Blastocyst development in assisted reproductive technologies: a narrative review evaluating its role as a surrogate marker for pregnancy outcomes and live birth success

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There is an urgent global need to improve in vitro fertilization success rates and expand access to services. Specific to the in vitro fertilization laboratory, challenges such as standardization and a shortage of trained embryologists hinder quality and limit service availability. Current standards for product approval rely on demonstrating comparable pregnancy rates, requiring extensive patient involvement and time-consuming trials, which may be further hindered by patient reluctance to participate in clinical trials. Efficient assessment of new protocols and devices for assessing human assisted reproductive technology requires considering intermediate endpoints and markers to complement conventional endpoints. This review explores blastocyst development as a potential surrogate marker for pregnancy. It examines the correlation between blastocyst development and implantation potential, evaluates how culture conditions and other factors affect outcomes, and discusses the evidence supporting an absence of adverse effects of embryo culture on perinatal and offspring health. The conclusion strongly suggests that blastocyst development could serve as a valuable surrogate for establishing equivalency of pregnancy and live births in new assisted reproductive technology protocols. This review underscores the need for a surrogate marker of quality and presents evidence supporting the utility of blastocyst use rate as a sufficient indicator. (F S Rev® 2025;6:100094. ©2025 by American Society for Reproductive Medicine.)

Key Words: Assisted reproductive technology, innovation, blastocyst development

ESSENTIAL POINTS

- Blastocyst development rate is sensitive to suboptimal conditions, occurring after embryonic genome activation and correlating strongly with both aneuploidy and implantation potential.
- Blastocyst development rates show consistent statistical inference with pregnancy outcomes—specifically, reduced blastocyst rates are correlated with reduced pregnancy rates, whereas equivalent or increased rates are associated with equivalent pregnancy rates.
- Using blastocyst development as a surrogate endpoint for regulatory approval of new devices and protocols could accelerate innovation in assisted reproduction while maintaining safety standards, particularly for automation technologies aimed at improving laboratory standardization and efficiency.

Fertility clinics face significant challenges in delivering high-quality care due to increasing demand coupled with a shortage of skilled embryological professionals. Factors such as delayed childbearing and increasing infertility rates contribute to the growing demand for fertility services, straining resources and leading to longer wait times for treatment. Meanwhile, high costs associated with in vitro fertilization (IVF) procedures create disparities in access, which is exacerbated by a shortage of specialized healthcare professionals. Technological innovation in the IVF laboratory provides a strategy for addressing these challenges, by developing tools

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to standardize and optimize protocols (1–4), thereby improving access (5), and extending the fertility care workforce (2, 5). Most products used in the IVF laboratory were established before 2000, and newer introductions often reference the Food and Drug Administration (FDA)'s Final Rule, 63 FR48428 for 510K approval. Notably, protocols such as intracytoplasmic sperm injection (ICSI) (6) and embryo cryopreservation (7) have evolved over time with minimal FDA oversight. In contrast, novel device development necessitates de novo applications to the FDA, with safety and efficacy traditionally evaluated on the basis of live birth rates, a metric requiring substantial sample sizes and protracted timelines for recruitment and evaluation. Given the burdens—financial and emotional—borne by patients with infertility, participation in clinical trials is often constrained, impeding innovation in new product development and the opportunity for enhancement of fertility treatment.

Innovations in assisted reproductive technology (ART) can be categorized into several distinct groups, each with varying potential to benefit from blastocyst development as a surrogate marker. Culture system technologies include the following: culture media formulations (conventional single-step/sequential media, protein supplements, and media with molecules such as antioxidants); physical culture parameters (oxygen concentration, temperature, pH, and humidity); and culture vessels and accessories (dishes, oil overlays, and incubation systems). Automation technologies represent a rapidly developing sector encompassing oocyte handling systems (retrieval and denudation), sperm processing and ICSI automation, embryo culture systems (time-lapse imaging), and cryopreservation automation (vitrification devices). Diagnostic and selection tools include noninvasive embryo assessment (morphokinetics, metabolomics, and spent media analysis), genetic testing methodologies (preimplantation genetic testing for aneuploidy and emerging noninvasive genetic testing), and artificial intelligence and machine learning platforms for embryo selection. Automation technologies are particularly relevant for the use of blastocyst development as a surrogate marker because these innovations primarily aim to replicate current manual processes with equivalent outcomes rather than necessarily improving pregnancy rates. Establishing equivalence rather than superiority is the appropriate standard for these technologies.

