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Minireview

The Human Endometrium as a Sensor of Embryo Quality[1]

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ABSTRACT

Human reproduction is characterized by a high degree of embryo wastage, which is largely ascribed to a high prevalence of embryo aneuploidy. It is proposed that maternal strategies have evolved that prevent inappropriate investment in invasive, but poorly viable embryos. Key to this is the emerging concept of the endometrium as biosensor, first identified in human in vitro embryo/decidualized stromal cell coculture systems and recently confirmed in an in vivo mouse model. In this review, the growing supporting experimental evidence for the biosensor component of decidualized endometrium is outlined, and recent insights into the nature of the embryo-derived signal detected by the endometrium and the biological processes by which this signal is thought to be converted into a go or no-go endometrial response are described. Finally, the clinical implications of this new paradigm of the choosy uterus are addressed.

decidualization, embryo, endometrium, implantation, selection

INTRODUCTION

Compared with other mammalian species, human reproductive efficiency is not impressive. The probability of achieving a pregnancy within one menstrual cycle, defined as the monthly fecundity rate (MFR), is 20%–30%. In contrast, the MFR may be as high as 80% in baboons and 90% in rabbits [1–3]. Those concerned about the impact of the rising population on the Earth's resources may find some reassurance in this observation, but for couples suffering from the distress of reproductive failure and their clinicians it is an exercising source of frustration. Progress in assisted reproductive techniques has led to the majority of couples undergoing in vitro fertilization (IVF) treatment obtaining embryos for transfer, but only around half implant, and up to half of these are lost soon after [4]. However, this high rate of peri-implantation loss is not solely a feature of assisted conception treatment. The first indications of the high peri-implantation attrition that characterizes human reproduction were revealed by Hertig and colleagues 1950s exploration of 210 postovulation hysterectomy specimens where only 34 yielded fertilized ova, good, bad, and indifferent [5]. The development of sensitive urinary human chorionic gonadotropin (hCG) assays led to the landmark study of Wilcox et al. [6] in which serial urinary samples collected over 6 mo or until a positive pregnancy test were collected from 221 women trying to conceive. Urinary hCG measurements revealed that implantation occurred in 19% of cycles, but more than a third were then lost, a majority before any clinical sign of conception had presented. When considered in conjunction with subsequent studies, it has been estimated that around 50% of human conceptions fail to progress to an ongoing pregnancy [7]. A greater understanding of the etiology and teleology of peri-implantation loss is key to improving reproductive outcomes, but human studies are marred by ethical and technical challenges. However, a combination of informative animal models and novel in vitro systems is beginning to throw new light on what has previously been considered the implantation ''black box''.

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THE NEED TO BE DISCERNING

The exceptional rate of early pregnancy loss that characterizes human reproduction is now thought to derive from two key features of human embryos: their intrinsic invasiveness and their high prevalence of chromosomal abnormalities. Genome-wide screening of individual blastomeres taken from high-quality cleavage-stage embryos IVF has shown that around 70% have complex chromosomal abnormalities, mostly arising from mitotic rather than meiotic nondisjunction [8–10]. This rate of aneuploidy, estimated to be at least 10 times greater than that observed in other mammalian species, appears to decrease during development to the blastocyst stage. Fluorescent in situ hybridization analysis of 10 chromosomes in individual blastomeres from viable human IVF embryos has shown that the prevalence of aneuploidy decreases from 83% on Day 4 of development to 42% on Day 8 [11]. Consistent with these findings, microarray comparative genomic hybridization analysis of 5 to 10 trophectoderm cells from 1046 blastocysts identified a total of 1113 distinct aneuploidies in 608 (58%) embryos [10], emphasizing that peri-implantation human embryos are intrinsically chromosomally diverse and predominantly mosaic.

Such observations raise a number of pressing questions. What is the biological significance of this high prevalence of aneuploidy and mosaicism, and what is the evolutionary advantage conferred by embryo aneuploidy? Remarkably little attention is given to these questions, perhaps because the prevailing thinking in assisted reproductive technology circles remains that the optimal embryo for transfer must be uniformly diploid.

An obvious teleological explanation for the genetic diversity of human embryos is that it renders implantation events intrinsically adaptable to a changing ecology, which arguably is essential for procreation and thus survival of a species that exhibits relatively few implantation events over their lifespan. In this context, emerging data are challenging the view that aneuploidy is uniformly detrimental for cells and organisms. For example, a variety of stress signals can induce chromosomal instability in budding yeast, but, importantly, the resultant aneuploidy then drives rapid phenotypic evolution and confers stress resistance [12, 13].

Considering that the cytogenetic anomalies in human embryos resemble closely those found in cancer cells [9], it is also not inconceivable that a degree of genomic instability in embryos may confer an implantation advantage or even compensate for loss of key nidation genes [14]. If so, it follows that the perfectly diploid human blastocyst may have maximal developmental potential but perhaps lack intrinsic implantation competence, not unlike mouse embryos. These conjectures clearly require further testing. However, it is incontrovertible that the rather peculiar human embryo imposes an important reproductive challenge: how to facilitate implantation while simultaneously safeguarding the mother against prolonged investment in potentially developmentally abnormal embryos [15]. In recent years, experimental evidence has emerged indicating that spontaneous decidualization of the endometrium coupled to cyclic menstruation and regeneration represents an ingenious strategy to meet this challenge.

