-stimulating factor 1 receptor, monocyte differentiation antigen CD14	↑	Acute-phase proteins	
PDBCM	↑	Degradation product of HERC2	[51]
Activated leukocyte cell adhesion molecule, ephrin type B receptor 4, E-selectin, CC-chemokine ligand 24	↑	Trophoblast migration, invasion, and adhesion	[52]
FAS receptor	↑	Apoptosis	
Junctional adhesion molecule A	↑	Organogenesis	
Platelet-derived growth factor subunit A	↑	Angiogenesis, trophoblast migration	
PECAM-1, tissue inhibitor of metalloproteinase-4	↑	Embryonic development	
Paraoxonase 3	↑	Antioxidant function	
Cystatin B, bleomycin hydrolase, von Willebrand factor	↑	Function unknown	

(except von Willebrand factor, cystatin B, bleomycin hydrolase, whose role in blastocyst development is still poorly understood) play important roles in trophoblast migration, invasion, and adhesion during implantation and embryonic development [53–61].

Several novel techniques for grading embryos and assessing their implantability can be developed based on the analysis of culture fluid, due to the diversity of proteins and metabolites produced by the blastocyst during its growth *in vitro*. However, all of the above techniques require further research and standardization.

CONCLUSION

In current clinical practice, assisted reproductive technology specialists still lack highly effective techniques for pre-implantation embryo selection for IVF. The morphologic blastocyst grading of Gardner et al. is the only visual grading technique currently used in practice. Although this technique is still considered the gold standard, it is relatively subjective: selection of an implanted high-grade embryo depends solely on an embryologist's visual assessment, and the percentage of successful IVF attempts using this technique is still less than 50%. Double-checking is also necessary because the vitrification process can affect the embryo grade. Grading of morphologically identical embryos remains a challenge, even for an experienced specialist in assisted reproductive technologies. Using a slow-motion system opens up new possibilities for evaluating embryonic morphology and detecting new morphological structures. Machine learning can also help distinguish morphologically identical embryos and analyze the proteome and metabolome of the culture medium to optimize the embryologist's workflow. Levels of substances secreted into the medium can be used for embryo grading. Whenever possible, every assisted reproductive technology laboratory should use liquid chromatography with tandem mass spectrometry to analyze culture fluid and assess embryo metabolic activity. Implementing a comprehensive approach to embryo grading can reduce the cost of the procedure and improve the patient's quality of life by reducing the number of IVF failures.

ADDITIONAL INFORMATION

Authors' contribution. D.D. Abasheva: literature review, collection and analysis of literature sources, manuscript preparation and writing; E.E. Rudenko: literature review, collection and analysis of literature sources, manuscript preparation and writing; N.S. Trifonova: collection and analysis of literature sources, manuscript preparation and writing, manuscript editing; S.E. Korolenko: literature review, collection and analysis of literature sources, manuscript writing. Y.I. Utkina: literature review, collection and analysis of literature sources, manuscript writing, manuscript editing. P.I. Tikhomirova: literature review, collection and analysis of literature sources, manuscript preparation and writing. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made a significant contribution to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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