Molecular Markers of Embryo Quality

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Evaluation of the optimal number of embryos, their quality, and the precise timing for transfer are critical determinants in reproductive success, although still remaining one of the main challenges in assisted reproduction technologies (ART). Indeed, the success of in vitro fertilization (IVF) treatments relies on a multitude of events and factors involving both the endometrium and the embryo. Despite concerted efforts on both fronts, the overall success rates of IVF techniques continue to range between 25% and 30%. The role of the endometrium in implantation has been recognized, leading to the hypothesis that both the “soil” and the “seed” play a central role in a successful pregnancy. In this respect, identification of the molecular signature of endometrial receptivity together with the selection of the best embryo for transfer become crucial in ART.

Keywords: IVF ; endometrial receptivity ; implantation ; blastocyst ; embryo ; ART ; AI ; extracellular vesicles

1. Introduction

Implantation in mammals relies on the activation of spatially and temporally regulated signals from both the embryo and the endometrium. Synchronization of the embryo-endometrium dialogue represents a limiting step for a successful pregnancy, and the rate of clinical pregnancy in normal cycles only reaches about 30–40% [1]. Despite the improvement of IVF techniques and preimplantation genetic testing (PGT) to assess embryo euploidy, implantation failure remains a challenge. Approximately 10–30% of patients referred to IVF clinics experience implantation failure [2], and attention has been focused on measures to improve pregnancy outcomes. Among these, the identification of difficult procedures of embryo transfer [3], methods of creating optimal embryo cultures [4][5], the standardization of morphological criteria to classify blastocyst competency [6][7], the improvement of PGT [8], and measures to identify the optimal day for embryo transfer [9][10][11], have been reported. Nevertheless, despite efforts in both endometrial analysis and embryonic evaluation, our capacity for assessment remains limited. One of the main complications in assisted reproductive techniques is recurrent implantation failure (RIF), defined as the failure to achieve pregnancy after the transfer of at least 3 good-quality embryos [12]. While embryo quality can be assessed using morphological and molecular parameters, the evaluation of proper endometrial competency/receptivity is more challenging. Due to its role in limiting embryo implantation, the concept of the endometrium as the guardian of pregnancy has been proposed [13]. Indeed, the embryo can efficiently implant in any tissue, independent of the stage of the cycle, with great invasion ability, while in the endometrium, it can only implant during a short period of time called the window of implantation (WOI) or window of endometrial receptivity [14][15]. In a 28-day normal cycle, the WOI occurs around 6–10 days after the LH surge and lasts about 3–6 days [14][16][17]. In cases of artificial cycles, the WOI occurs 4–7 days after the administration of progesterone [2]. The WOI is finely regulated by a plethora of factors, which include hormones, such as estrogen and progesterone, cytokines, and growth and immunomodulatory factors, all driving a series of morphological and molecular changes fundamental for a correct blastocyst-endometrial dialogue. Although the boundaries of the WOI in a 28-day cycle have been identified, the accurate assessment of endometrial receptivity in women with irregular cycles undergoing IVF is needed in order to timely transfer the embryo and reduce the risk of RIF [18]. The routine procedure to identify the day of embryo transfer in IVF is mainly based on the measurement of endometrial thickness by ultrasound, which can be ineffective as a method of predicting the risk of RIF [19][20]. Histological dating according to the Noyes criteria has been previously exploited to evaluate the morphological changes in stromal and glandular compartments along the proliferative and secretory phase of the menstrual cycle, and to identify the WOI [21]. These parameters are no longer considered predictive of endometrial receptivity, mainly due to their operator dependency [22][23]. More recently, omics approaches have been proposed as tools to identify the WOI [24][25][26][27][28]. Among these, the Endometrial Receptivity Assay (ERA) has been developed to assess the gene expression signature characterizing the receptive endometrium. However, the limits of the ERA include the assumption of signature reproducibility among cycles, the high costs, and the relatively small number of patients used to validate the assay [29][30][31].

2. Morphological Criteria to Identify the Best Embryo

2.1. Static and Morphological Embryonic Features–Cleavage Stage Embryo

Assessment of a cleavage stage embryo by using morphological characteristics considers several parameters, including cell number, the degree of fragmentation, the presence of multinucleation, and blastomere size and symmetry. Numerous studies have been conducted on the correlation between the morphology of embryos at the cleavage stage and their subsequent implantation outcomes [7]. According to the Istanbul consensus [32], the characteristics of 'good' embryos included 4 blastomeres on Day 2, and at least 8 blastomeres on Day 3, depending on the time elapsed post-insemination. Furthermore, blastomeres should be even sized. Moreover, embryos had to exhibit <10% fragmentation and show no signs of multinucleation. On the other hand, some studies reported a positive correlation between the live birth rate and an increment in cell count up to 8, while noting a reduction in live birth rates in embryos with more than 8 cells [33][34] Therefore, there is a general consensus about the reduced developmental potential of slow-cleaving embryos, but the developmental competence of fast-cleaving embryos remains a controversial issue.

The presence of multinucleated blastomeres in human embryos is widely recognized as a factor associated with a diminished potential of embryo development, manifested by reduced blastocyst formation, significantly lower implantation, and decreased live birth rates [35][36][37][38][39]. Therefore, the recording of this morphological characteristic should be integrated into embryo grading schemes.

2.2. Static and Morphological Embryonic Features–Blastocyst

The evaluation of embryo morphology is the predominant method for assessing human blastocysts worldwide [40][41][42]. This grading system incorporates various parameters such as blastocyst expansion and hatching, the appearance of the inner cell mass (ICM), and trophectoderm (TE) cohesiveness [32]. Significantly, many studies have identified a correlation between the chromosomal status of the embryo and the blastocyst morphology, with higher-quality ICM and TE being linked to increased rates of euploidy [43][44][45][46][47][48][49][50][51]. Conversely, poor quality ICM and TE are associated with elevated rates of complex aneuploidy, affecting multiple chromosomes [43][44]. In the context of embryo transfers involving vitrified-warmed embryos without genetic testing, factors such as blastocyst expansion and the grading of the TE and ICM have been linked to pregnancy outcomes. However, there remains a lack of consensus regarding the predictive value of each of these parameters, with studies yielding conflicting results about which parameter serves as the strongest predictor. Some studies showed that ICM grade had the best predictive effect [52][53][54], while others indicated that expansion stage and TE grade were stronger predictors [55][56][57]. A recent systematic review and meta-analysis revealed that embryos with a grade C ICM were correlated with a decreased rate of live births per euploid transfer compared to those with grade A/B ICM. Similarly, embryos with a grade C TE exhibited a lower live birth rate per euploid transfer compared to those with grade A/B TE. Additionally, poor quality blastocysts (<BB) were associated with reduced live birth rates per euploid transfer in comparison to high-quality blastocysts [58].

2.3. Morphokinetic Embryonic Features

The implementation of time-lapse technology (TLT) has facilitated an enhancement in both the frequency of observations and the dynamic monitoring of embryo development [59]. Various timings are recorded, mainly following ESHRE guidelines [60], such as the time of pronuclear fading (tPNf) and cleavage times at various stages (t2, t3, t4, etc.). Consequently, the durations of the initial three cell cycles (CC1, CC2, and CC3), as well as the period of blastocyst expansion, can be deduced from these observations. Numerous studies have explored whether these developmental timings are indicative of embryonic competence. Rienzi and coworkers observed that the duration until morulation and the quality of TE are substantial indicators for predicting live births following the transfer of euploid embryos [61]. Moreover, a recent meta-analysis, including 58 studies and over 40,000 embryos, examined a potential link between ploidy status and morphokinetic characteristics observed via TLT [62]. It was noted that aneuploid blastocysts exhibited extended durations for t8, t9, and the initiation of expansion (tEB), in addition to higher grades of fragmentation, persistent multinucleation at the four-cell stage, and blastocyst contractions. Nevertheless, due to the diverse nature of these results and the low quality of evidence, the authors recommended further research. Lastly, a retrospective study examined timings such as tPNf, t2, t3, t4, t8, tM, and tB in relation to 192 euploid single embryo transfers. Embryos resulting in live birth, euploid pregnancy loss, or no pregnancy have nearly identical morphokinetic parameters after monitoring with TLT [63].