THE SELECTIVE DECIDUA

Endometrial decidualization, a process imperative for pregnancy in all species with invading embryos, is characterized by the transformation of stromal fibroblasts into secretory decidual cells [16]. In contrast to most species, the human endometrium decidualizes in response to endocrine rather than embryonic cues. Hence, decidualization is a feature of the midluteal phase in all ovulatory cycles, whether they lead to conception or not. The term decidua refers in part to the intrinsic ability of differentiated stromal cells to self-destruct and slough off in response to falling progesterone levels, which in turn accounts for cyclic menstruation in women [17–19]. Duplication of an ancestral β-luteinizing hormone gene enabled human embryos to avoid guaranteed maternal destruction by ensuring continuous ovarian progesterone production in the early stages of pregnancy through the production of beta hCG [20]. Hence, decidual cells are a priori programmed to select against embryos that are perceived to lack fitness because of insufficient hCG production. Similarly, the transition from histiotrophic nutrition of the early conceptus to active maternal perfusion of the placenta toward the end of the first trimester of pregnancy causes dramatic changes in local oxygen tension and production of free radicals [21, 22]. Arguably, this transition could be viewed as imposing a stress test on the feto-maternal interface, thus increasing the likelihood that a failing pregnancy is disposed of in a timely manner. From both an evolutionary and clinical perspective, the later the pregnancy is lost in gestation, the greater the harm to maternal fitness and health.

While the mechanisms outlined above may account for clinical miscarriages, they do not explain the high attrition rate during the peri-implantation period. They also do not explain why complex aneuploidies and large-scale complex chromosomal errors that are prevalent in preimplantation embryos have rarely been reported in miscarriage tissues [10]. Recently, additional sophisticated and dynamic functions have been uncovered that position the decidua as the key determinant of successful implantation and as the active component of the maternal strategy to cope discerningly with genomically unstable blastocysts.

The first evidence supporting the concept of active human embryo selection at implantation emerged from coculture studies, consisting of single hatched human blastocysts cultured for 3 days on a layer of primary human endometrial stromal cells that had been decidualized with a cyclic AMP analog and medroxyprogesterone acetate [23]. While it might be considered unlikely that 50 000 decidualizing cells would produce a measureable response to a single human embryo, profound changes in the production of a number of key cytokines and growth factors were observed. Moreover, the nature of this response was opposite to what had been expected. The pervasive embryo-centric paradigm predicted that developmentally competent embryos would signal their viability to the decidualized stromal cells, which would respond by up-regulating production of proimplantation modulators, such as interleukin-1 beta (IL-1β), heparin-binding EGF-like growth factor (HB-EGF), or leukemia inhibitory factor (LIF). However, in comparison to control cultures, high-grade embryos had little impact on the supernatant concentrations of 14 factors measured by multiplex immunoassay. By contrast, embryos that showed morphological signs of developmental impairment elicited a strong response in decidualizing cells, characterized by selective inhibition of IL-1β, -6, -10, -17, and -18, as well as eotaxin (CCL11) and HB-EGF secretion (Fig. 1). This putative biosensor function resides in the decidual phenotype because human embryos did not trigger a response when cocultured with undifferentiated endometrial stromal cells [23].

The mechanism underlying this novel concept of human embryo selectivity was unclear and difficult to study directly. To address these challenges, a study was designed in which decidualizing cells were exposed for 12 h to pooled conditioned medium from human embryos deemed insufficient for uterine transfer and high-quality embryos that resulted in an on-going pregnancy after single embryo transfer, thus providing unequivocal proof of their developmental competence. A third pool of unconditioned embryo culture media was applied as a control. Genomewide expression profiling uncovered only 15 decidual genes responsive to soluble signals from competent human embryos. In contrast, and consistent with the previously observed cytokine response [23], 449 maternal genes were deregulated in response to medium conditioned by poor-quality embryos. Gene ontology studies showed half of these genes to be associated with the broad biological process of transport, translation, and cell cycle regulation [14].

This concept of the decidualized endometrium responding more profoundly to the developmentally incompetent than the competent embryo is consistent with previous studies asserting that the healthy embryo, needing only to invest energy in growth and development, is metabolically quiet in comparison to less viable embryos that must additionally engage in repair and apoptosis [24–26]. This concept introduces the possibility that an embryo may be so quiet that it fails to trigger any

