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Emerging *in vitro* platforms and omics technologies for studying the endometrium and early embryo-maternal interface in humans

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ABSTRACT

Human reproductive success relies on the intricate interplay between the developing embryo and the maternal endometrium. These highly-coordinated interactions facilitate implantation, setting in motion a series of developmental programs to establish a sustained fetal-maternal interface. Understanding endometrial function and the early human embryo-maternal dialogue is thus an important prerequisite for refining clinical approaches to alleviate implantation failure, early pregnancy loss and other obstetric complications. Yet, many mediators of implantation remain elusive. Driven by endocrine factors, interactions at the embryo-maternal interface are tightly regulated and highly complex. Coupled to the inaccessibility of the *in vivo* environment and scarcity of research material, studying human implantation remains exceptionally challenging. Nevertheless, the field continues to gain momentum. Cutting-edge omics technologies and high-resolution imaging have revealed important structural and functional insights into endometrial biology, while emerging bioengineering tools are enhancing our ability to model the synergies and individual features of the embryo-maternal environment. Novel *in vitro* platforms using human cells and embryos are considerably more accessible and easier to manipulate compared to *in vivo* approaches, enhancing our ability to capture specific stages of implantation. This review aims to showcase current and emerging technologies used to study human endometrial biology and the early embryo-maternal interface, including single cell omics approaches, bioengineered endometrial models and embryo-endometrium co-culture platforms. We highlight the value of these approaches and provide our perspective on the current challenges faced by the field. Recognizing the physiological scope of these emerging technologies will be key for utilizing their full potential and driving future innovation.

1. Introduction

Reciprocal molecular exchange between the human embryo and the maternal environment is imperative for human reproductive success. These intricate interactions require synchrony and active contribution from both the mother and the embryo, equally indispensable for the establishment and progression of a normal pregnancy. Accordingly, disruptions to this early dialogue lead to poor pregnancy outcomes, including implantation failure, placental insufficiency and other obstetric complications [1]. Human implantation is highly precarious, with fewer than 30% of all fertilization events resulting in a successful pregnancy, even for young, fertile couples (reviewed in [2]). Staggeringly, data gleaned from medically assisted reproduction indicate that two-thirds of pregnancies are lost due to the failure of the embryo to implant [3]. This figure remains remarkably consistent despite tremendous progress in the field [4]. While a significant proportion of preclinical losses may be attributed to embryonic chromosomal abnormalities [5], implantation failure following the transfer of good-quality euploid embryos remains an unresolved obstacle ([6,7], reviewed in [8]).

Despite its fundamental and clinical significance, the cellular and molecular mechanisms underlying this critical period of human development remain poorly understood. For ethical and practical reasons, human implantation cannot be adequately studied *in vivo*, while *ex vivo* and *in vitro* studies also present significant challenges. Limited research material, biological variation, and the inability to recapitulate the morphology and molecular landscape of the embryo-maternal

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Abbreviations

2D	two-dimensional
3D	three-dimensional
D	days post fertilization
ECM	extracellular matrix
EV	extracellular vesicle
ICM	inner cell mass
scRNAseq	single cell RNA sequencing
TE	trophectoderm

environment undoubtedly hamper success. Animal models, including rodents and non-human primates, have been valuable for inferring certain aspects of human implantation (reviewed in [9,10]), but pose a different set of trade-offs.

Capturing the complexity of the cellular and molecular microenvironment at the early embryo-maternal interface will be paramount for uncovering the landscape of events surrounding human embryo implantation. This review aims to showcase current and emerging approaches to study these processes. We discuss recent insights into endometrial biology gained through single cell omics, state-of-the-art strategies to recapitulate the human endometrium, as well as 2D and 3D culture platforms for modelling human embryo implantation events. Ultimately, the convergence of these approaches will be mutually beneficial. By considering significant contributions in the field, we aim to identify promising new tools for understanding the early embryo-maternal microenvironment, whilst also providing a critical overview of the current challenges faced by the field. While new technologies do promise to improve the translational limitations of conventional animal models and traditional *in vitro* culture systems, they too only capture particular aspects of physiology. Understanding these inherent limitations will be critical for driving meaningful progress in elucidating the early embryo-maternal interface and generating accurate, predictive and biologically relevant data.