To expedite new procedural and device development for fertility treatments, the adoption of an intermediate endpoint capable of accurately assessing risk and efficacy holds promise in shortening approval timelines, thereby accelerating generalized introduction of new fertility treatments to clinical care. Surrogate endpoints, well-established alternatives, streamline trial efficiency while furnishing requisite safety and efficacy data for FDA scrutiny (8, 9). Over the past 3 decades, numerous biomarkers or intermediate outcomes have gained acceptance as surrogate endpoints in regulatory approvals for pharmaceuticals and medical devices (10, 11). Notably, research in IVF routinely uses surrogate endpoints for live birth, such as blastocyst development and clinical/ongoing pregnancy rates (12–17). In this review, we consider whether blastocyst development holds promise as a suitable endpoint for clinical trials.

Piantadosi (18) outlines key attributes of a valuable surrogate endpoint: ease of measurement without invasive procedures; relevance to the causal pathway for the preferred endpoint; consistent statistical inference; and responsiveness to treatment effects. Mere correlation between a surrogate and clinical outcome falls short; instead, the intervention's impact on the surrogate should reliably predict its effect on the clinical outcome. Considering the critical stages of human embryo development, this review concentrates on blastocyst development as a pivotal stage and probes its potential as a surrogate endpoint for pregnancy rates, applying Piantadosi's (18) criteria for evaluation.

This narrative review examines the evidence supporting blastocyst development as a surrogate endpoint for pregnancy and live birth in ART using literature identified through searches of PubMed/MEDLINE using combinations of terms related to assisted reproduction ("in vitro fertilization" and "IVF"), blastocyst development ("blastocyst" and "embryo culture"), clinical outcomes ("pregnancy rate" and "live birth"), and surrogate endpoints ("surrogate marker" and "validation"). Additional relevant studies were identified through reference list screening and citation tracking of key articles. The review incorporates 3 decades of research and clinical experience in human ART, critically evaluating the strength of evidence supporting blastocyst development as a surrogate marker. Although animal studies are referenced where relevant, particularly regarding developmental biology and safety assessment, the primary focus is on human clinical outcomes. Evidence is assessed for quality and relevance, with careful distinction made between established findings and expert interpretation. This approach aims to provide a comprehensive evaluation of whether blastocyst development could serve as a reliable surrogate endpoint while maintaining the high safety standards essential in reproductive medicine.

BLASTOCYST DEVELOPMENT: AN EASILY MEASURED SURROGATE THAT IS PART OF THE CAUSAL PATHWAY OF IMPLANTATION AND CAN BE ASSESSED NONINVASIVELY WITHOUT IMPAIRING FUTURE POTENTIAL FOR SUCCESS

Clinical IVF laboratories are unique in medicine in that they provide the safe, ex vivo, passage of gametes and embryos—the foundations of generational health. Although the original protocols and products used in clinical IVF were nearly exclusively developed in animal models with few safety studies in humans (19), today's modern IVF laboratory evaluates different protocols and products for quality improvement initiatives as part of a broader quality management system (QMS) (20–23). Markers of efficacy vary from those specific to a developmental stage (e.g., oocyte maturation rate and fertilization rate) to markers that reflect the entire ecosystem of the IVF laboratory (e.g., blastocyst development). The goal of a QMS approach to the

assessment and implementation of change in the laboratory is to establish equivalence before the introduction into routine practice, without the need to wait for live birth rates (24).

Laboratory automation, whereby an automated method replaces a manual one, also requires proof of equivalence. For instance, some intermediate markers such as accuracy of automated semen analysis (25), recovery rate of microfluidic sperm isolation (26), or survival after embryo automated vitrification (27) are essential to confirm safety and efficacy before implementation. However, these intermediate measures do not assess the impact of the change on the entire process and, ultimately, on success of fertility treatment.

The rate at which fertilized oocytes develop into usable blastocysts, or the blastocyst use rate (BUR), is the most comprehensive measure of an embryo culture ecosystem. Unlike other intermediate measures, such as fertilization rate and cleavage stage development, usable blastocyst rate depends on embryonic genome activation (28) and is responsive to aneuploidy (i.e., blastocyst rate is reduced for aneuploid embryos (29)). Although there is a direct relationship between blastocyst grade (30), speed of development (31), and implantation rate for a single embryo transfer, the development of a cohort of fertilized oocytes from each patient provides a robust measure of overall laboratory performance.