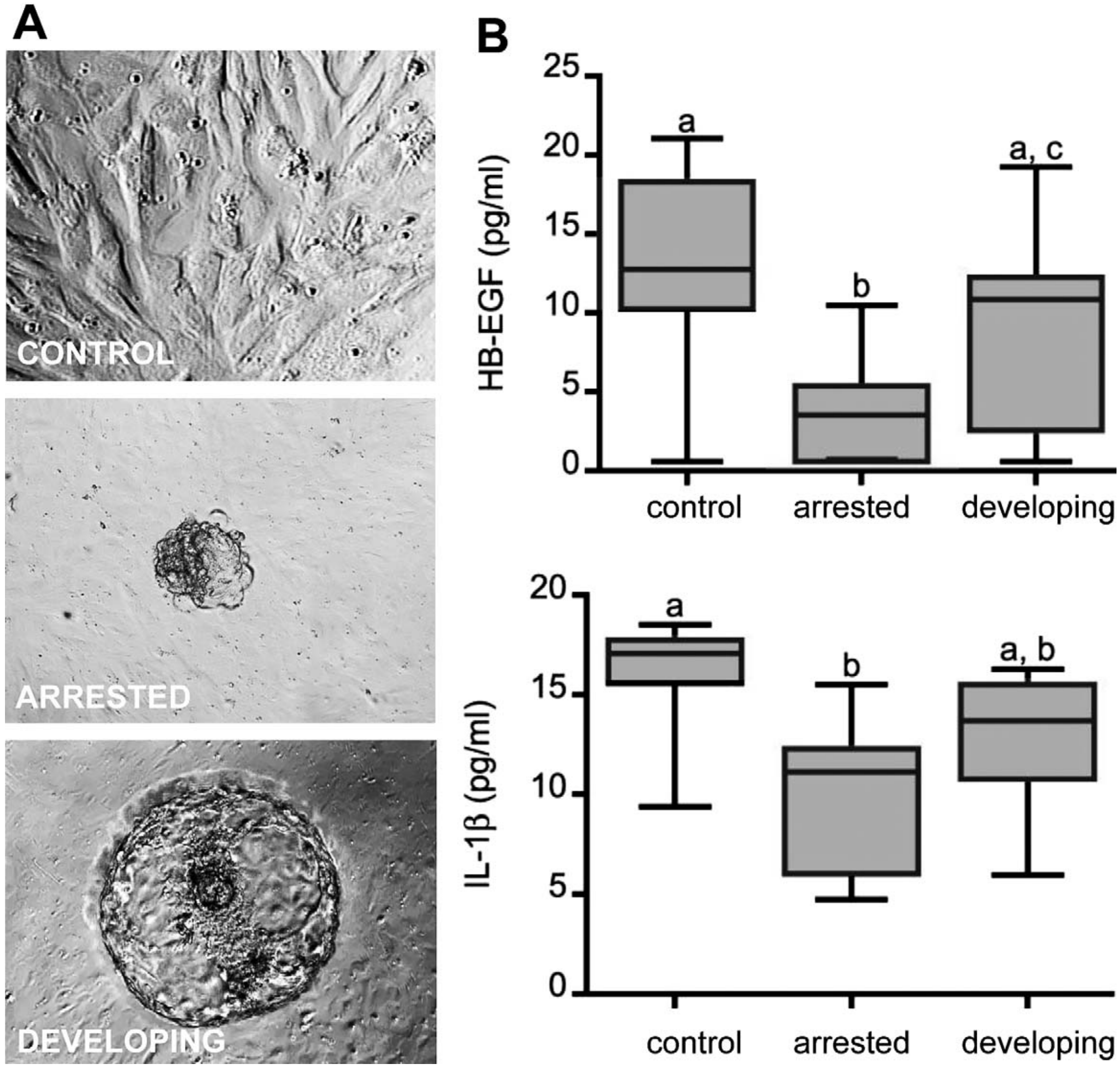

FIG. 1. **A**) Primary human endometrial stromal cells were first decidualized for 5 days and then cocultured with human embryos (magnification ×40) or not (control, magnification ×100). Over the 72-h coculture period, 30 embryos arrested (arrested) whereas 11 continued to develop normally (developing). **B**) Analysis of the culture supernatants revealed that the presence of an arresting embryo inhibited the secretion of a number of factors. Data for HB-EGF and Il-1β are shown (median and interquartile range: box = 25%–75%, whiskers = 10%–90%) The letters above the box plots indicate significant differences between groups ($P < 0.01$). Adapted from Teklenburg et al. [23] with permission.

supportive maternal response, a hypothesis that would be consistent with the conjecture that some degree of aneuploidy may confer certain advantages to the implanting embryo. The converse notion to that of the high-quality, quiet embryo is that of the struggling, metabolically noisy embryo producing a source of putative signals to which the decidualized stroma responds by deregulating a repertoire of genes implicated in implantation.

In response to signals present in conditioned media derived from impaired human embryos, the most down-regulated gene in the array analysis of exposed decidualized stromal cells was *HSPA8*, which encodes a ubiquitously and constitutively expressed member of the heat shock protein 70 family of molecular chaperones involved in protein assembly and folding [27]. HSPA8 is a multifaceted molecular chaperone that can represent up to 1% of total cellular protein content. It is involved in clatherin-mediated endocytosis, assembly of multiprotein complexes, transport of nascent polypeptides, and regulation of protein folding. It is also a major regulator of chaperone-mediated autophagy [28, 29]. Knockdown of *HSPA8* in decidualizing cells compromised the secretion of two sensitive differentiation markers, prolactin and insulin-like growth factor binding protein 1, and induced endoplasmic reticulum (ER) stress [14]. In keeping with the role of HSPA8 as a pivotal sensor molecule for embryonic signals, transfection of a luciferase reporter that becomes activated under ER stress conditions confirmed that soluble signals from developmentally impaired human embryos induce a proteotoxic stress response in decidualizing cells.

These in vitro studies provided evidence for a putative mechanism by which the decidualized stromal cells sense developmentally compromised embryos. However, in order to elucidate whether this mechanism could be identified in vivo, the uteri of mice were flushed with naive or conditioned human embryo culture medium. Analysis of the uterine transcriptome confirmed the findings of the in vitro studies, with around six times as many genes showing altered expression in response to media conditioned by impaired versus competent human embryos [14]. Intriguingly, signals emanating from competent human embryos triggered a very specific transcriptional response in the mouse uterus, characterized by the induction of multiple metabolic genes. Moreover, in excess of 30% of

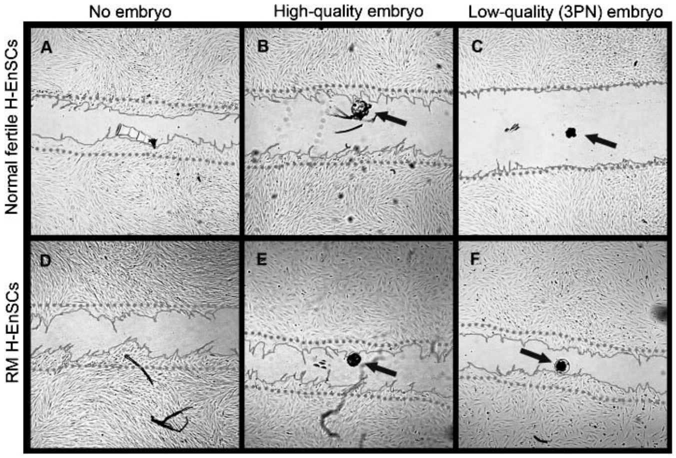

FIG. 2. The migration zone after adding a high-quality, low-quality, or no embryo. The migratory response of decidualized human endometrial stromal cells (H-EnSCs) from normally fertile (**A**–**C**) and RM women (**D**–**F**) was analyzed in the absence of a human embryo (**A** and **D**), in presence of a high-quality embryo (**B** and **E**), or a low-quality embryo (**C** and **F**). Phase contrast pictures were taken 18 h after creating the migration zone. The dotted line represents the front of the migration zone directly after its creation. As a reference for the position of the embryo, the bottom of the plate was marked. The arrows indicate the position of the embryo. All the pictures were taken with 25× magnification. (Reprinted from Weimar et al. [37]) with permission.

