Embryo implantation: biology, evaluation, and enhancement

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Purpose of review
Implantation is an essential step in the development of a pregnancy, but often fails in humans. In assisted reproductive technologies, implantation failure continues to impair treatment outcomes, with distressing results for patients and physicians.

Recent findings
Morphokinetics, comprehensive chromosome screening, and the analysis of embryo-derived products detectable in spent culture media offer new means of assessing embryo viability. However, all await validation in randomized controlled trials. Genomic, transcriptomic, and secretomic technologies are similarly being exploited to define specific biomarkers of endometrial receptivity with the aim of identifying novel therapeutic interventions. However, to date no single, clinically relevant molecular marker capable of indicating endometrial receptivity has been reported. Recent work continues to describe the key signalling pathways which result in acceptance or rejection of the implanting embryo. In-vitro studies have revealed that the decidualized endometrium plays an important role in natural embryo selection, which could change our understanding of the aetiology and treatment of reproductive failure.

Summary
Recent developments in analytical techniques have initiated a search for biomarkers of embryo quality and endometrial receptivity, and in-vitro studies have revealed novel roles for the decidualized endometrium as a biosensor of embryo quality.

Keywords
embryo, endometrium, implantation, receptivity

INTRODUCTION

Implantation is an essential step in the development of a pregnancy. It depends on three factors: the quality of the embryo, the receptivity of the endometrium, and the communication between the two. The embryo must be viable and present in the uterine cavity during the putative receptive window of implantation (WOI), which is limited to the mid-secretory phase. Subsequently the embryo and endometrium need to accomplish a complicated cross-talk involving numerous cytokines, growth factors, proteins, receptors, and mediators, so the embryo can adhere and then pass through the endometrial lining and invade into the stromal layer. In the human, this process frequently fails, and this is considered to primarily reflect the high rate of chromosomal abnormalities observed in human embryos.

Although important steps have been made towards increasing our knowledge of implantation, several key factors remain to be elucidated before we really understand this enigmatic process. The high rate of implantation failure occurring after the transfer of a morphologically good quality embryo remains a source of frustration for patients and their carers. The availability of large-scale analytical techniques has led to a search for biomarkers of embryo quality and endometrial receptivity. These biomarkers may improve both embryo selection and point to new tools to enhance receptivity,

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KEY POINTS

- Implantation often fails in the human. Recently, it has been shown that this may represent the appropriate prevention of nonviable embryos from implanting, which when disturbed may result in reproductive failure.
- Morphokinetics, CCS, and secretomic approaches are improving embryo selection, but their clinical value is not yet proven.
- Novel methods of assessing endometrial receptivity are revealing new potential pathways for intervention. RCTs of many of these new approaches, with live birth rate as main outcome, are awaited.

thus increasing the effectiveness of fertility treatments.

In this review, recent concepts in the biology of implantation, new developments in embryo quality assessment, and on markers of endometrial receptivity will be described, together with current approaches to optimize the chance of successful implantation in assisted reproductive technology (ART).

NEW CONCEPTS IN THE BIOLOGY OF IMPLANTATION

A review of the biology of implantation is beyond the scope of this article. However, current clinical interest is focused on the role of uterine natural killer (uNK) cells, and novel roles ascribed to the decidualized endometrium as an embryo biosensor.

Recent human data derived from in-vitro modelling suggest that the decidualized endometrium is capable of rejecting incompetent embryos and, therefore, plays an important role in the natural selection of good quality embryos [1–3]. In reaction to signals from the embryo, endometrial stromal cells tend to migrate towards a developing embryo, whereas this is not observed in presence of an arrested embryo. In women with recurrent miscarriage, the interval between clinical pregnancies is shorter and recent work has shown this is associated with impaired-cyclic decidualization [4▪]. It is proposed that this underlies reduction in discrimination between good and poor quality embryos [5], allowing poorly viable embryos to implant long enough to present as a clinical pregnancy and miscarriage, rather than being lost in the early preclinical phase.

uNK cells are highly specialized components of the decidualized endometrium which produce a number of pro and anti-inflammatory cytokines considered to play an important role in implantation. Higher numbers of uNK cells have been described in different cases of pregnancy failure, including implantation failure. Although early studies reported that certain populations of uNK cells are increased in women with pregnancy failure and implantation failure, this has not always been confirmed [6] and numbers appear to be subject to significant cycle to cycle variation [7]. Although risk interpretation models have been developed in which uNK subset counts seem predictive of pregnancy outcome [8], the clinical impact of therapeutic interventions aimed at modulating NK subsets remains to be demonstrated.

EVALUATION OF EMBRYO IMPLANTATION POTENTIAL

The cornerstone of embryo selection remains the assessment of morphological characteristics, which correlate with viability of the embryo. This approach is well tolerated, noninvasive and does not require sophisticated and expensive technologies. However, it is based on the inherently 'subjective' analysis of snapshot views. The development of time-lapse imaging has created the possibility to follow embryo development over time, opening the new field of morphokinetics. Systems are now available which enable 24 h video monitoring, removing the need to displace the embryo from its ideal culture environment for assessment [9]. A number of studies using this technique have been performed aimed at identifying novel morphokinetic variables predictive of embryo viability [10–16]. Duration of the first cleavage stage, duration of the two and three cell stage, and the time from intracytoplasmic sperm injection (ICSI) to five cell stage were parameters identified as predictors of reaching blastocyst stage [12–14]. Although more prospective data are awaited, pregnancy outcomes appear to be improved in patients for whom embryos were selected with the time-lapse monitoring, rather than standard morphological analysis [17].