To be a trusted surrogate marker for equivalency of a treatment, blastocyst development rate requires a standardized definition. The Alpha/European Society of Human Reproduction and Embryology consensus on embryo grading provides 2: number of good-quality blastocysts on day 5 and total blastocysts on day 5 (32, 33). These key performance indicators require further development for 2 reasons: they do not incorporate total blastocyst development rate to day 6 and/or 7, and they do not provide a definition for "good quality." Most publications address the issue of quality by presenting the percentage of top-quality blastocyst (34), typically those with grade A inner cell mass and/or trophectoderm (TE) on the basis of the Gardner grading scheme (35). Similarly, the total number of blastocysts is based on the number used, either transferred or cryopreserved (36). Although blastocyst grading is subjective (37) and definitions of usability vary among clinics (38), good-quality blastocyst formation, usable development rate, and overall BUR are effective when comparing 2 or more methods *within* a laboratory. Although different measures have merit, this review will use the term BUR as a general term for rate of blastocyst development.

Several potential surrogate markers for pregnancy and live birth, which can be obtained easily and noninvasively, are present during human embryo in vitro culture's developmental timepoints. Most preimplantation developmental stages have been correlated with implantation potential (39–44). However, an effective surrogate should occur after human embryonic genome activation, which occurs during the cleavage stages of development (days 1–3 (45)). Furthermore, as blastocyst culture has become a part of routine clinical practice, evidence suggests that predictive features before embryo compaction reflect an embryo's ability to develop in vitro and that these early features lose predictive ability for implantation after blastocyst formation (46, 47).

The speed and quality of blastocyst development have been repeatedly demonstrated to be strongly correlated with the chance of live birth. Blastocysts that develop "on time" or early (48) have significantly higher implantation rates than those that develop on day 6, which are higher than those developing on day 7 (31, 49–53). Coupled with speed, blastocyst morphology is associated with implantation potential, with the TE grade showing the highest correlation with live birth (54–56). Blastocyst size (55, 57, 58) and inner cell mass grade (59, 60) also have associations with live birth. These 3 variables are compiled into 1 score, for example, the Gardner blastocyst grade (61), and, when coupled with developmental speed, provide meaningful prognostic insights into a patient's chance for a live birth (29). The relationship between blastocyst quality and implantation is robust, leading to development of artificial intelligence-based embryo selection systems (62–70).

The rate of euploid blastocyst development could be considered a better surrogate endpoint than BUR without genetic testing; however, it is invasive and, thus, does not meet one of Piantadosi's (18) criteria. Preimplantation genetic testing for aneuploidy is an objective diagnostic assessment of an embryo's developmental potential. It provides insights into blastocyst potential, and thus, euploid blastocyst rates could also as serve as a surrogate endpoint for pregnancy. Furthermore, assessment of embryo genetics in relation to pregnancy outcomes would be beneficial, particularly if such assessments could be obtained noninvasively (71–73).

Many early developmental milestones that are correlated with blastocyst development are also correlated with aneuploidy. For instance, morphokinetic algorithms show good prediction of both aneuploidy and pregnancy (74–76). A strong relationship exists between aneuploidy and blastocyst developmental speed and quality (77). Aneuploidy is higher for slow growing day 6 and 7 blastocysts (49, 78, 79) and for lower-grade blastocysts (80). It is unlikely that earlier developmental stages provide additional prognostic value over blastocyst development alone or euploid blastocyst development.

The number of blastocysts or euploid blastocysts obtained from mature oocytes or zygotes is the euploid BUR. The euploid BUR is likely the best surrogate for pregnancy when testing a proposed or alternate procedure or intervention because it can detect mitotic aneuploidies that occur during embryo culture, aneuploidies that may be the result of an intervention (81). Although promising in theory, evidence for an impact of culture on rates of aneuploidy is currently limited (81, 82). Furthermore, until a noninvasive genetic test is developed, this metric requires an invasive TE biopsy. Although blastocyst quality and speed of development are associated with aneuploidy, implantation potential of euploid blastocysts diminishes the impact of quality and speed of development on implantation rates (78, 83).

In summary, the rate of good-quality blastocyst development per oocyte/zygote is independently the strongest, and perhaps the only, noninvasive predictor of implantation

TABLE 1

Summary of retrospective and prospective studies of associations between chemical and physical parameters and blastocyst rate and pregnancy rate, adapted from the study by Bartolacci et al. (90).