3. Developmental Timing to Identify the Best Blastocyst

Full blastocyst expansion should be assessed at 116 ± 2 h post-insemination [32]. However, it has been observed that a significant number of blastocysts continue to develop beyond Day 5, with their development extending up to Day 7. Some

systematic review and meta-analyses reported that clinical pregnancy and live birth rates were significantly higher following transfers of fresh or frozen-thawed blastocysts developed on Day 5 compared to Day 6/Day 7, demonstrating that blastocysts with a slower development can be of top morphological grade, euploid, and result in a healthy live birth [58][64][65]. A summary of the morphological criteria so far discussed is reported in **Table 1**.

Table 1. Summary of the morphological criteria available to identify the best embryo and for endometrial receptivity assessment.

	Criteria	Description	Evidence in Support	Evidence Against
Embryo	Good embryo according to Istanbul consensus	At least 8 blastomeres even sized on Day 3, <10% fragmentation and no signs of multinucleation	[7][33]	[34]
	Multinucleated blastomeres	A multinucleation in Day 2 and Day 3 cleavage embryos		[35][36][37] [38][39]
	ICM grading	The grading scale for ICM quality of the blastocyst	[52][53][54]	
	Expansion stage and TE grading	The grading scale for expansion and TE quality of the blastocyst	[55][56][57]	
	Developmental timing	Full blastocyst expansion should be assessed at 116 ± 2 h post-insemination	[58][64][65]	
Endometrium	Endometrial Thickness (EndT)	Optimal thickness for receptive endometrium of about 16–18 mm (evaluated by transvaginal ultrasound)	[66][67][68][69] [70]	[71][72][73] [74][75]
	Noyes Criteria	Histological criteria identifying gland mitosis and tortuosity, apical position of secretory vesicles in cells of the glandular epithelium, secreted material in the glandular lumen, stromal edema, pseudo-decidual reaction, and leukocyte infiltration	[76]	[18][22][23] [77][78][79]
	Pinopodes	Evaluation of density and morphology of plasma membrane protrusions on epithelial cells projecting toward the uterine lumen on days 20–22 of a natural menstrual cycle	[80][81][82][83] [84][85]	[86][87]

4. Molecular Markers of Embryo Quality

4.1. Mitochondrial DNA (mtDNA)

The concentration of mitochondrial DNA (mtDNA) within embryonic cells has been postulated to play a key role in determining embryonic competence. Given that mitochondria originate from the oocyte and considering the established influence of oocyte quality on early embryonic development, it is plausible to suggest that mitochondrial function could significantly impact embryonic competence. This perspective aligns with the hypothesis that elevated mtDNA levels may indicate suboptimal energy production and compromised homeostasis within the embryo [88]. In fact, Fragouli et al. [89] observed a significant correlation between increased levels of mtDNA content above a threshold level and dramatically diminished clinical outcomes among euploid blastocysts. Concurrently, Diez-Juan et al. [90] confirmed these findings, suggesting a model where rising mtDNA copy numbers correlate with diminishing implantation potential.

However, these observations are not universally accepted, as other studies have reported contrasting results [91]. Victor and coworkers [92] were unable to find a relationship between mtDNA content and clinical outcomes in euploid embryos. Likewise, other studies did not identify any implantation benefits in embryos with reduced mtDNA content [93][94][95]. Moreover, the analysis of TE samples from 615 euploid human blastocysts showed that mtDNA content was not predictive of euploid human embryo reproductive competence [96]. Recently, an attempt was made to conduct a meta-analysis, but the heterogeneity in study designs, characteristics of experimental groups, analytical methodologies, and outcome measures hindered direct comparisons across studies and a real understanding of the impact of mtDNA levels on the reproductive competence of embryos [58]. These data do not support the use of mitochondrial DNA copy number in clinical decision making when selecting which embryo to transfer.

4.2. Cumulus Cells or Spent Media Molecular Analyses

Cumulus cells (CCs) are somatic cells closely associated with the oocyte, playing crucial roles in metabolic and signaling functions during folliculogenesis and oocyte maturation [97]. Given that oocyte competence is achieved through

bidirectional signaling between the oocyte and the surrounding cumulus cells [98], and that these cells are typically discarded after oocyte retrieval, they represent a compelling and non-invasive focal point for in-depth investigation into the factors influencing preimplantation embryo quality.

Looking for molecular markers of oocyte competence in CCs constitutes a way to enhance the predictive value of conventional embryo selection. To enhance the predictive value currently obtained from standard embryo morphology assessments, these molecular markers should be able to identify oocytes which, after ART, have progressed to the blastocyst stage. More specifically, these markers should have the ability to discern those oocytes that have not only reached the blastocyst stage but are also capable of successfully establishing a pregnancy. Some studies have indicated a correlation between CCs function and embryo development. Seven genes related to CCs metabolism (CCND2, CXCR4, GPX3, CTNND1 DHCR7, DVL3, HSPB1, and TRIM28) were found to be altered at the cleavage stage in genome-wide gene expression studies [99]. Moreover, ANG, RGS2, and PLIN2 were indicated as potential predictors of blastocyst development [100]. Scarica and coworkers [101] investigated the association of CCs-related expression of a selected cluster of genes (PTGS2, CAMK1D, HAS2, STC1, and EFNB2) with embryo development to blastocyst. In particular, a strong association between the CAMK1D expression level and blastocyst formation was observed [102][103].

Other studies have explored gene expression in CCs and its relation to embryonic competence. EFNB2 and CAMK1D were suggested to be promising genes that could help to choose EFNB2 and CAMK1D were suggested to be promising genes that could help to select for transfer the embryo with the highest chance to give a pregnancy [104]. Assou et al. [105] associated NFIB reduction and BCL2L11 and PCK1 upregulation with CCs of embryos resulting in live births. Moreover, the upregulation of VCAN, PTGS2, GREM1 and PFKP in CCs of oocytes was observed in embryos leading to successful pregnancy [106]. Similarly, Wathlet and colleagues [104] described an association between pregnancy success and EFNB2, CAMK1D, STC1, and STC2 gene expression in CCs of embryos leading to successful pregnancy. Lastly, prediction models based on CCs gene expression showed upregulation of FGF12, GPR137B, SLC2A9, ARID1B, NR2F6, ZNF132, and FAM36A, and down-regulation of ZNF93, RHBDL2, DNAJC15, MTUS1, and NUP133 in the CCs of oocytes that resulted in a successful pregnancy after IVF [107].

It is noteworthy that these studies assessed pregnancy outcomes after multiple embryo transfers, without adequately accounting for the ploidy status of the embryos, limiting the applicability of these findings to current practices. In contrast, two studies conducted transcriptomic analysis on CCs from oocytes that developed into either implanting or non-implanting euploid blastocysts [108][109]. One study examined five cases per group, while another investigated 17 double embryo transfers of sibling blastocysts, yielding conflicting outcomes. Both studies identified several differentially expressed genes, but none reached statistical significance, so these genes cannot serve as reliable biomarkers of blastocyst competence.

The failure to identify transcriptome biomarkers in this analysis aligns with the results reported by Burnik Papler et al. [110]. This study, conducted using a microarray platform, similarly found no discernible differences in gene expression that could predict either oocyte fertilization or embryo implantation [110]. Moreover, recently, Sachs and colleagues [111] compared the transcriptome of CCs obtained from oocytes that resulted in pregnancy, did not result in pregnancy, led to live birth, or did not result in live birth. Although the RNA sequencing analysis did not uncover differentially expressed genes (DEGs) when comparing the transcriptomic profiles of the groups “no pregnancy” with “pregnancy”, they identified 139 DEGs when comparing the subset of “pregnancy only” with “live birth”. Notably, 28 of these differentially expressed genes were associated with clusters crucial for successful ART outcomes, such as CTGF, SERPINE2, PCK1, HHIP, HS3ST, and BIRC5 [111].

Emerging omics methodologies, including proteomics and metabolomics, are increasingly revealing distinct molecular signatures in viable gametes and embryos. These unique profiles offer potential biomarkers that may be harnessed for the purposes of developmental or viability assessment and selection. Of particular interest in ART is the secretome, those proteins that are produced within the embryo and secreted into the surrounding environment. Defining the embryonic secretome will also provide a deeper understanding of the distinctive series of events crucial for successful implantation, encompassing the essential prerequisites of the blastocyst. Given the intricate and diverse nature of the human embryo, it appears rational to anticipate a collaborative ‘omics’ approach in characterizing the human embryonic secretome [112].

Moreover, in recent years an expanding body of literature has emerged to explore the clinical applicability of spent embryo culture media (SCM) in the context of PGT-A [113][114][115][116]. Different studies demonstrated the ability to detect, extract, and amplify cell-free DNA (cfDNA) from SCM at both the cleavage and blastocyst stages. Belandres and colleagues [117] suggested enhancements to increase the precision and sensitivity of the assay prior to integrating PGT-A with SCM into clinical practice.