regulated genes coded for known implantation factors, including COX-2, cytochrome p450 26a1, and osteopontin. Hence, the endometrial response to embryonic signals in this model was not limited to negative selection but also demonstrated a component consistent with the established paradigm of a competent embryo evoking a supportive intrauterine environment [14, 30–32]. A dual-phase response of the endometrium can therefore be proposed consisting of recognition and selection. The ability of the luminal epithelium to recognize a high-quality embryo and modulate the decidual response it encounters on breaching the epithelium would aid subsequent nurturing and development in the postimplantation phase. This supportive implantation response is intrinsically lacking in the presence of a developmentally impaired embryo. However, chromosomal instability, invasiveness, and the inherent unpredictability of low-quality embryos could mean that many will breach the luminal epithelium but are then selected against and disposed of as a consequence of the ensuing decidual stress response.

EMBRYO SELECTION: THE ROLE OF DECIDUAL MIGRATION

Once the embryo has successfully breached the luminal epithelium, it is thought to continue its journey into the endometrial stroma as the active party, invading through what has been considered a mechanically passive decidual matrix. However, recent studies indicate that active decidual cell migration and encapsulation of the conceptus are integral steps in the implantation process. By placing human or mouse blastocysts on a monolayer of decidualized stromal cells, Grewal et al. [33] observed that stromal cell motility is obligatory for blastocyst implantation and trophoblast outgrowth. Moreover, time-lapse recordings revealed that the stromal cells moved around the embryo to accommodate its expansion [33, 34]. Decidualizing stromal cells have been shown to provide a more favorable matrix for trophoblast expansion than do undifferentiated cells [35], indicating that the choreography of stromal cell movement actively assists expansion. Further work showing trophoblast spheroids to expand to a greater extent when cultured on a monolayer of decidualized versus undifferentiated stromal cells provided more evidence supporting directed cell migration as a key element of the decidual phenotype [36].

Further confirmation for this comes from recent imaging studies, which revealed that decidualizing endometrial stromal cells are programmed to migrate toward implantation-competent blastocysts [37]. This chemotactic and invasive migration of endometrial stromal cells is triggered by signals emanating from the trophoblast, especially platelet-derived growth factor-AA (PDGF-AA) [36, 38–40]. Transcriptome analysis of trophectoderm cells from human blastocysts and matched endometrial biopsies confirmed that secretion of embryo-derived PDGF-AA occurs in tandem with increased expression of its receptor, PDGF-Rα, in endometrial cells [41]. Other local growth factors that may serve to fine-tune directed and nondirected migration of decidual cells at the implantation site include PDGF-BB and HB-EGF [39]. The active participation of the decidualized stroma in the encapsulation of the embryo illustrated by these studies raises the question as to whether invasion with its implications of offensive incursion is the appropriate term to describe this phase of human implantation. A more appropriate term that better reflects this closely choreographed pas de deux may be embedding.

A modified wound assay, consisting of a monolayer of decidualizing cells with a single human embryo placed in the migratory zone, has been used to further test the biosensor hypothesis. Weimar et al. [37] observed that the intrinsic propensity of decidual cells to migrate toward a conceptus was confined to high-quality human embryos and entirely inhibited in the presence of chromosomally abnormal tripronuclear embryos (Fig. 2). Taken together, these data indicate that decidual cells have the capacity to actively hinder invasion and outgrowth of abnormal human embryos that have breached the luminal epithelium.

EMBRYO-DERIVED COMPETENCE SIGNALS

How human embryos signal their developmental competence remains an unresolved question that if answered could have considerable clinical implications for embryo selection and modulation of endometrial receptivity. Recently, evidence has emerged from both human and murine studies that implicates embryo-derived serine-proteases as candidate signals. Elegant studies in the mouse uterus have demonstrated that trypsin produced by the implanting blastocyst cleaves and activates epithelial Na^+ channels present on the apical border of luminal epithelial cells (14, 42]. This leads in turn to an inward current, membrane depolarization, and Ca^{2+} entry into epithelial cells though voltage-gated L-type Ca^{2+} channels. The resultant Ca^{2+} transients then help promote phosphorylation and activation of transcription factor CREB, which in turn up-regulates COX-2 and leads to PGE2 release [42]. Conditioned medium from human embryos also trigger Ca^{2+} oscillations in human endometrial epithelial cells (EECs). Importantly, the Ca^{2+} transients induced in EECs by developmentally competent embryos (DCEs) are very short-lived, lasting approximately 5 min. In contrast, low-quality human embryos trigger prolonged and disorganized Ca^{2+} oscillations in EECs, leading to a uterine stress response (Fig. 3) [14]. The precise nature and origins of embryo-derived serine proteases are not clear. Furthermore, whether or not protease activity in embryo culture medium could lead to