Parameter	Comparison	Reference group	Studies	RCTs with both BR and PR	Studies with statistical inference for equivalence
Temperature	37 vs. <37 °C	37 °C	5 (91–95)	3	3 (93–95)
Oxygen	5% vs. 20%	5%	9 (96–104)	5	5 (100–104)
Oxygen	Biphasic (5-2%) vs. 5%	Biphasic	9 (105–113)	3	3 (111–113)
Humidity	Humid vs. dry	Humid	5 (114–118)	1	1 (114)
Incubator	Time-lapse vs. standard	Time-lapse	7 (119–125)	3	3 (122–124)

Note: BR = blastocyst rate; PR = pregnancy rate; RCT = randomized controlled trial.

Morbeck. Blastocyst rate - a surrogate marker. F S Rev 2025.

within the causal pathway of in vitro embryo development. Only the addition of invasive genetic testing of blastocysts potentially yields a better surrogate.

BLASTOCYST RATE YIELDS THE SAME STATISTICAL INFERENCE AS PREGNANCY RATE AND IS RESPONSIVE TO TREATMENT EFFECTS

Assessing a surrogate marker involves determining whether the statistical analysis of the surrogate provides the same inference about the treatment effect on the true endpoint. Several tests and conditions gauge the degree of similar inference for surrogate endpoints, including the proportion of treatment effect explained (84), consistency of effect across studies (85), and presence of consistency over time for longitudinal surrogates (86). However, the last criterion is nonapplicable in this context because blastocyst rate is not a longitudinal intermediate measure.

A treatment intervention is typically assessed for either equivalence or superiority. For most laboratory process changes or improvements, whether changing to a new culture medium, culture dish, incubator, or automation, establishing equivalence is essential and a requirement of the QMS. In this context, automation—that is, the replacement of a manual method—similarly should establish equivalence and is not required to establish superiority. This is a critical distinction, because the ability to improve pregnancy and live birth rates is constrained by the complexity of the system, which includes variables such as age, the quality and number of gametes, effectiveness of the laboratory, embryo transfer, and uterine receptivity. This concept applies to other areas of medicine, such as the approval of biosimilar drugs, where equivalence, not superiority, is required (87, 88).

When assessing the effect of an intervention on ART outcome, we propose that BUR provides statistical inference for equivalence. Simply put, a treatment leading to similar or better BUR yields similar or better pregnancy rates, whereas a treatment leading to a lower BUR reduces pregnancy rates. This argument applies to cumulative live birth rate from 1 oocyte retrieval and not pregnancy rate per transfer because the surrogate is a measure of the embryo creation cycle. Blastocyst use rate is a critical measure of an intervention's effect on a patient's entire embryo cohort (89), and thus, an effect on embryo quality that reduces the overall cohort may not be detected by simply following the outcome of the first embryo transfer.

Numerous examples exist that illustrate the relationship between BUR and pregnancy outcomes (Table 1) (90). Chemical and physical features of embryo culture have been assessed in randomized controlled trials, providing examples of the causal relationship between BUR and pregnancy (90). These studies illustrate that the relationship between BUR and pregnancy rate is strong and 1-sided: a reduction in BUR has statistical inference for pregnancy or live birth rate, whereas an equivalent or increased BUR assures an equivalent pregnancy rate (91–125).

Table 1 (90) confirms that numerous laboratory interventions demonstrate a causal relationship for equivalence between blastocyst development and pregnancy rates (90, 126, 127). Although the review (90) included studies on the effects of light (128), features of the oil overlay (115), and pH of culture media (129) and the statistical inference held true, the studies did not include sufficient detail to be included in the summary. Importantly, to our knowledge, there are no examples where an intervention lowers pregnancy rates while not adversely affecting blastocyst development.

Comparing different culture conditions is the most studied laboratory intervention in IVF. Typically, the impact of a change has been assessed using blastocyst development as a primary endpoint and pregnancy outcomes as the secondary endpoints. Culturing in reduced (5%) vs. ambient (20%) oxygen yields more blastocysts and either equivalent or improved pregnancy rates (101, 103, 113, 130, 131). Application of a biphasic oxygen culture, where O_2 is changed from 5% to 2 % on day 3, did not change BUR or CPR (111). Culturing embryos in groups vs. individually similarly increases the number and quality of blastocysts while either not affecting or improving pregnancy rates (132, 133). Culturing at a reduced temperature (<37 vs. 37 °C) and in dry conditions (vs. humidified incubation) also impacts outcomes and can lead to fewer blastocysts and either equivalent (134) or reduced pregnancy rates (114).