Additionally, it has been shown that microRNA (miRNAs) can be detected in IVF culture media, and that some of them are differentially expressed according to the fertilization method, chromosomal status, and pregnancy outcome, which makes them potential biomarkers for predicting euploidy as well as IVF success [118][119][120].

Three studies focused on miRNAs released in the SCM of euploid blastocysts, comparing those that implanted to those that did not [121][122][123]. Initially, a study involving 53 euploid single embryo transfers (SETs) found increased expression of miR-20a and miR-30c in the SCM of implanted blastocysts [122]. Nevertheless, a subsequent multicenter study that employed a tailored plate and protocol for the analysis of 10 miRNAs in 221 euploid SETs did not corroborate these findings. Although the latter study reported significant differences between non-implanted and implanted euploid blastocysts in terms of both miRNA detection and relative quantitation, when the data were adjusted for embryo morphology and day of biopsy, no significant association was confirmed [123]. The expression of miR-372 and miR-191 in embryo culture medium was found to be related to implantation failure [118], while miR-661 was successfully detected in embryonic blastocyst medium, with a higher expression in blastocysts that failed to implant [119]. Moreover, Borges and coworkers found that the expression of miR-142-3p was higher in successfully implanted embryos compared with embryos that failed to implant [124].

Recent studies have focused on the possibility of conducting PGT-A on SCM, aiming to set up a workflow to conduct non-invasive aneuploidy testing [125]. Two studies assessed outcomes following the SET of blastocysts classified as euploid via PGT-A of TE biopsy, but as either euploid or aneuploid in the SCM analysis [126][127]. A recent meta-analysis showed that SCM reported as aneuploid or euploid were associated with similar live birth and miscarriage rates per clinical pregnancy.

An additional study adopted a similar approach but complemented TE analysis with the outcome of DNA amplification from blastocoel fluid collected via blastocentesis [128]. Intriguingly, among 53 euploid SETs, the detection of DNA in the blastocoel correlated with a significantly lower live birth rate (31.5% versus 67.6%), although the miscarriage rate remained comparable. The authors suggested that this cost-effective analysis might act as a biomarker of embryo reproductive potential, indirectly revealing the impact of apoptosis or necrosis in embryonic cells, which release DNA into the blastocoel fluid. However, further research is required to substantiate this hypothesis.

In the context of spent media, extracellular vesicles (EVs) have been identified as interesting candidates able to modulate embryo development, as well as to be released by the embryos [129]. These small, membrane-bound entities released by cells have been identified in various bodily fluids, including the spent media from human embryos [130]. EVs have the capability to transport regulatory molecules such as miRNAs, mRNAs, lipids, metabolites, and proteins [131][132], reflecting the genetic makeup of the originating cells, such as the developing embryo. The membrane of EVs effectively shields enclosed cargo contents, rendering miRNAs derived from EVs more stable and reliable than free-floating miRNAs, owing to protection against RNase present in the medium [133][134].

Until now, only a few studies conducted on animal models investigated miRNAs isolated from conditioned media generated by group cultured blastocysts or degenerated embryos. These studies suggested a link with embryo quality and development [120][135]. Recent studies by Pavani et al. [136] have shed light on the selective enrichment of specific miRNAs in EVs secreted by bovine embryos reaching the blastocyst stage. The administration of synthetic forms of these miRNAs significantly enhanced the hatching capacity of blastocysts, showcasing the potential of EV-associated genetic material in influencing embryonic development. Moreover, in a study conducted by Giacomini and coworkers [130], it was demonstrated that EVs derived from human embryos obtained from ICSI carry a distinct molecular cargo, and they are internalized by endometrial cells. Additionally, EVs released by individually cultured preimplantation bovine embryos can alter the gene expression of oviduct epithelial cells [137] and endometrial cells [138]. Masoumeh Es-Haghi and colleagues demonstrated that three RNA transcripts in EVs secreted by human trophoblast spheroids were directly transferred to endometrial cells [139]. These data underline the crucial role of embryo-derived EVs in embryo–embryo and embryo-maternal communication and in the establishment of endometrial receptivity. Considering the implications for genetic diagnostics, the cargo within EVs secreted by embryos becomes a valuable source for potential biomarkers indicative of genetic health and abnormalities.

In the context of PGT, the genetic content encapsulated within EVs could provide a non-invasive and informative means of assessing the genetic status of embryos, potentially enhancing the accuracy and comprehensiveness of PGT results. By leveraging the molecular cargo of EVs secreted by human embryos, researchers may unveil new possibilities for advancing genetic diagnostics in the field of assisted reproductive technologies, paving the way for more precise and insightful genetic assessments during the preimplantation phase.

References

1. Macklon, N.S.; Geraedts, J.P.M.; Fauser, B.C.J.M. Conception to Ongoing Pregnancy: The “black Box” of Early Pregnancy Loss. Hum. Reprod. Update 2002, 8, 333–343.
2. Maziotis, E.; Kalampokas, T.; Giannelou, P.; Grigoriadis, S.; Rapani, A.; Anifantakis, M.; Kotsifaki, A.; Pantou, A.; Triantafyllidou, O.; Tzanakaki, D.; et al. Commercially Available Molecular Approaches to Evaluate Endometrial Receptivity: A Systematic Review and Critical Analysis of the Literature. Diagnostics 2022, 12, 2611.
3. Galati, G.; Reschini, M.; Mensi, L.; Di Dio, C.; Somigliana, E.; Muzii, L. The Impact of Difficult Embryo Transfer on the Success of IVF: A Systematic Review and Meta-Analysis. Sci. Rep. 2023, 13, 22188.
4. Mani, S.; Mainigi, M. Embryo Culture Conditions and the Epigenome. Semin. Reprod. Med. 2018, 36, 211–220.
5. Simopoulou, M.; Sfakianoudis, K.; Rapani, A.; Giannelou, P.; Anifandis, G.; Bolaris, S.; Pantou, A.; Lambropoulou, M.; Pappas, A.; Deligeoroglou, E.; et al. Considerations Regarding Embryo Culture Conditions: From Media to Epigenetics. In Vivo 2018, 32, 451–460.
6. Kirkegaard, K.; Agerholm, I.E.; Ingerslev, H.J. Time-Lapse Monitoring as a Tool for Clinical Embryo Assessment. Hum. Reprod. 2012, 27, 1277–1285.
7. Gardner, D.K.; Balaban, B. Assessment of Human Embryo Development Using Morphological Criteria in an Era of Time-Lapse, Algorithms and “OMICS”: Is Looking Good Still Important? Mol. Hum. Reprod. 2016, 22, 704–718.
8. Cimadomo, D.; Rienzi, L.; Capalbo, A.; Rubio, C.; Innocenti, F.; García-Pascual, C.M.; Ubaldi, F.M.; Handyside, A. The Dawn of the Future: 30 Years from the First Biopsy of a Human Embryo. The Detailed History of an Ongoing Revolution. Hum. Reprod. Update 2020, 26, 453–473.
9. Kontopoulos, G.; Simopoulou, M.; Zervomanolakis, I.; Prokopakis, T.; Dimitropoulos, K.; Dedoulis, E.; Grigorakis, S.; Agapitou, K.; Nikitos, E.; Rapani, A.; et al. Cleavage Stage versus Blastocyst Stage Embryo Transfer in Oocyte Donation Cycles. Medicina 2019, 55, 293.
10. Simopoulou, M.; Sfakianoudis, K.; Tsioulou, P.; Rapani, A.; Maziotis, E.; Giannelou, P.; Grigoriadis, S.; Pantou, A.; Nikolettos, K.; Vlahos, N.; et al. Should the Flexibility Enabled by Performing a Day-4 Embryo Transfer Remain as a Valid Option in the IVF Laboratory? A Systematic Review and Network Meta-Analysis. J. Assist. Reprod. Genet. 2019, 36, 1049–1061.
11. Li, Y.-X.; Wang, J.; Sun, T.-Z.; Lv, M.-Q.; Ge, P.; Li, H.-N.; Zhou, D.-X. Pregnancy Outcomes after Day 5 versus Day 6 Blastocyst-Stage Embryo Transfer: A Systematic Review and Meta-Analysis. J. Obstet. Gynaecol. Res. 2020, 46, 595–605.
12. Coughlan, C.; Ledger, W.; Wang, Q.; Liu, F.; Demirol, A.; Gurgan, T.; Cutting, R.; Ong, K.; Sallam, H.; Li, T.C. Recurrent Implantation Failure: Definition and Management. Reprod. Biomed. Online 2014, 28, 14–38.
13. Macklon, N.S.; Brosens, J.J. The Human Endometrium as a Sensor of Embryo Quality. Biol. Reprod. 2014, 91, 98.
14. Psychoyos, A. Uterine Receptivity for Nidation. Ann. N. Y. Acad. Sci. 1986, 476, 36–42.
15. Ma, W.; Song, H.; Das, S.K.; Paria, B.C.; Dey, S.K. Estrogen Is a Critical Determinant That Specifies the Duration of the Window of Uterine Receptivity for Implantation. Proc. Natl. Acad. Sci. USA 2003, 100, 2963–2968.
16. Navot, D.; Scott, R.T.; Droesch, K.; Veeck, L.L.; Liu, H.C.; Rosenwaks, Z. The Window of Embryo Transfer and the Efficiency of Human Conception In Vitro. Fertil. Steril. 1991, 55, 114–118.
17. Donaghay, M.; Lessey, B.A. Uterine Receptivity: Alterations Associated with Benign Gynecological Disease. Semin. Reprod. Med. 2007, 25, 461–475.
18. Cohen, A.M.; Ye, X.Y.; Colgan, T.J.; Greenblatt, E.M.; Chan, C. Comparing Endometrial Receptivity Array to Histologic Dating of the Endometrium in Women with a History of Implantation Failure. Syst. Biol. Reprod. Med. 2020, 66, 347–354.
19. Järvelä, I.Y.; Sladkevicius, P.; Kelly, S.; Ojha, K.; Campbell, S.; Nargund, G. Evaluation of Endometrial Receptivity during In-Vitro Fertilization Using Three-Dimensional Power Doppler Ultrasound. Ultrasound Obstet. Gynecol. 2005, 26, 765–769.
20. Bonilla-Musoles, F.; Raga, F.; Osborne, N.G.; Castillo, J.C.; Bonilla, F. Endometrial Receptivity: Evaluation with Ultrasound. Ultrasound Q. 2013, 29, 3–20.
21. Noyes, R.W.; Hertig, A.T.; Rock, J. Dating the Endometrial Biopsy. Am. J. Obst. Gynecol. 1975, 122, 262–263.
22. Smith, S.; Hosid, S.; Scott, L. Endometrial Biopsy Dating. Interobserver Variation and Its Impact on Clinical Practice. J. Reprod. Med. 1995, 40, 1–3.