2. Setting the stage: early human development and implantation *in vivo*

During the first days of human development, the fertilized zygote undergoes a series of specialized cell divisions that ultimately lead to the formation of the blastocyst around 5 days post fertilization, D5. The D5 blastocyst consists of two main groups of cells: a smaller compact inner cell mass (ICM) and an outer epithelial layer, the trophectoderm (TE) (Fig. 1A). Prior to implantation, the ICM begins to differentiate into two further lineages, pluripotent epiblast cells and hypoblast cells [11,12]. Cells of the ICM give rise to the fetus and several extraembryonic cell types of the placenta, amnion, chorion, yolk sac and umbilical cord. TE

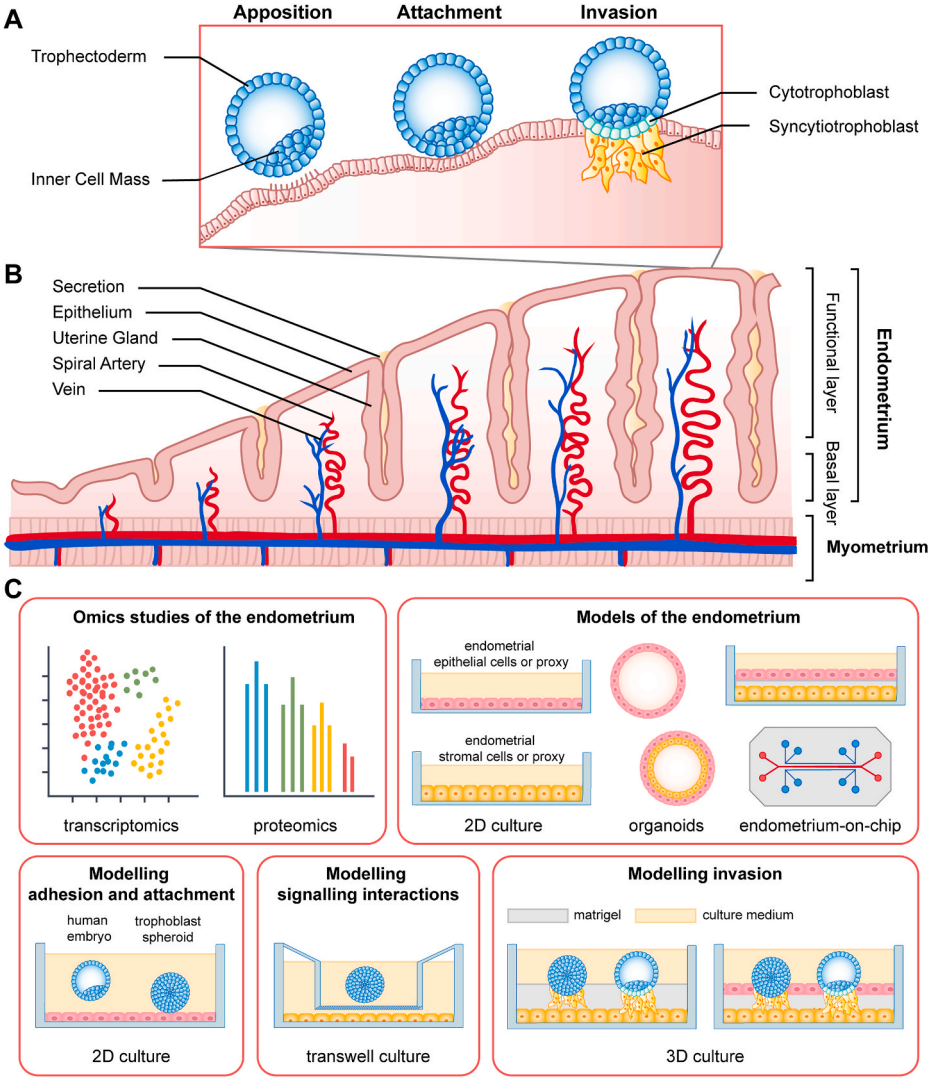

Fig. 1. Human implantation, the endometrium and emerging technologies to study them. (A) Phases of early human implantation as the human blastocyst undergoes apposition, attachment and invasion of the endometrium. During invasion, the trophectoderm (TE) at the embryonic pole (polar TE) is induced to proliferate and forms multinucleated terminally differentiated syncytiotrophoblast cells. The TE cells surrounding the blastocyst cavity or blastocoel, remain mononucleated and constitute the cytotrophoblast. **(B)** The endometrium is formed by a basal layer adjacent to the myometrium, and a transient functional layer closest to the uterine cavity. The functional layer is further composed of a surface columnar endometrial epithelium overlying glands (glandular epithelium), which extends into the underlying stroma. The thickness of the functional layer is determined by the phase of the menstrual cycle. During menstruation, both the epithelial and stromal compartments are shed. Regeneration begins almost instantaneously driven by the rising levels of estrogen, resulting from the growth of ovarian follicles and the proliferative phase is marked by proliferation of the endometrial epithelium, stroma, glands and vasculature. The surge in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary subsequently induce ovulation. This marks the beginning of the secretory phase, which fosters a stable endometrium for blastocyst implantation. In the absence of a competent blastocyst, the rapid decrease in progesterone resets the cycle, initiating menstruation. **(C)** Cartoon illustrating different technologies used to study the endometrium (top part) and the different stages of early implantation (bottom part).

cells do not contribute directly to the fetus, but are critical for implantation as they give rise to the outer layers of the placenta: the cytotrophoblast (that contributes to the formation of the extravillous trophoblast cells that anchor and invade the maternal endometrium) and the syncytiotrophoblast (the multinucleated outer layer of the placental villi that interfaces with the maternal blood). Together with the maternal uterus, the invading TE-derived cells form the fetal-maternal interface (reviewed in [13,14]). By D6, the blastocyst will hatch out of the zona pellucida, interact with the endometrium, and ultimately implant (Fig. 1A). Notably, the narrow window of endometrial receptivity, 7–10 days post-ovulation, restricts the period for embryo-maternal interactions [15,16], beyond which the blastocyst cannot implant.