The composition of culture media varies considerably among the many brands used for clinical IVF (135–137). Comparisons of the effect of culture media on outcomes

present a similar pattern to studies on culture conditions: an impact on blastocyst development with or without an impact on pregnancy rates (138). Few studies provide cumulative outcomes, making the direct link between embryo quality and pregnancy outcomes difficult. Only a single study demonstrated a reduced day 5 blastocyst rate with 1 culture medium that also corresponded to lower pregnancy rates for both the first transfer and cumulative rates (36).

The final feature indicating similar statistical inference to the endpoint is consistency of the effect across studies and populations. The studies presented in Table 1 (90) and in this discussion are from diverse research groups that consistently show that BUR provides statistical inference for equivalence, highlighting blastocyst development's significance in ART.

REVIEWING THE RISKS OF LONG-TERM EFFECTS OF GAMETE AND EMBRYO HANDLING IN VITRO

Although live birth with the absence of neonatal complications is the current standard endpoint for clinical trials in IVF, long-term impacts on health of ART-conceived offspring remain a crucial area of research (139–141). In fact, neonatal outcomes are important surrogate markers for potential long-term health because preterm birth, small for gestational age, and large for gestational age (LGA) are all associated with childhood and adult health (142–145). Offspring conceived through ART exhibit higher rates of preterm birth, small for gestational age, and LGA (146–151).

Assessing the risks associated with embryo culture and laboratory interventions in the context of neonatal outcomes remains an important area of research. Factors that may contribute to adverse outcomes include maternal/paternal age, underlying infertility, ovarian stimulation, laboratory conditions, and the uterine environment after embryo transfer (146). Underlying infertility plays a significant role in observed differences. For instance, birth defects are increased by 30% for ART offspring (152, 153) compared with 20% for offspring from subfertile women not requiring IVF or ICSI (154). Overall, the absolute increase in the risk of adverse outcomes is small, and a direct link between laboratory conditions has not been definitively established. A theoretical monitoring effect, whereby pregnancies and children resulting from ART are closely monitored and seek healthcare more frequently, has also been proposed (155). In general, IVF-conceived children are healthy and develop normally (140, 156).

Despite challenges in identifying causative factors amid underlying infertility, certain laboratory interventions, such as type of culture media (157), extended culture to the blastocyst stage (158), and embryo cryopreservation (151), have been associated with neonatal outcomes (159). Results remain inconclusive, however, with some studies reporting differences in birth weight associated with culture media (160, 161), whereas others find no significant impact (146). The incidence of LGA is higher after blastocyst and frozen embryo transfers (151), which has been attributed to the lack of a corpus luteum during programmed replacement cycles (162) and the duration of embryo culture (163). The impact of extended culture to the blastocyst stage remains an active area of debate (158, 164–166).

Longitudinal studies examining the impact of changes in clinical practice on birth weight provide evidence that culture media and duration of culture are unlikely to influence neonatal outcomes. In 2 studies from Boston tracking IVF outcomes over a 18-24 year period, birth weights and other neonatal outcomes remained unaffected by significant changes, such as oxygen concentration, culture media type (changing from a simple human tubal fluid solution to a single-step complete medium), and type of incubator (167, 168). A similar study in the United Kingdom found a gradual increase in birth weight over a 25-year period and an association between culture media type and live birth rate; however, no association was found between culture media and birth weight (168, 169). Regarding epigenetic causation, one of the culture medium studies observed deoxyribonucleic acid methylation differences at birth that resolved by the age of 9 years (159), whereas the second study found no difference in methylation status of newborns from the 2 culture media (170).

The theory that laboratory conditions during human ART have long-term consequences remains a question with insufficient high-quality evidence. Two main factors contribute to the hypothesis that conditions during preimplantation embryo development are linked to adverse neonatal and long-term outcomes: first, the epigenome undergoes extensive reprogramming after fertilization and before implantation (171); second, convincing evidence from animal models, such as ruminants (172) and mice (173), demonstrates the adverse impact of culture on epigenetic changes with enduring effects. The critical reprogramming that occurs during this stage of development, combined with evidence of sensitivity in various species, makes it intuitively appealing to suggest that a link to human parallels exist. A reported increase in imprinting disorders in ART-conceived children provides evidence for a direct cause and effect (174, 175). However, the theory remains unproven due to the absence of case reports providing clear evidence of a direct link and the general challenge of registry-based epidemiological studies for disorders with very low incidence (176).