Human embryos show a high rate of chromosomal aneuploidy, and this is considered to be the most important cause of implantation failure [18]. Comprehensive chromosome screening (CCS) techniques like single nucleotide polymorphisms arrays, quick polymerase chain reaction analysis, and comparative genomic hybridization (CGH) arrays can provide a complete picture of the genetic blueprint of the cell studied, and have replaced fluorescence in situ-hybridization (FISH) which only enabled analysis of a limited number of chromosomes. CGH analysis can be performed on

polar bodies, single blastomeres from cleavage stage embryos, and trophectoderm cells from blastocysts. As blastocysts show less mosaicism than cleavage stage embryos [19], and biopsy seems less harmful to embryo viability at the blastocyst stage [20▪], this approach is becoming more widespread, and pregnancy results after trophectoderm biopsies appear promising [21–23]. Using this approach, aneuploidy was detected in 45% of blastocysts in IVF populations [24] and in 60% of blastocysts from patients experiencing recurrent implantation failure (RIF) [25]. The high aneuploidy rate among biopsied blastocysts highlights the inherent imprecision of embryo transfer when conventional morphology evaluation is used. When the outcome of CCS was not used for embryo selection, but retrospectively linked to IVF outcome, 96% of the aneuploid-predicted embryos failed to implant, compared to 41% of the euploid embryos [26].

Using FISH to select embryos did not prove any benefits on pregnancy rate [27]. As with early FISH studies, preliminary reports using CGH analysis show improvement of pregnancy rate [24,28,29]. Retrospective studies have shown an ongoing pregnancy rate after CGH analysis and single embryo transfer of 55%, compared to 42% in the morphology-only group ($P < 0.01$) [24]. A small prospective randomized controlled trial (RCT) reported an ongoing pregnancy rate of 69 versus 42% in controls ($P = 0.009$) [28]. A significant reduction in miscarriage rate is also being reported. Very recently, the first study appeared in which morphokinetics were linked to ploidy testing of an embryo. Aneuploid embryos were delayed at the initiation of compaction, initiation of blastulation, and the time to reach full blastocyst stage compared with euploid embryos [30]. However, additional studies are needed to verify these pilot data and confirm the place of both morphokinetics and CGH assessment of ploidy in IVF/ICSI treatment.

A further means of assessing embryo viability is to measure its metabolic activity [31,32]. A lower metabolic turnover has been reported in viable developing embryos, whereas a struggling nonviable embryo has been shown to exhibit a high rate of metabolism as it seeks to repair and survive [31]. This metabolically 'quiet' embryo hypothesis forms the basis for noninvasive means of selecting embryos on the basis of the molecular footprint they leave in their culture media.

Combining genomic and secretomic information, an initial protein secretome study reported significant differences between individual euploid and aneuploid blastocysts [33]. Nine novel candidate biomarkers correlating with chromosome aneuploidy were then identified, with lipocalin-1 marked as the first potential biomarker for noninvasive aneuploidy screening [34]. Culture medium analysis by mass spectrometry identified implanting and nonimplanting embryos with 100 and 70% accuracy, respectively [35]. However, in a Dutch RCT, live birth rate was not significantly different in the group in which embryos were selected on metabolic profile of the culture media using near infrared spectroscopy, compared to morphology only [36]. Despite this the search for the best predictive markers of the embryo's 'implantation potential' and chromosomal normality is going on in full speed. Recently, oocyte quality markers in the follicular fluid have been investigated to search for signals of predictive viability of the evolving embryo [37]. Although potentially helpful in improving selection of the single embryo with best implantation potential, no selection technique actually improves embryo quality or the chance of implantation for a specific embryo. This will require improvement in culture media and endometrial receptivity.

EVALUATION OF ENDOMETRIAL RECEPTIVITY

The putative WOI represents a period of just 4–5 days, in which the endometrial tissue acquires the functional receptive status that permits embryo adhesion and invasion. Genomic, transcriptomic, and secretomic technologies are assisting in teasing out the complex molecular pathways involved, defining specific biomarkers and profiles of receptive endometrium. However, to date no single, clinically relevant molecular marker capable of indicating endometrial receptivity has been identified. Indeed, it is likely that a high degree of molecular redundancy is present, and that profiles of a series of markers will be of more clinical value than a single marker.