23. Murray, M.J.; Meyer, W.R.; Lessey, B.A.; Zaino, R.J.; Novotny, D.B.; Fritz, M.A. Endometrial Dating Revisited: A Randomized Systematic Study of Secretory Phase Histologic Characteristics in Normally Cycling Fertile Women. Fertil. Steril. 2002, 78, S67.

24. Díaz-Gimeno, P.; Horcajadas, J.A.; Martínez-Conejero, J.A.; Esteban, F.J.; Alamá, P.; Pellicer, A.; Simón, C. A Genomic Diagnostic Tool for Human Endometrial Receptivity Based on the Transcriptomic Signature. Fertil. Steril. 2011, 95, 50–60.e15.

25. Díaz-Gimeno, P.; Ruiz-Alonso, M.; Blesa, D.; Bosch, N.; Martínez-Conejero, J.A.; Alamá, P.; Garrido, N.; Pellicer, A.; Simón, C. The Accuracy and Reproducibility of the Endometrial Receptivity Array Is Superior to Histology as a Diagnostic Method for Endometrial Receptivity. Fertil. Steril. 2013, 99, 508–517.

26. Garrido-Gómez, T.; Ruiz-Alonso, M.; Blesa, D.; Diaz-Gimeno, P.; Vilella, F.; Simón, C. Profiling the Gene Signature of Endometrial Receptivity: Clinical Results. Fertil. Steril. 2013, 99, 1078–1085.

27. Ruiz-Alonso, M.; Blesa, D.; Díaz-Gimeno, P.; Gómez, E.; Fernández-Sánchez, M.; Carranza, F.; Carrera, J.; Vilella, F.; Pellicer, A.; Simón, C. The Endometrial Receptivity Array for Diagnosis and Personalized Embryo Transfer as a Treatment for Patients with Repeated Implantation Failure. Fertil. Steril. 2013, 100, 818–824.

28. Ruiz-Alonso, M.; Galindo, N.; Pellicer, A.; Simon, C. What a Difference Two Days Make: “Personalized” Embryo Transfer (pET) Paradigm: A Case Report and Pilot Study. Hum. Reprod. 2014, 29, 1244–1247.

29. Hashimoto, T.; Koizumi, M.; Doshida, M.; Toya, M.; Sagara, E.; Oka, N.; Nakajo, Y.; Aono, N.; Igarashi, H.; Kyono, K. Efficacy of the Endometrial Receptivity Array for Repeated Implantation Failure in Japan: A Retrospective, Two-Centers Study. Reprod. Med. Biol. 2017, 16, 290–296.

30. Bassil, R.; Casper, R.; Samara, N.; Hsieh, T.-B.; Barzilay, E.; Orvieto, R.; Haas, J. Does the Endometrial Receptivity Array Really Provide Personalized Embryo Transfer? J. Assist. Reprod. Genet. 2018, 35, 1301–1305.

31. Tan, J.; Kan, A.; Hitkari, J.; Taylor, B.; Tallon, N.; Warraich, G.; Yuzpe, A.; Nakhuda, G. The Role of the Endometrial Receptivity Array (ERA) in Patients Who Have Failed Euploid Embryo Transfers. J. Assist. Reprod. Genet. 2018, 35, 683–692.

32. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul Consensus Workshop on Embryo Assessment: Proceedings of an Expert Meeting. Hum. Reprod. 2011, 26, 1270–1283.

33. Racowsky, C.; Stern, J.E.; Gibbons, W.E.; Behr, B.; Pomeroy, K.O.; Biggers, J.D. National Collection of Embryo Morphology Data into Society for Assisted Reproductive Technology Clinic Outcomes Reporting System: Associations among Day 3 Cell Number, Fragmentation and Blastomere Asymmetry, and Live Birth Rate. Fertil. Steril. 2011, 95, 1985–1989.

34. Pons, M.C.; Carrasco, B.; Parriego, M.; Boada, M.; González-Foruria, I.; Garcia, S.; Coroleu, B.; Barri, P.N.; Veiga, A. Deconstructing the Myth of Poor Prognosis for Fast-Cleaving Embryos on Day 3. Is It Time to Change the Consensus? J. Assist. Reprod. Genet. 2019, 36, 2299–2305.

35. Van Royen, E.; Mangelschots, K.; Vercruyssen, M.; De Neubourg, D.; Valkenburg, M.; Ryckaert, G.; Gerris, J. Multinucleation in Cleavage Stage Embryos. Hum. Reprod. 2003, 18, 1062–1069.

36. Meriano, J.; Clark, C.; Cadesky, K.; Laskin, C.A. Binucleated and Micronucleated Blastomeres in Embryos Derived from Human Assisted Reproduction Cycles. Reprod. Biomed. Online 2004, 9, 511–520.

37. Yakin, K.; Balaban, B.; Urman, B. Impact of the Presence of One or More Multinucleated Blastomeres on the Developmental Potential of the Embryo to the Blastocyst Stage. Fertil. Steril. 2005, 83, 243–245.

38. Ambroggio, J.; Gindoff, P.R.; Dayal, M.B.; Khaldi, R.; Peak, D.; Frankfurter, D.; Dubey, A.K. Multinucleation of a Sibling Blastomere on Day 2 Suggests Unsuitability for Embryo Transfer in IVF-Preimplantation Genetic Screening Cycles. Fertil. Steril. 2011, 96, 856–859.

39. Balakier, H.; Sojecki, A.; Motamedi, G.; Librach, C. Impact of Multinucleated Blastomeres on Embryo Developmental Competence, Morphokinetics, and Aneuploidy. Fertil. Steril. 2016, 106, 608–614.e2.

40. Gardner, D.K.; Schoolcraft, W.B. Culture and Transfer of Human Blastocysts. Curr. Opin. Obstet. Gynecol. 1999, 11, 307–311.

41. Schoolcraft, W.B.; Gardner, D.K.; Lane, M.; Schlenker, T.; Hamilton, F.; Meldrum, D.R. Blastocyst Culture and Transfer: Analysis of Results and Parameters Affecting Outcome in Two In Vitro Fertilization Programs. Fertil. Steril. 1999, 72, 604–609.