Although clinical ART continuously strives to develop treatments that improve outcomes, instances where culture conditions were suboptimal or even embryotoxic are opportunities to determine whether poor conditions during preimplantation development can have adverse neonatal outcomes. For instance, as of 2014, most IVF clinics still cultured with atmospheric oxygen (177), yet there is no significant effect of the oxidative environment on offspring when culturing at atmospheric oxygen vs. 5% oxygen (178). Similarly, the metabolic stress of culture in a simple salt solution—what would now be considered a suboptimal culture medium (e.g., Fujifilm Irvine's HTF)—has not shown adverse effects on birth weight (157, 167). Pregnancies from cycles where poor air quality reduced available blastocysts have not produced case series or reports on adverse outcomes (179). Additionally, several recalls of embryotoxic mineral oil containing

peroxides have occurred in the past 2 decades (180, 181) and likely more cases where affected lots were used but not recalled, with no reports of long-term adverse outcomes.

The absence of severe impacts from adverse culture conditions aligns with the all-or-none hypothesis of embryo toxicity, suggesting either that the preimplantation embryo succumbs to poor conditions and does not result in a live birth or that the preimplantation embryo survives without long-term effects on offspring (182). This hypothesis is based on the concept that teratogenicity occurs later in development, during organogenesis (183). Although reassuring, further investigation is needed to fully understand the implications of this hypothesis.

In summary, although suboptimal culture conditions influence preimplantation embryo viability, evidence suggests that laboratory factors do not significantly impact the health of offspring conceived through ART. Continued research will provide a more complete understanding of possible long-term effects and direct mitigation of any potential risks associated with ART procedures.

SURROGATE ENDPOINTS FOR DEVELOPMENT OF DEVICES AND BIOLOGICS

Innovation stands as a pivotal force in enhancing the success rates of IVF procedures (184) and streamlining laboratory operations for heightened efficiency and more consistent quality. Although culture media have seen minimal evolution in recent decades (136, 137, 185), potential improvements, such as the incorporation of growth factors (186, 187), could address existing gaps. Similarly, despite the introduction of time-lapse incubators (188), many ART laboratory procedures remain manual and lack standardization.

Various innovations drive the need for distinct and appropriate endpoints for safety assessment. Notably, interventions targeting gene transcription, such as the addition of growth factors or cytokines (189), demand rigorous testing due to potential epigenetic implications. For instance, the inclusion of serum in preimplantation bovine embryo culture has led to well-documented cases of large offspring syndrome (190), emphasizing the need for stringent evaluation of potent transcription-altering factors in culture media. Although some advancements, such as the incorporation of granulocyte-macrophage colony-stimulating factor, have undergone thorough randomized controlled trials with associated live birth rates (187), others, such as antioxidants, have been introduced on the basis of clinical trials using ongoing pregnancy as the endpoint (191).

In addition to optimization of culture conditions, automation of laboratory procedures holds promise for enhancing efficiency and standardization, potentially assessed using surrogate endpoints such as blastocyst development. The lengthy and hands-on nature of embryologist training limits the workforce available to meet increasing demand. Automation of critical laboratory steps could simultaneously improve reproducibility while significantly reducing training time (and possibly processing time) and facilitating standardization and improved quality of care. Several automation initiatives are underway, encompassing procedures such as oocyte retrievals (192), cumulus cell removal (193, 194), ICSI (195, 196), embryo culture, and oocyte and embryo cryopreservation (27, 197).

Blastocyst development emerges as a feasible surrogate endpoint for automation due to several factors. The variability in laboratory procedures underscores the adaptability in gamete and embryo manipulation techniques. Automation aims to replicate human actions while maintaining optimal conditions, using components already prevalent in the laboratory setting. Many of the components are already in use in the laboratory, and those that are not can be tested with well-validated embryo toxicity assays (198).