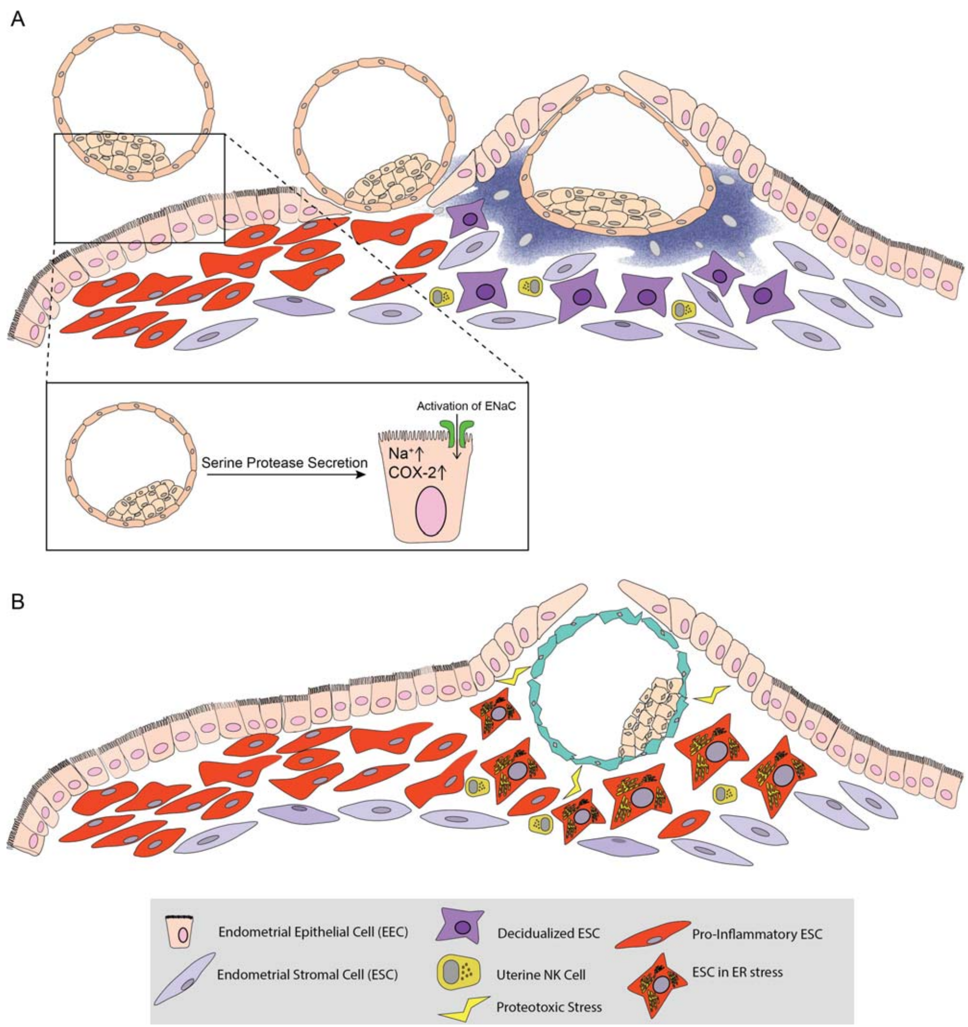

FIG. 3. Positive and negative mechanisms contribute to active selection of human embryos at implantation. **A**) Developmentally competent human embryos secrete evolutionarily conserved serine proteases that activate epithelial Na+ channel (ENaC) expressed on luminal epithelial cells (box), triggering calcium signaling and, ultimately, induction of factors involved in implantation and postimplantation embryo development, such as cyclooxygenase-2 (COX-2). In this model, the luminal surface epithelium amplifies and relays embryonic signals to the underlying stroma to optimize postimplantation development. **B**) By contrast, the luminal epithelium may not be much of a barrier for developmentally compromised embryos that are characterized by excessive protease production. However, expansion of the endoplasmic reticulum (ER) in underlying decidualizing cells renders them particularly sensitive to aberrant embryonic signals, leading to proteotoxic stress, active withdrawal of key decidual factors, tissue breakdown, and, ultimately, elimination of an unwanted conceptus.

improved selection of embryos for transfer warrants further investigation.

A REPRODUCTIVE TRADE-OFF: RECEPTIVITY VERSUS SELECTIVITY

A further test of the validity of the selective biosensor function of decidualized endometrium is to consider the likely clinical manifestations caused by decidual selection. A predictable consequence of a reduced ability to recognize embryonic signals would be increased frequency of implantation of impaired embryos that embark on a developmental trajectory destined to fail as a clinical miscarriage. Conversely, failure to respond to signals of high-quality embryos will result in a suboptimal environment for subsequent development and placenta formation, thus also increasing the risk of miscarriage.

If this supposition is correct, persistently impaired endometrial selectivity is predicted to result in recurrent early pregnancy loss in conjunction with paradoxical superfertility. This hypothesis was supported by a study on the time-to-pregnancy (TTP) intervals in 560 women presenting with a history of three of more consecutive miscarriages. Women with recurrent miscarriage (RM) were shown to be highly fecund, with TTP further decreasing in those with a history of five or more miscarriages. The observed incidence of achieving three or more pregnancies within 6 mo was much higher than would be predicted given a normal MFR of 20%. Indeed 40% of women with recurrent pregnancy loss could be considered superfertile when defined by a mean TTP of 3 mo or less. On the other hand, prolonged endometrial receptivity rather than impaired selectivity would also shorten the interpregnancy interval as well as the miscarriage rate by promoting out-of-phase implantation. This conjecture is also supported indirectly by clinical observations. For example, in the aforementioned study of Wilcox and colleagues [43], 84% of conceptions were detected between 8 to 10 days after ovulation. Among the 102 pregnancies identified on Day 9 after ovulation, 13% ended in an early pregnancy loss. This proportion rose to 26% with implantation on Day 10, to 52% on Day 11, and to 82% on Day 12 and beyond. Taken together, these clinical observations