42. Gardner, D.K.; Lane, M.; Stevens, J.; Schlenker, T.; Schoolcraft, W.B. Blastocyst Score Affects Implantation and Pregnancy Outcome: Towards a Single Blastocyst Transfer. Fertil. Steril. 2000, 73, 1155–1158.

43. Alfarawati, S.; Fragouli, E.; Colls, P.; Stevens, J.; Gutiérrez-Mateo, C.; Schoolcraft, W.B.; Katz-Jaffe, M.G.; Wells, D. The Relationship between Blastocyst Morphology, Chromosomal Abnormality, and Embryo Gender. Fertil. Steril. 2011, 95, 520–524.

44. Capalbo, A.; Rienzi, L.; Cimadomo, D.; Maggiulli, R.; Elliott, T.; Wright, G.; Nagy, Z.P.; Ubaldi, F.M. Correlation between Standard Blastocyst Morphology, Euploidy and Implantation: An Observational Study in Two Centers Involving 956 Screened Blastocysts. Hum. Reprod. 2014, 29, 1173–1181.

45. Fragouli, E.; Alfarawati, S.; Spath, K.; Wells, D. Morphological and Cytogenetic Assessment of Cleavage and Blastocyst Stage Embryos. Mol. Hum. Reprod. 2014, 20, 117–126.

46. Minasi, M.G.; Colasante, A.; Riccio, T.; Ruberti, A.; Casciani, V.; Scarselli, F.; Spinella, F.; Fiorentino, F.; Varricchio, M.T.; Greco, E. Correlation between Aneuploidy, Standard Morphology Evaluation and Morphokinetic Development in 1730 Biopsied Blastocysts: A Consecutive Case Series Study. Hum. Reprod. 2016, 31, 2245–2254.

47. Barash, O.O.; Ivani, K.A.; Willman, S.P.; Rosenbluth, E.M.; Wachs, D.S.; Hinckley, M.D.; Pittenger Reid, S.; Weckstein, L.N. Association between Growth Dynamics, Morphological Parameters, the Chromosomal Status of the Blastocysts, and Clinical Outcomes in IVF PGS Cycles with Single Embryo Transfer. J. Assist. Reprod. Genet. 2017, 34, 1007–1016.

48. Guzman, L.; Nuñez, D.; López, R.; Inoue, N.; Portella, J.; Vizcarra, F.; Noriega-Portella, L.; Noriega-Hoces, L.; Munné, S. The Number of Biopsied Trophectoderm Cells May Affect Pregnancy Outcomes. J. Assist. Reprod. Genet. 2019, 36, 145–151.

49. Hernandez-Nieto, C.; Lee, J.A.; Duke, M.; Stein, D.E.; Copperman, A.B.; Sandler, B.; Mukherjee, T. Direct Correlation between B-HCG Levels and Trophectoderm Morphology Quality in Single Euploid Embryo Transfer Cycles. Fertil. Steril. 2019, 112, e49.

50. Kim, M.K.; Park, J.K.; Jeon, Y.; Choe, S.-A.; Lee, H.J.; Kim, J.; Chang, E.M.; Kim, J.W.; Lyu, S.W.; Kim, J.Y.; et al. Correlation between Morphologic Grading and Euploidy Rates of Blastocysts, and Clinical Outcomes in In Vitro Fertilization Preimplantation Genetic Screening. J. Korean Med. Sci. 2019, 34, e27.

51. Viñals Gonzalez, X.; Odia, R.; Naja, R.; Serhal, P.; Saab, W.; Seshadri, S.; Ben-Nagi, J. Euploid Blastocysts Implant Irrespective of Their Morphology after NGS-(PGT-A) Testing in Advanced Maternal Age Patients. J. Assist. Reprod. Genet. 2019, 36, 1623–1629.

52. Richter, K.S.; Harris, D.C.; Daneshmand, S.T.; Shapiro, B.S. Quantitative Grading of a Human Blastocyst: Optimal Inner Cell Mass Size and Shape. Fertil. Steril. 2001, 76, 1157–1167.

53. Boynukalin, F.K.; Gultomruk, M.; Cavkaytar, S.; Turgut, E.; Findikli, N.; Serdarogullari, M.; Coban, O.; Yarkiner, Z.; Rubio, C.; Bahceci, M. Parameters Impacting the Live Birth Rate per Transfer after Frozen Single Euploid Blastocyst Transfer. PLoS ONE 2020, 15, e0227619.

54. Ai, J.; Jin, L.; Zheng, Y.; Yang, P.; Huang, B.; Dong, X. The Morphology of Inner Cell Mass Is the Strongest Predictor of Live Birth after a Frozen-Thawed Single Embryo Transfer. Front. Endocrinol. 2021, 12, 621221.

55. Ahlström, A.; Westin, C.; Reismer, E.; Wikland, M.; Hardarson, T. Trophectoderm Morphology: An Important Parameter for Predicting Live Birth after Single Blastocyst Transfer. Hum. Reprod. 2011, 26, 3289–3296.

56. Honnma, H.; Baba, T.; Sasaki, M.; Hashiba, Y.; Ohno, H.; Fukunaga, T.; Endo, T.; Saito, T.; Asada, Y. Trophectoderm Morphology Significantly Affects the Rates of Ongoing Pregnancy and Miscarriage in Frozen-Thawed Single-Blastocyst Transfer Cycle In Vitro Fertilization. Fertil. Steril. 2012, 98, 361–367.

57. Chen, X.; Zhang, J.; Wu, X.; Cao, S.; Zhou, L.; Wang, Y.; Chen, X.; Lu, J.; Zhao, C.; Chen, M.; et al. Trophectoderm Morphology Predicts Outcomes of Pregnancy in Vitrified-Warmed Single-Blastocyst Transfer Cycle in a Chinese Population. J. Assist. Reprod. Genet. 2014, 31, 1475–1481.

58. Cimadomo, D.; Rienzi, L.; Conforti, A.; Forman, E.; Canosa, S.; Innocenti, F.; Poli, M.; Hynes, J.; Gemmell, L.; Vaiarelli, A.; et al. Opening the Black Box: Why Do Euploid Blastocysts Fail to Implant? A Systematic Review and Meta-Analysis. Hum. Reprod. Update 2023, 29, 570–633.

59. Meseguer, M.; Herrero, J.; Tejera, A.; Hilligsøe, K.M.; Ramsing, N.B.; Remohí, J. The Use of Morphokinetics as a Predictor of Embryo Implantation. Hum. Reprod. 2011, 26, 2658–2671.

60. ESHRE Working Group on Time-Lapse Technology; Apter, S.; Ebner, T.; Freour, T.; Guns, Y.; Kovacic, B.; Le Clef, N.; Marques, M.; Meseguer, M.; Montjean, D.; et al. Good Practice Recommendations for the Use of Time-Lapse Technology. Hum. Reprod. Open 2020, 2020, hoaa008.

61. Rienzi, L.; Cimadomo, D.; Delgado, A.; Minasi, M.G.; Fabozzi, G.; Gallego, R.D.; Stoppa, M.; Bellver, J.; Giancani, A.; Esbert, M.; 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, 1080–1093.e1.

62. Bamford, T.; Barrie, A.; Montgomery, S.; Dhillon-Smith, R.; Campbell, A.; Easter, C.; Coomarasamy, A. Morphological and Morphokinetic Associations with Aneuploidy: A Systematic Review and Meta-Analysis. Hum. Reprod. Update 2022, 28, 656–686.

63. McQueen, D.B.; Mazur, J.; Kimelman, D.; Confino, R.; Robins, J.C.; Bernardi, L.A.; Yeh, C.; Zhang, J.; Pavone, M.E. Can Embryo Morphokinetic Parameters Predict Euploid Pregnancy Loss? Fertil. Steril. 2021, 115, 382–388.

64. Bourdon, M.; Pocate-Cheriet, K.; Finet de Bantel, A.; Grzegorczyk-Martin, V.; Amar Hoffet, A.; Arbo, E.; Poulain, M.; Santulli, P. Day 5 versus Day 6 Blastocyst Transfers: A Systematic Review and Meta-Analysis of Clinical Outcomes. Hum. Reprod. 2019, 34, 1948–1964.