An example of the inherent heterogeneity in the IVF laboratory and the utility of blastocyst development as a surrogate endpoint is the relatively recent evolution and broad application of oocyte freezing. Early success was achieved with slow freezing protocols (199). Since then, vitrification has become standard for both oocytes and embryos (7). During the past 30 years, countless variations of cryoprotectants (200, 201), temperature (202), exposure times (203–205), and devices (202, 206, 207) have been developed and used, most using oocyte survival, fertilization, and embryo development as surrogate endpoints. A recent study even demonstrated that only 2 minutes is needed for equilibration before vitrification (208), a major change from the long-held belief that at least 10 minutes is required for complete equilibration of vitrification solutions (209). The improvement in efficiency for the laboratory is driving a rapid change in clinical practice, and commercially available products that incorporate these changes are now available, all on the basis of predicate applications.

Establishing the safety of materials is a necessary first step, followed by demonstrating that automated processes yield comparable blastocyst development rates, quality, and speed as manual techniques. Given the strong correlation between blastocyst development and pregnancy outcomes, efficacy confirmation using this surrogate endpoint paves the way for broader approval and adoption of automated processes in ART laboratories. Continuous monitoring of pregnancy and birth outcomes remains essential to ensure ongoing safety and efficacy.

REASONS FOR CAUTION WHEN CONSIDERING BLASTOCYST RATE AS A SURROGATE FOR PREGNANCY

Although blastocyst development demonstrates promising correlations with pregnancy outcomes, several factors warrant consideration when evaluating its suitability as a surrogate endpoint for clinical trials and regulatory approval. The relationship between blastocyst formation and successful pregnancy is influenced by multiple factors beyond the developmental competence captured by blastocyst rate alone. Euploid blastocyst implantation rates typically range from 50% to 70% (210, 211), indicating a significant gap between blastocyst formation and pregnancy success. Factors that contribute to this gap include embryonic factors beyond standard morphological assessment (210), maternal factors such as endometrial receptivity (212), paternal contributions to

embryo competence (213, 214), and various clinical and laboratory variables (210). These additional determinants of pregnancy success highlight the complex nature of implantation that may not be fully captured by blastocyst development metrics.

The methodology of studies evaluating extended culture also affects outcome interpretation. Reports analyzing results on a per-transfer basis may yield different conclusions than intention-to-treat analyses that include all cycle starts (215). Cycle cancellations due to poor embryo development or lack of blastocyst formation are important outcomes that should be considered when evaluating the overall impact of laboratory interventions. Regulatory frameworks should account for how study design influences the apparent predictive value of blastocyst development and ensure that selection bias does not lead to overestimation of clinical efficacy.

Laboratory context also plays a significant role in blastocyst development outcomes. Studies have shown that blastocyst formation rates vary across laboratory settings despite similar patient demographics (81, 216). These variations arise from differences in culture systems, incubation conditions, and technical expertise. When evaluating blastocyst rate as a surrogate endpoint, the transferability of findings across different laboratory environments merits attention to ensure that regulatory decisions on the basis of blastocyst outcomes will translate reliably across diverse clinical settings.

Despite these considerations, blastocyst development remains a valuable biomarker of embryo competence with demonstrated correlation to pregnancy outcomes. When considering its role as a surrogate endpoint for regulatory purposes, these additional factors provide important context for determining the appropriate framework. Although initial regulatory decisions may reasonably rely on equivalent blastocyst quality and numbers as an endpoint, postapproval surveillance and/or additional postapproval studies may be an appropriate means to expeditiously approve new technologies while more carefully confirming safety and efficacy with larger data that can be obtained in a typical phase II/III study. This balanced approach may leverage blastocyst rate as an initial indicator of efficiency and safety for laboratory innovations, particularly those aimed at standardization and automation, while maintaining appropriate monitoring of clinical pregnancy and live birth outcomes to confirm that laboratory improvements translate to meaningful benefits for patients.

SUMMARY

Innovation in ART holds tremendous potential for enhancing overall success rates, streamlining laboratory operations and improving access to treatment. From advancements in culture media to the automation of laboratory procedures, these innovations aim to address existing gaps in efficiency and quality. However, ensuring the safety and efficacy of these innovations requires meticulous evaluation, leveraging surrogate endpoints such as blastocyst development. Blastocyst development is a promising surrogate endpoint for assessing both the safety and efficacy of automated processes in ART laboratories. As ART continues to evolve, ongoing monitoring and evaluation remain crucial to ensure the safety and effectiveness of these innovations in improving fertility treatments.

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Dean E. Morbeck: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Michael P. Diamond:** Writing – review & editing, Writing – original draft, Formal analysis.

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During the preparation of this work the authors used Anthropic's Claude to provide critical editing of grammar and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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