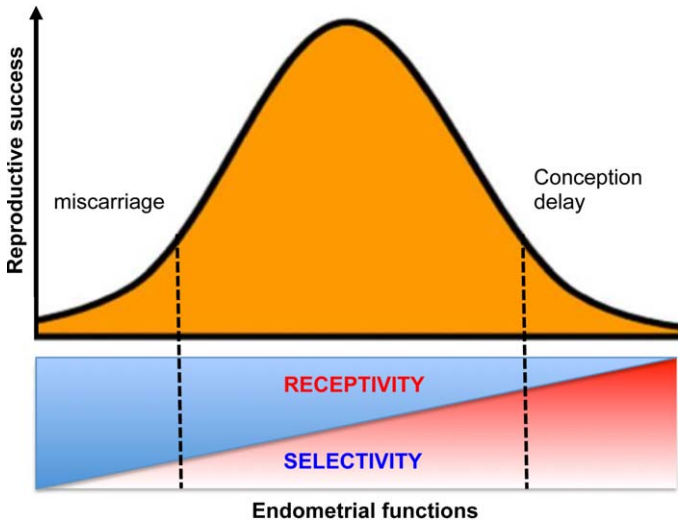

FIG. 4. Balancing endometrial receptivity versus selectivity. Human preimplantation embryos are intrinsically diverse. To be reproductively successful, the maternal endometrium must be receptive as well as selective, meaning acquiring the ability to mount a secretory response that is tailored to an individual embryo. The purpose of a tailored maternal response is either to support further development of high-quality embryos or to trigger early disposal of an unwanted conceptus. A prolonged or unopposed receptive phenotype will lead to miscarriage and all but the most developmentally competent embryos. Conversely, premature or excessive decidualization will increase the barrier function of the endometrium and reduce the likelihood of pregnancy. Notably, as maternal age advances, the incidence of embryonic aneuploidy increases, thus requiring a greater need for endometrial selection in order to avoid recurrent pregnancy failure.

indicate that unrestrained endometrial receptivity and lack of embryo selection both contribute to subsequent pregnancy failure. This concept has been further supported by in vitro wound healing assays in which the suppression of stromal cell migration observed in the presence of a developmentally incompetent embryo did not occur when the decidual cells had been obtained from women with RM (Fig. 2) [37].

Emerging evidence indicates that an aberrant decidual response both prolongs receptivity phenotype of the endometrium and impairs the ability of the endometrium to engage in embryo quality control. Decidualization is often depicted as an all or nothing static phenotype, defined by the induction of marker genes, such as *PRL* or *IGFBP1*. This perception is misguided because the expression of several decidual gene networks change profoundly as the differentiation process unfolds. For example, a recent PCR array analysis of 84 inflammatory mediators identified 70 up-regulated cytokines, interleukins, and their receptors in cells decidualized for 2 days compared to undifferentiated control cells [44]. By 8 days of differentiation, only 12 transcripts remained elevated while 34 other mRNAs were now expressed below the level of that seen in undifferentiated cells. This suggests that decidual transformation is at least a biphasic process, characterized initially by an acute-phase inflammatory response, which is followed by a profound anti-inflammatory response [44]. Arguably, the anti-inflammatory phenotype coincides with the marked expansion of the decidual ER, up-regulation of various molecular chaperones, including HSPA8, and thus the ability of the cell to sense and respond to embryonic signals in a tailored fashion.

The biological relevance of the transient inflammatory decidual initiation response was examined by flushing mouse uteri with conditioned medium of undifferentiated and decidualizing primary human endometrial stromal cell cultures prior to embryo transfer. Strikingly, strong expression of the receptivity genes and effective embryo implantation were only observed if mice were exposed to the inflammatory secretome that marks the early stages of the decidual process. Compared to control cultures, this initial proinflammatory decidual response was both prolonged and disordered in primary cultures established from RM patients. Furthermore, when flushed through the mouse uterus, secreted factors from decidualizing RM cultures not only prolonged the window of receptivity but also increased the incidence of pathological implantation sites, characterized by focal bleeding, immune cell infiltration, and fetal demise [44]. Additional studies have shown that differentiating human endometrial stromal cells from RM patients are characterized by lower induction of decidual marker genes, increased vulnerability to oxidative apoptosis, aberrant responses to hCG, and failure to discriminate between high- and low-quality human embryos in cell migration assays [37, 45, 46].

Taken together, these observations indicate that the transition of decidual cells from the proinflammatory initiation phase to a fully secretory anti-inflammatory phenotype balances the receptivity versus selectivity traits of the human endometrium. Thus, an excessive decidual response will curtail the window of receptivity and increase the disposal efficacy of embryos, thereby reducing the incidence of miscarriages but also increasing the likelihood of conception delay or recurrent implantation failure after IVF. Conversely, a disordered decidual response will increase the likelihood of both pregnancy as well as miscarriage by facilitating out-of-phase implantation [47] (Fig. 4). Importantly, spontaneous decidualization is inextricably linked to menstruation and cyclic regeneration; a process that involves recruitment of extrauterine stem cells and activation of local progenitor cells. Thus, the quality of the decidual process, that is, the balance of the receptive versus selective traits, will vary in response to the cumulative impact of menstruations and other reproductive events, such as miscarriage and parturition.

CONCLUSIONS

From a clinical perspective, RM and implantation failure have remained frustratingly devoid of effective therapeutic strategies. However, the emerging evidence that the decidualizing endometrium is an active participant and key determinant of successful implantation promises to open up new therapeutic avenues. Moreover, the recognition that endometrial function can be considered in terms of both the epithelial feature of receptivity or ability to recognize and respond supportively to a high-quality embryo and the decidual function of selectivity offers a new paradigm for the investigation and management of early reproductive failure.