65. Corti, L.; Cermisoni, G.C.; Alteri, A.; Pagliardini, L.; Ambrosini, G.; Andrisani, A.; Papaleo, E.; Viganò, P.; Noventa, M. Clinical Outcomes Deriving from Transfer of Blastocysts Developed in Day 7: A Systematic Review and Meta-Analysis of Frozen-Thawed IVF Cycles. Reprod. Sci. 2022, 29, 43–53.

66. Chen, S.-L.; Wu, F.-R.; Luo, C.; Chen, X.; Shi, X.-Y.; Zheng, H.-Y.; Ni, Y.-P. Combined Analysis of Endometrial Thickness and Pattern in Predicting Outcome of In Vitro Fertilization and Embryo Transfer: A Retrospective Cohort Study. Reprod. Biol. Endocrinol. 2010, 8, 30.

67. Kuć, P.; Kuczyńska, A.; Topczewska, M.; Tadejko, P.; Kuczyński, W. The Dynamics of Endometrial Growth and the Triple Layer Appearance in Three Different Controlled Ovarian Hyperstimulation Protocols and Their Influence on IVF Outcomes. Gynecol. Endocrinol. 2011, 27, 867–873.

68. Liu, K.E.; Hartman, M.; Hartman, A.; Luo, Z.-C.; Mahutte, N. The Impact of a Thin Endometrial Lining on Fresh and Frozen-Thaw IVF Outcomes: An Analysis of over 40,000 Embryo Transfers. Hum. Reprod. 2018, 33, 1883–1888.

69. Liao, S.; Wang, R.; Hu, C.; Pan, W.; Pan, W.; Yu, D.; Jin, L. Analysis of Endometrial Thickness Patterns and Pregnancy Outcomes Considering 12,991 Fresh IVF Cycles. BMC Med. Inform. Decis. Mak. 2021, 21, 176.

70. Wang, L.; Lv, S.; Mao, W.; Pei, M.; Yang, X. Assessment of Endometrial Receptivity during Implantation Window in Women with Unexplained Infertility. Gynecol. Endocrinol. 2020, 36, 917–921.

71. Schild, R.L.; Knobloch, C.; Dorn, C.; Fimmers, R.; van der Ven, H.; Hansmann, M. Endometrial Receptivity in an In Vitro Fertilization Program as Assessed by Spiral Artery Blood Flow, Endometrial Thickness, Endometrial Volume, and Uterine Artery Blood Flow. Fertil. Steril. 2001, 75, 361–366.

72. Ng, E.H.Y.; Chan, C.C.W.; Tang, O.S.; Yeung, W.S.B.; Ho, P.C. The Role of Endometrial and Subendometrial Blood Flows Measured by Three-Dimensional Power Doppler Ultrasound in the Prediction of Pregnancy during IVF Treatment. Hum. Reprod. 2006, 21, 164–170.

73. Kasius, A.; Smit, J.G.; Torrance, H.L.; Eijkemans, M.J.C.; Mol, B.W.; Opmeer, B.C.; Broekmans, F.J.M. Endometrial Thickness and Pregnancy Rates after IVF: A Systematic Review and Meta-Analysis. Hum. Reprod. Update 2014, 20, 530–541.

74. Griesinger, G.; Trevisan, S.; Cometti, B. Endometrial Thickness on the Day of Embryo Transfer Is a Poor Predictor of IVF Treatment Outcome. Hum. Reprod. Open 2018, 2018, hox031.

75. Cruz, F.; Bellver, J. Live Birth after Embryo Transfer in an Unresponsive Thin Endometrium. Gynecol. Endocrinol. 2014, 30, 481–484.

76. Li, Y.; Li, X.F.; Liao, J.N.; Fan, X.X.; Hu, Y.B.; Gan, R.; Lu, G.; Lin, G.; Gong, F. Clinical Value of Histologic Endometrial Dating for Personalized Frozen-Thawed Embryo Transfer in Patients with Repeated Implantation Failure in Natural Cycles. BMC Pregnancy Childbirth 2020, 20, 527.

77. Acosta, A.A.; Elberger, L.; Borghi, M.; Calamera, J.C.; Chemes, H.; Doncel, G.F.; Kliman, H.; Lema, B.; Lustig, L.; Papier, S. Endometrial Dating and Determination of the Window of Implantation in Healthy Fertile Women. Fertil. Steril. 2000, 73, 788–798.

78. Murray, M.J.; Meyer, W.R.; Zaino, R.J.; Lessey, B.A.; Novotny, D.B.; Ireland, K.; Zeng, D.; Fritz, M.A. A Critical Analysis of the Accuracy, Reproducibility, and Clinical Utility of Histologic Endometrial Dating in Fertile Women. Fertil. Steril. 2004, 81, 1333–1343.

79. Alfer, J.; Fattahi, A.; Bleisinger, N.; Krieg, J.; Behrens, R.; Dittrich, R.; Beckmann, M.W.; Hartmann, A.; Classen-Linke, I.; Popovici, R.M. Endometrial Dating Method Detects Individual Maturation Sequences During the Secretory Phase. In Vivo 2020, 34, 1951–1963.

80. Aghajanova, L.; Stavreus-Evers, A.; Nikas, Y.; Hovatta, O.; Landgren, B.-M. Coexpression of Pinopodes and Leukemia Inhibitory Factor, as Well as Its Receptor, in Human Endometrium. Fertil. Steril. 2003, 79, 808–814.

81. Nikas, G. Pinopodes as Markers of Endometrial Receptivity in Clinical Practice. Hum. Reprod. 1999, 14, 99–106.

82. Stavreus-Evers, A.; Nikas, G.; Sahlin, L.; Eriksson, H.; Landgren, B.-M. Formation of Pinopodes in Human Endometrium Is Associated with the Concentrations of Progesterone and Progesterone Receptors. Fertil. Steril. 2001, 76, 782–791.

83. Jin, X.Y.; Zhao, L.J.; Luo, D.H.; Liu, L.; Dai, Y.D.; Hu, X.X.; Wang, Y.Y.; Lin, X.; Hong, F.; Li, T.C.; et al. Pinopode Score around the Time of Implantation Is Predictive of Successful Implantation Following Frozen Embryo Transfer in Hormone Replacement Cycles. Hum. Reprod. 2017, 32, 2394–2403.

84. Qiong, Z.; Jie, H.; Yonggang, W.; Bin, X.; Jing, Z.; Yanping, L. Clinical Validation of Pinopode as a Marker of Endometrial Receptivity: A Randomized Controlled Trial. Fertil. Steril. 2017, 108, 513–517.e2.

85. D'Ippolito, S.; Di Nicuolo, F.; Papi, M.; Castellani, R.; Palmieri, V.; Masciullo, V.; Arena, V.; Tersigni, C.; Bernabei, M.; Pontecorvi, A.; et al. Expression of Pinopodes in the Endometrium from Recurrent Pregnancy Loss Women. Role of Thrombomodulin and Ezrin. J. Clin. Med. 2020, 9, 2634.

86. Quinn, C.; Ryan, E.; Claessens, E.A.; Greenblatt, E.; Hawrylyshyn, P.; Cruickshank, B.; Hannam, T.; Dunk, C.; Casper, R.F. The Presence of Pinopodes in the Human Endometrium Does Not Delineate the Implantation Window. Fertil. Steril. 2007, 87, 1015–1021.

87. Quinn, C.E.; Casper, R.F. Pinopodes: A Questionable Role in Endometrial Receptivity. Hum. Reprod. Update 2009, 15, 229–236.

88. Fragouli, E.; Wells, D. Mitochondrial DNA Assessment to Determine Oocyte and Embryo Viability. Semin. Reprod. Med. 2015, 33, 401–409.

89. Fragouli, E.; Spath, K.; Alfarawati, S.; Kaper, F.; Craig, A.; Michel, C.-E.; Kokocinski, F.; Cohen, J.; Munne, S.; Wells, D. Altered Levels of Mitochondrial DNA Are Associated with Female Age, Aneuploidy, and Provide an Independent Measure of Embryonic Implantation Potential. PLoS Genet. 2015, 11, e1005241.

90. Diez-Juan, A.; Rubio, C.; Marin, C.; Martinez, S.; Al-Asmar, N.; Riboldi, M.; Díaz-Gimeno, P.; Valbuena, D.; Simón, C. Mitochondrial DNA Content as a Viability Score in Human Euploid Embryos: Less Is Better. Fertil. Steril. 2015, 104, 534–541.e1.

91. Podolak, A.; Woclawek-Potocka, I.; Lukaszuk, K. The Role of Mitochondria in Human Fertility and Early Embryo Development: What Can We Learn for Clinical Application of Assessing and Improving Mitochondrial DNA? Cells 2022, 11, 797.