Many transcriptomic studies of the endometrium have been published up till now and these are reviewed elsewhere [38▪]. In a review of six studies in which early secretory and mid-secretory expression profiles were identified, the consistency of genes found to be representive of the receptive endometrium was poor. Indeed only two genes were shared [osteopontin and interleukin 15 (IL 15)], whereas 770 distinct genes were identified by the individual groups [39▪]. Diverse biopsy methods, variance in micro-array assays and laboratory conditions, as well as variety in populations could be the reason. This inconsistency may also represent differential expression in the various cellular compartments of the endometrium. This issue has

recently been addressed by a laser microdissection study of glandular and stromal cell gene expression. Micro-array analysis showed distinct RNA expression profiles, each dependent on the day of the cycle [40].

Only one group has sought to translate endometrial transcription analysis into a commercially available test of receptivity. The endometrial receptivity array uses an array of 238 genes to describe the receptive endometrium. Although prospective controlled studies demonstrating its value in a clinical setting are awaited, reproducibility appears high, and when used to assess receptivity in women with RIF after ART, those who underwent embryo transfer after a 'receptive diagnosis' demonstrated a 38% implantation rate [38▪].

The limitations of tissue-based invasive genomic approaches may be overcome by analysing the molecular content of uterine secretions [41]. Two studies have indicated that aspiration of endometrial fluid immediately before embryo transfer does not interfere with implantation [42,43]. This approach provides the opportunity to link the mediators present around the time the embryo arrives in the womb directly to implantation outcome. Multiplex immunoassays have identified IL-1β and tumour necrosis factor-α (TNF-α) [44] and vascular endothelial growth factor (VEGF) and proprotein convertase type 6 (PC6) as promising markers [45,46]. However, results from different studies using the more broad high-throughput proteomic analysis are yet to identify a reproducible protein profile [47–49]. The variance is explained by differences in sample selection and methods, similar to discongruence observed in genomics studies. Proteomics and secretomics have been combined, identifying known decidualization markers and new biomarkers [50]. This is the first step towards a more integrated method to find key markers of a receptive endometrium. The systems biology approach further integrates genome expression analyses of human embryos and human endometrium cells to identify the specific gene expression profiles and the biological pathways activated during the implantation window [51,52].

CLINICAL INTERVENTIONS AIMED AT IMPROVING IMPLANTATION

The high proportion of IVF cycles which fail to result in pregnancy despite the transfer of apparently viable embryos has led to the widespread adoption of adjuvant therapies aimed at improving endometrial receptivity and implantation rates. However, the underlying rationale is often weak and clinical evidence for their efficacy mostly lacking. One example is the administration of glucocorticoids, which has been proposed to improve the intrauterine environment by acting as immunomodulators on uNK cells and cytokine expression. The recently updated Cochrane meta-analysis shows no such benefit on live birth or pregnancy rate in IVF/ICSI treatments [53]. Although a positive effect of glucocorticoid administration is seen in a subgroup analysis of IVF patients (rather than ICSI) [odds ratio 1.5, 95% confidence interval (CI) 1.05–2.13], the studies meeting the criteria for inclusion were small. Further well designed randomized trials are required to reveal if this therapy has effect in well defined IVF populations.

The use of aspirin or heparin on IVF outcome has been similarly debated. Heparin is suggested to cause modulation of growth factors, cytokines, and adhesion molecules important in blastocyst adherence and invasion, whereas aspirin is proposed to act by reducing vasoconstriction in the decidualized endometrium, favouring implantation. However, in a systematic review including five small RCTs on heparin therapy, no benefit on pregnancy or live birth rate was observed [54]. A recent systematic review on aspirin treatment in ART analysed 17 RCTs. Meta-analysis led to the conclusion that aspirin therapy did not show a substantial efficacy on the live birth after IVF/ICSI [55].

In contrast to this litany of apparently rational but ineffective interventions, attention has recently turned to a treatment which appears irrational but does appear effective. Therapeutic endometrial injury by endometrial biopsy, hysteroscopy, or curettage has been shown to increase the chance of embryo implantation in a subsequent cycle. Two systematic reviews including meta-analysis have been published. The first included six RCTs, and showed a beneficial effect of the intervention on clinical pregnancy rate [relative risk (RR) 1.86, 95% CI 1.46–2.38] [56▪▪]. The second, a meta-analysis in women with RIF, included two RCTs and two non-randomized studies on endometrial biopsy, which also showed a positive effect on clinical pregnancy rate (RR 2.32, 95% CI 1.72–3.13). The three hysteroscopy studies revealed a benefit as well [57] as did the two studies reporting on live birth rate [58,59]. The underlying mechanism by which endometrial injury enhances endometrial receptivity remains unclear. It is hypothesized that injury could improve decidualization, or cause a wound-healing effect which is associated with a significant increase in cytokine secretion introducing an environment more suitable for embryo implantation; injury may alternatively act to ameliorate the advanced

maturation caused by an ovarian stimulation in ART cycles which can render the uterus less receptive to the transferred embryo.

CONCLUSION

Implantation remains the rate-limiting step in conceiving a pregnancy, either spontaneously or after IVF. However, new tools are being developed which are likely to open the black box of human implantation failure. Morphokinetics, CCS, and analysis of embryo-conditioned media are providing new information, which may improve embryo selection in IVF. Moreover, the molecular and functional characteristics of the receptive and appropriately selective endometrium are now being elucidated.

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Conflicts of interest

There are no conflicts of interest.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 340).

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