Similar to its previously proposed role in the bovine species [47], the decidualized human endometrium is emerging as an active gatekeeper to implantation in the human. This novel model requires further validation but addresses a number of imperatives characteristic of human reproduction. The remarkable prevalence of aneuploidy in human embryos demands maternal investment in effective means of preventing invasive but chromosomally chaotic embryos from establishing a clinical pregnancy destined to fail. Menstruation, a process triggered by spontaneous decidualization of the endometrium in an embryo-independent manner and almost unique to humans among mammalian species, may have emerged as a strategy for early detection and active rejection of developmentally abnormal embryos that have breached the luminal epithelium. At the same time, cyclic regeneration of the

endometrium provides a mechanism to continuously rebalance the receptivity and selectivity traits of the endometrium, thus increasing the likelihood of reproductive success.

It is likely that the response of the luminal epithelium serves to transduce and amplify signals coming from competent embryos in a way that renders the underlying decidual layer more receptive to invasion. Conversely, in the presence of a poor-quality embryo, evidence from recent studies suggests that the network of supportive decidual processes is not activated. Instead, decidual cells mount a stress response, leading to withdrawal of key implantation factors and active withdrawal of maternal support for a developmentally compromised embryo. An economic and evolutionarily conserved signaling system by which the embryo indicates its competence may be represented by embryonic serine proteases [14].

Taken together, there is accumulating experimental and clinical evidence to support a bimodal and biphasic endometrial response to the implanting embryo characterized by recognition and then selective elements. The clinical implications of this are potentially far reaching. To date, clinical interventions aimed at treating RM and implantation failure have been characterized by their similarity and disappointing efficacy. Further understanding of these dual processes may reveal that recurrent implantation failure and RM are caused by unbalancing of these functional endometrial traits; the former reflecting a net overselective phenotype, and the latter an inadequately discerning endometrium. A key challenge now is to unravel the molecular processes that control the timely transition from a proinflammatory, receptive decidual phenotype to a fully secretory and selective decidual phenotype. Once fully elucidated, new and effective approaches to modulate implantation await.

REFERENCES

1. Chard T. Frequency of implantation and early pregnancy loss in natural cycles. Baillieres Clin Obstet Gynaecol 1991; 5:179–189.
2. Foote RH, Carney EW. Factors limiting reproductive efficiency in selected laboratory animals. Ann NY Acad Sci 1988; 541:683–696.
3. Stevens VC. Some reproductive studies in the baboon. Hum Reprod Update 1997; 3:533–540.
4. Boomsma CM, Kavelaars A, Eijkemans MJ, Lentjes EG, Fauser BC, Heijnen CJ, Macklon NS. Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. Hum Reprod 2009; 24: 1427–1435.
5. Hertig AT, Rock J, Adams EX, Menkin MC. Thirty-four fertilized human ova, good, bad and indifferent, recovered from 210 women of known fertility; a study of biologic wastage in early human pregnancy. Pediatrics 1959; 23:202–211.
6. Wilcox, AJ Weinberg, CR, O'Connor, JF, Baird, DD, Schlatterer, JP, Canfield, RE, Armstrong, EG, Nisula. BC. Incidence of early loss of pregnancy. N Engl J Med 1988; 319:189–194.
7. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum Reprod Update 2002; 8: 333–343.
8. Mertzanidou A, Wilton L, Cheng J, Spits C, Vanneste E, Moreau Y, Vermeesch JR, Sermon K. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. Hum Reprod 2013; 28:256–264.
9. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Fryns JP, Verbeke G, et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med 2009; 15:577–583.
10. Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, Wells D. The origin and impact of embryonic aneuploidy. Hum Genet 2013; 132: 1001–1013.
11. Santos MA, Teklenburg G, Macklon NS, Van Opstal D, Schuring-Blom GH, Krijtenburg PJ, de Vreeden-Elbertse J, Fauser BC, Baart EB. The fate of the mosaic embryo: chromosomal constitution and development of Day 4, 5 and 8 human embryos. Hum Reprod 2010; 25:1916–1926.
12. Chen G, Bradford WD, Seidel CW, Li R. Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy. Nature 2012; 29:482: 246–250.
13. Oromendia AB, Dodgson SE, Amon A. Aneuploidy causes proteotoxic stress in yeast. Genes Dev 2012; 26:2696–2708.
14. Brosens JJ, Salker MS, Teklenburg G, Nautiyal J, Salter S, Lucas ES, Steel JH, Christian M, Chan YW, Boomsma CM, Moore JD, Hartshorne GM, et al. Uterine selection of human embryos at implantation. Sci Rep 2014; 6: 3894.
15. Haig D. Genetic conflicts in human pregnancy. Q Rev Biol 1993; 68: 495–532.
16. Gellersen B, Brosens IA, Brosens JJ. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Semin Reprod Med 2007; 25:445–453.
17. Brosens JJ, Gellersen B. Death or survival—progesterone-dependent cell fate decisions in the human endometrial stroma. J Mol Endocrinol 2006; 36:389–398.
18. Finn CA. Menstruation: a nonadaptive consequence of uterine evolution. Q Rev Biol 1998; 73:163–173.
19. Emera D, Romero R, Wagner G. The evolution of menstruation: a new model for genetic assimilation: explaining molecular origins of maternal responses to fetal invasiveness. Bioessays 2012; 34:26–35.
20. Talmadge K, Boorstein WR, Fiddes JC. The human genome contains seven genes for the beta-subunit of chorionic gonadotropin but only one gene for the beta-subunit of luteinizing hormone. DNA 1983; 2:281–289.
21. Jauniaux E, Watson AL, Hempstock J, Bao YP, Skepper JN, Burton GJ. Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. Am J Pathol 2000; 157: 2111–2122.
22. Hempstock J, Jauniaux E, Greenwold N, Burton GJ. The contribution of placental oxidative stress to early pregnancy failure. Hum Pathol 2003; 34: 1265–1275.
23. Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, Roelen BA, Quenby S, et al. Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. PLoS One 2010; 5:e10258.
24. Leese HJ. Quiet please, do not disturb: a hypothesis of embryo metabolism and viability. Bioessays 2002; 24:845–849.
25. Brison DR, Houghton FD, Falconer D, Roberts SA, Hawkhead J, Humpherson PG, Lieberman BA, Leese HJ. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. Hum Reprod 2004; 19:2319–2324.
26. Sturmey RG, Hawkhead JA, Barker EA, Leese HJ. DNA damage and metabolic activity in the preimplantation embryo. Hum Reprod 2009; 24: 81–91.
27. Hartl FU, Hayer-Hartl M. Converging concepts of protein folding in vitro and in vivo. Nat Struct Mol Biol 2009; 16:574–581.
28. Agarraberes FA, Dice JF. A molecular chaperone complex at the lysosomal membrane is required for protein translocation. J Cell Sci 2001; 114:2491–2499.
29. Stricher F, Macri C, Ruff M, Muller S. HSPA8/HSC70 chaperone protein: structure, function, and chemical targeting. Autophagy 2013; 9: 1937–1954.
30. Das SK, Wang XN, Paria BC, Damm D, Abraham JA, Klagsbrun M, Andres GK, Dey SK. Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. Development 1994; 120:1071–1083.
31. Dey SK, Lim H, Das SK, Reese BC, Paria T, Daikoku T, Wang H. Molecular clues to implantation. Endocr Rev 2004; 25:341–373.
32. Singh H, Nardo L, Kimber SJ, Aplin JD. Early stages of implantation as revealed by an in vitro model. Reproduction 2010; 139:905–914.
33. Grewal S, Carver JG, Ridley AJ, Mardon HJ. Implantation of the human embryo requires Rac1-dependent endometrial stromal cell migration. Proc Natl Acad Sci U S A 2008. 21:105(42):16189–161094.
34. Grewal S, Carver JG, Ridley AJ, Mardon HJ. Human endometrial stromal cell rho GTPases have opposing roles in regulating focal adhesion turnover and embryo invasion in vitro. Biol Reprod 2010; 83:75–82.
35. Gonzalez M, Neufeld J, Reimann K, Wittmann S, Samalecos A, Wolf A, Bamberger AM, Gellersen B. Expansion of human trophoblastic spheroids is promoted by decidualized endometrial stromal cells and enhanced by heparin-binding epidermal growth factor-like growth factor and interleukin-1 β. Mol Hum Reprod 2011; 17:421–433.
36. Gellersen B, Wolf A, Kruse M, Schwenke M, Bamberger AM. Human endometrial stromal cell-trophoblast interactions: mutual stimulation of