92. Victor, A.R.; Brake, A.J.; Tyndall, J.C.; Griffin, D.K.; Zouves, C.G.; Barnes, F.L.; Viotti, M. Accurate Quantitation of Mitochondrial DNA Reveals Uniform Levels in Human Blastocysts Irrespective of Ploidy, Age, or Implantation Potential. Fertil. Steril. 2017, 107, 34–42.e3.

93. Treff, N.R.; Zhan, Y.; Tao, X.; Olcha, M.; Han, M.; Rajchel, J.; Morrison, L.; Morin, S.J.; Scott, R.T. Levels of Trophectoderm Mitochondrial DNA Do Not Predict the Reproductive Potential of Sibling Embryos. Hum. Reprod. 2017, 32, 954–962.

94. Podolak, A.; Liss, J.; Kiewisz, J.; Pukszta, S.; Cybulska, C.; Rychlowski, M.; Lukaszuk, A.; Jakiel, G.; Lukaszuk, K. Mitochondrial DNA Copy Number in Cleavage Stage Human Embryos-Impact on Infertility Outcome. Curr. Issues Mol. Biol. 2022, 44, 273–287.

95. Lukaszuk, K.; Podolak, A. Does Trophectoderm Mitochondrial DNA Content Affect Embryo Developmental and Implantation Potential? Int. J. Mol. Sci. 2022, 23, 5976.

96. Scott, R.T.; Sun, L.; Zhan, Y.; Marin, D.; Tao, X.; Seli, E. Mitochondrial DNA Content Is Not Predictive of Reproductive Competence in Euploid Blastocysts. Reprod. Biomed. Online 2020, 41, 183–190.

97. Robert, C. Nurturing the Egg: The Essential Connection between Cumulus Cells and the Oocyte. Reprod. Fertil. Dev. 2021, 34, 149–159.

98. Richani, D.; Dunning, K.R.; Thompson, J.G.; Gilchrist, R.B. Metabolic Co-Dependence of the Oocyte and Cumulus Cells: Essential Role in Determining Oocyte Developmental Competence. Hum. Reprod. Update 2021, 27, 27–47.

99. van Montfoort, A.P.A.; Geraedts, J.P.M.; Dumoulin, J.C.M.; Stassen, A.P.M.; Evers, J.L.H.; Ayoubi, T.A.Y. Differential Gene Expression in Cumulus Cells as a Prognostic Indicator of Embryo Viability: A Microarray Analysis. Mol. Hum. Reprod. 2008, 14, 157–168.

100. Feuerstein, P.; Puard, V.; Chevalier, C.; Teusan, R.; Cadoret, V.; Guerif, F.; Houlgatte, R.; Royere, D. Genomic Assessment of Human Cumulus Cell Marker Genes as Predictors of Oocyte Developmental Competence: Impact of Various Experimental Factors. PLoS ONE 2012, 7, e40449.

101. Scarica, C.; Cimadomo, D.; Dovere, L.; Giancani, A.; Stoppa, M.; Capalbo, A.; Ubaldi, F.M.; Rienzi, L.; Canipari, R. An Integrated Investigation of Oocyte Developmental Competence: Expression of Key Genes in Human Cumulus Cells, Morphokinetics of Early Divisions, Blastulation, and Euploidy. J. Assist. Reprod. Genet. 2019, 36, 875–887.

102. McKenzie, L.J.; Pangas, S.A.; Carson, S.A.; Kovanci, E.; Cisneros, P.; Buster, J.E.; Amato, P.; Matzuk, M.M. Human Cumulus Granulosa Cell Gene Expression: A Predictor of Fertilization and Embryo Selection in Women Undergoing IVF. Hum. Reprod. 2004, 19, 2869–2874.

103. Cillo, F.; Brevini, T.A.L.; Antonini, S.; Paffoni, A.; Ragni, G.; Gandolfi, F. Association between Human Oocyte Developmental Competence and Expression Levels of Some Cumulus Genes. Reproduction 2007, 134, 645–650.

104. Wathlet, S.; Adriaenssens, T.; Segers, I.; Verheyen, G.; Janssens, R.; Coucke, W.; Devroey, P.; Smitz, J. New Candidate Genes to Predict Pregnancy Outcome in Single Embryo Transfer Cycles When Using Cumulus Cell Gene Expression. Fertil. Steril. 2012, 98, 432–439.e4.

105. Assou, S.; Haouzi, D.; Mahmoud, K.; Aouacheria, A.; Guillemin, Y.; Pantesco, V.; Rème, T.; Dechaud, H.; De Vos, J.; Hamamah, S. A Non-Invasive Test for Assessing Embryo Potential by Gene Expression Profiles of Human Cumulus Cells: A Proof of Concept Study. Mol. Hum. Reprod. 2008, 14, 711–719.

106. Gebhardt, K.M.; Feil, D.K.; Dunning, K.R.; Lane, M.; Russell, D.L. Human Cumulus Cell Gene Expression as a Biomarker of Pregnancy Outcome after Single Embryo Transfer. Fertil. Steril. 2011, 96, 47–52.e2.

107. Iager, A.E.; Kocabas, A.M.; Otu, H.H.; Ruppel, P.; Langerveld, A.; Schnarr, P.; Suarez, M.; Jarrett, J.C.; Conaghan, J.; Rosa, G.J.M.; et al. Identification of a Novel Gene Set in Human Cumulus Cells Predictive of an Oocyte's Pregnancy Potential. Fertil. Steril. 2013, 99, 745–752.e6.

108. Parks, J.C.; Patton, A.L.; McCallie, B.R.; Griffin, D.K.; Schoolcraft, W.B.; Katz-Jaffe, M.G. Corona Cell RNA Sequencing from Individual Oocytes Revealed Transcripts and Pathways Linked to Euploid Oocyte Competence and Live Birth. Reprod. Biomed. Online 2016, 32, 518–526.

109. Green, K.A.; Franasiak, J.M.; Werner, M.D.; Tao, X.; Landis, J.N.; Scott, R.T.; Treff, N.R. Cumulus Cell Transcriptome Profiling Is Not Predictive of Live Birth after In Vitro Fertilization: A Paired Analysis of Euploid Sibling Blastocysts. Fertil. Steril. 2018, 109, 460–466.e2.

110. Papler, T.B.; Bokal, E.V.; Maver, A.; Kopitar, A.N.; Lovrečić, L. Transcriptomic Analysis and Meta-Analysis of Human Granulosa and Cumulus Cells. PLoS ONE 2015, 10, e0136473.

111. Sachs, M.K.; Makieva, S.; Velasco Gil, A.; Xie, M.; Ille, F.; Salvadori, V.; Schmidhauser, M.; Saenz-de-Juano, M.D.; Ulbrich, S.E.; Leeners, B. Transcriptomic Signature of Luteinized Cumulus Cells of Oocytes Developing to Live Birth after Women Received Intracytoplasmic Sperm Injection. F&S Sci. 2024, 5, 24–38.

112. Katz-Jaffe, M.G.; McReynolds, S.; Gardner, D.K.; Schoolcraft, W.B. The Role of Proteomics in Defining the Human Embryonic Secretome. Mol. Hum. Reprod. 2009, 15, 271–277.

113. Capalbo, A.; Romanelli, V.; Patassini, C.; Poli, M.; Girardi, L.; Giancani, A.; Stoppa, M.; Cimadomo, D.; Ubaldi, F.M.; Rienzi, L. Diagnostic Efficacy of Blastocoel Fluid and Spent Media as Sources of DNA for Preimplantation Genetic Testing in Standard Clinical Conditions. Fertil. Steril. 2018, 110, 870–879.e5.

114. Ho, J.R.; Arrach, N.; Rhodes-Long, K.; Ahmady, A.; Ingles, S.; Chung, K.; Bendikson, K.A.; Paulson, R.J.; McGinnis, L.K. Pushing the Limits of Detection: Investigation of Cell-Free DNA for Aneuploidy Screening in Embryos. Fertil. Steril. 2018, 110, 467–475.e2.

115. Kuznyetsov, V.; Madjunkova, S.; Antes, R.; Abramov, R.; Motamedi, G.; Ibarrientos, Z.; Librach, C. Evaluation of a Novel Non-Invasive Preimplantation Genetic Screening Approach. PLoS ONE 2018, 13, e0197262.