chemotactic migration and promigratory roles of cell surface molecules CD82 and CEACAM1. Biol Reprod 2013; 88:80.
37. Weimar CH, Kavelaars A, Brosens JJ, Gellersen B, de Vreeden-Elbertse JM, Heijnen CJ, Macklon NS. Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. PLoS One 2012; 7:e41424.
38. Gellersen B, Reimann K, Samalecos A, Aupers S, Bamberger AM. Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. Hum Reprod 2010; 25:862–873.
39. Schwenke M, Knöfler M, Velicky P, Weimar CH, Kruse M, Samalecos A, Wolf A, Macklon NS, Bamberger AM, Gellersen B. Control of human endometrial stromal cell motility by PDGF-BB, HB-EGF and trophoblast-secreted factors. PLoS One 2013; 8:e54336.
40. Weimar CH, Macklon NS, Post Uiterweer ED, Brosens JJ, Gellersen B. The motile and invasive capacity of human endometrial stromal cells: implications for normal and impaired reproductive function. Hum Reprod Update 2013; 19:542–557.
41. Haouzi D, Dechaud H, Assou S, Monzo C, de Vos J, Hamamah S. Transcriptome analysis reveals dialogues between human trophectoderm and endometrial cells during the implantation period. Hum Reprod 2011; 26:1440–1449.
42. Ruan YC, Guo JH, Liu X, Zhang R, Tsang LL, Dong JD, Chen H, Yu MK, Jiang X, Zhang XH, Fok KL, Chung YW, et al. Activation of the epithelial Na+ channel triggers prostaglandin E_2 release and production required for embryo implantation. Nat Med 2012; 18:1112–1117.
43. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. N Engl J Med 1999; 340:1796–1799.
44. Salker MS, Nautiyal J, Steel JH, Webster Z, Sucurovic S, Nicou M, Singh Y, Lucas ES, Murakami K, Chan YW, James S, Abdallah Y, et al. Disordered IL-33/ST2 activation in decidualizing stromal cells prolongs uterine receptivity in women with recurrent pregnancy loss. PLoS One 2012; 7:e52252.
45. Salker MS, Christian M, Steel JH, Nautiyal J, Lavery S, Trew G, Webster Z, Al-Sabbagh M, Puchchakayala G, Föller M, Landles C, Sharkey AM, et al. Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. Nat Med 2011; 7(11): 1509–1513.
46. Salker M, Teklenburg G, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, Roelen BA, Quenby S, et al. Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. PLoS One 2010; 5:e10287.
47. Quenby S, Vince G, Farquharson R, Aplin J. Recurrent miscarriage: a defect in nature's quality control? Hum Reprod 2002; 17:1959–1963.
48. Mansouri-Attia N, Sandra O, Aubert J, Degrelle S, Everts RE, Giraud-Delville C, Heyman Y, Galio L, Hue I, Yang X, Tian XC, Lewin HA, et al. Endometrium as an early sensor of in vitro embryo manipulation technologies. Proc Natl Acad Sci U S A 2009; 106:5687–5692.