116. Vera-Rodriguez, M.; Diez-Juan, A.; Jimenez-Almazan, J.; Martinez, S.; Navarro, R.; Peinado, V.; Mercader, A.; Meseguer, M.; Blesa, D.; Moreno, I.; et al. Origin and Composition of Cell-Free DNA in Spent Medium from Human Embryo Culture during Preimplantation Development. Hum. Reprod. 2018, 33, 745–756.

117. Belandres, D.; Shamonki, M.; Arrach, N. Current Status of Spent Embryo Media Research for Preimplantation Genetic Testing. J. Assist. Reprod. Genet. 2019, 36, 819–826.

118. Rosenbluth, E.M.; Shelton, D.N.; Wells, L.M.; Sparks, A.E.T.; Van Voorhis, B.J. Human Embryos Secrete microRNAs into Culture Media—A Potential Biomarker for Implantation. Fertil. Steril. 2014, 101, 1493–1500.

119. Cuman, C.; Van Sinderen, M.; Gantier, M.P.; Rainczuk, K.; Sorby, K.; Rombauts, L.; Osianlis, T.; Dimitriadis, E. Human Blastocyst Secreted microRNA Regulate Endometrial Epithelial Cell Adhesion. EBioMedicine 2015, 2, 1528–1535.

120. Kropp, J.; Khatib, H. Characterization of microRNA in Bovine In Vitro Culture Media Associated with Embryo Quality and Development. J. Dairy Sci. 2015, 98, 6552–6563.

121. Mutia, K.; Wiweko, B.; Abinawanto, A.; Dwiranti, A.; Bowolaksono, A. microRNAs as A Biomarker to Predict Embryo Quality Assessment in In Vitro Fertilization. Int. J. Fertil. Steril. 2023, 17, 85–91.

122. Capalbo, A.; Ubaldi, F.M.; Cimadomo, D.; Noli, L.; Khalaf, Y.; Farcomeni, A.; Ilic, D.; Rienzi, L. MicroRNAs in Spent Blastocyst Culture Medium Are Derived from Trophectoderm Cells and Can Be Explored for Human Embryo

Reproductive Competence Assessment. Fertil. Steril. 2016, 105, 225–235.e3.

123. Cimadomo, D.; Rienzi, L.; Giancani, A.; Alviggi, E.; Dusi, L.; Canipari, R.; Noli, L.; Ilic, D.; Khalaf, Y.; Ubaldi, F.M.; et al. Definition and Validation of a Custom Protocol to Detect miRNAs in the Spent Media after Blastocyst Culture: Searching for Biomarkers of Implantation. Hum. Reprod. 2019, 34, 1746–1761.
124. Borges, E., Jr.; Setti, A.S.; Braga, D.P.A.F.; Geraldo, M.V.; de Cássia S Figueira, R.; Iaconelli, A., Jr. miR-142-3p as a Biomarker of Blastocyst Implantation Failure—A Pilot Study. JBRA Assist. Reprod. 2016, 20, 200–205.
125. Leaver, M.; Wells, D. Non-Invasive Preimplantation Genetic Testing (niPGT): The next Revolution in Reproductive Genetics? Hum. Reprod. Update 2020, 26, 16–42.
126. Rubio, C.; Rienzi, L.; Navarro-Sánchez, L.; Cimadomo, D.; García-Pascual, C.M.; Albricci, L.; Soscia, D.; Valbuena, D.; Capalbo, A.; Ubaldi, F.; et al. Embryonic Cell-Free DNA versus Trophectoderm Biopsy for Aneuploidy Testing: Concordance Rate and Clinical Implications. Fertil. Steril. 2019, 112, 510–519.
127. Yeung, Q.S.Y.; Zhang, Y.X.; Chung, J.P.W.; Lui, W.T.; Kwok, Y.K.Y.; Gui, B.; Kong, G.W.S.; Cao, Y.; Li, T.C.; Choy, K.W. A Prospective Study of Non-Invasive Preimplantation Genetic Testing for Aneuploidies (NiPGT-A) Using next-Generation Sequencing (NGS) on Spent Culture Media (SCM). J. Assist. Reprod. Genet. 2019, 36, 1609–1621.
128. Magli, M.C.; Albanese, C.; Crippa, A.; Tabanelli, C.; Ferraretti, A.P.; Gianaroli, L. Deoxyribonucleic Acid Detection in Blastocoelic Fluid: A New Predictor of Embryo Ploidy and Viable Pregnancy. Fertil. Steril. 2019, 111, 77–85.
129. Pavani, K.C.; Hendrix, A.; Van Den Broeck, W.; Couck, L.; Szymanska, K.; Lin, X.; De Koster, J.; Van Soom, A.; Leemans, B. Isolation and Characterization of Functionally Active Extracellular Vesicles from Culture Medium Conditioned by Bovine Embryos In Vitro. Int. J. Mol. Sci. 2018, 20, 38.
130. Giacomini, E.; Vago, R.; Sanchez, A.M.; Podini, P.; Zarovni, N.; Murdica, V.; Rizzo, R.; Bortolotti, D.; Candiani, M.; Viganò, P. Secretome of In Vitro Cultured Human Embryos Contains Extracellular Vesicles That Are Uptaken by the Maternal Side. Sci. Rep. 2017, 7, 5210.
131. Saadeldin, I.M.; Kim, S.J.; Choi, Y.B.; Lee, B.C. Improvement of Cloned Embryos Development by Co-Culturing with Parthenotes: A Possible Role of Exosomes/Microvesicles for Embryos Paracrine Communication. Cell. Reprogram. 2014, 16, 223–234.
132. Pavani, K.C.; Alminana, C.; Wydooghe, E.; Catteeuw, M.; Ramírez, M.A.; Mermillod, P.; Rizos, D.; Van Soom, A. Emerging Role of Extracellular Vesicles in Communication of Preimplantation Embryos In Vitro. Reprod. Fertil. Dev. 2016, 29, 66–83.
133. Théry, C.; Amigorena, S.; Raposo, G.; Clayton, A. Isolation and Characterization of Exosomes from Cell Culture Supernatants and Biological Fluids. Curr. Protoc. Cell Biol. 2006, 30, 3.22.1–3.22.29.
134. Vlaeminck-Guillem, V. Extracellular Vesicles in Prostate Cancer Carcinogenesis, Diagnosis, and Management. Front. Oncol. 2018, 8, 222.
135. Lin, X.; Beckers, E.; Mc Cafferty, S.; Gansemans, Y.; Joanna Szymańska, K.; Chaitanya Pavani, K.; Catani, J.P.; Van Nieuwerburgh, F.; Deforce, D.; De Sutter, P.; et al. Bovine Embryo-Secreted microRNA-30c Is a Potential Non-Invasive Biomarker for Hampered Preimplantation Developmental Competence. Front. Genet. 2019, 10, 315.
136. Pavani, K.C.; Meese, T.; Pascottini, O.B.; Guan, X.; Lin, X.; Peelman, L.; Hamacher, J.; Van Nieuwerburgh, F.; Deforce, D.; Boel, A.; et al. Hatching Is Modulated by microRNA-378a-3p Derived from Extracellular Vesicles Secreted by Blastocysts. Proc. Natl. Acad. Sci. USA 2022, 119, e2122708119.
137. Dissanayake, K.; Nõmm, M.; Lättekivi, F.; Ord, J.; Ressaissi, Y.; Godakumara, K.; Reshi, Q.U.A.; Viil, J.; Jääger, K.; Velthut-Meikas, A.; et al. Oviduct as a Sensor of Embryo Quality: Deciphering the Extracellular Vesicle (EV)-Mediated Embryo-Maternal Dialogue. J. Mol. Med. 2021, 99, 685–697.
138. Godakumara, K.; Ord, J.; Lättekivi, F.; Dissanayake, K.; Viil, J.; Boggavarapu, N.R.; Faridani, O.R.; Jääger, K.; Velthut-Meikas, A.; Jaakma, Ü.; et al. Trophoblast Derived Extracellular Vesicles Specifically Alter the Transcriptome of Endometrial Cells and May Constitute a Critical Component of Embryo-Maternal Communication. Reprod. Biol. Endocrinol. 2021, 19, 115.
139. Es-Haghi, M.; Godakumara, K.; Häling, A.; Lättekivi, F.; Lavrits, A.; Viil, J.; Andronowska, A.; Nafee, T.; James, V.; Jaakma, Ü.; et al. Specific Trophoblast Transcripts Transferred by Extracellular Vesicles Affect Gene Expression in Endometrial Epithelial Cells and May Have a Role in Embryo-Maternal Crosstalk. Cell Commun. Signal. 2019, 17, 146.