The female reproductive cycle is influenced by a complex cascade of fluctuating hormones allowing ovulation, fertilization and embryo implantation. The unique dynamic endometrial microenvironment is inherent to these cyclical changes (Fig. 1B). In preparation for implantation, the endometrium undergoes the process of decidualization, which involves morphological and biochemical reprogramming of the endometrial stromal compartment [17]. During this process, endometrial stromal fibroblasts transform into epithelial-like secretory cells, along with extracellular matrix (ECM), vascular remodeling, and local immune response regulation (reviewed in [18]). As decidualization proceeds, the uterine glands increase their production of uterine secretions (also known as 'uterine milk') [19]. If an embryo implants successfully, decidual cells promote the invasion of TE-derived cells, facilitating spiral-artery remodeling and conferring maternal immunotolerance towards the embryo. In the absence of implantation, the decidua is shed during menstruation. Interestingly, decidualizing endometrial stromal cells have been suggested to act as natural biosensors of embryo quality [20–22] (Table 1). Notably, humans are one of the few species in which decidualization is not triggered by the embryo. In mouse, for instance, decidualization is blastocyst-dependent, however it can also be elicited by physical means or by locally applied growth factors [23–25].

Only a few direct observations of early human peri-implantation have been made, with the collection from the Carnegie Department of Embryology [26], still serving as the foundation. The process of implantation has been described to involve sequential steps of apposition, attachment, and invasion (reviewed in [27]) (Fig. 1A). During apposition, the polar TE cells of the hatched human blastocyst initiate a weak interaction with the luminal epithelium of the endometrium. ECM proteins and adhesion molecules, such as L-selectin have been suggested to initiate implantation [28,29]. It is generally accepted that most human blastocysts attach to the endometrial epithelium around D7 through the mediation of bridging ligand interactions. During the adhesion phase, the competent blastocyst induces breakdown of the endometrial mucin layer at the implantation site to promote attachment [30]. Endometrial-derived factors at the embryo-maternal interface activate TE differentiation required for attachment and invasion of the underlying endometrial stroma. On the other hand, in response to signals derived from the invasive TE-derived cells, the endometrium undergoes local remodeling (reviewed in [31]).

3. Transcriptomics technologies to study the human endometrium

The endometrium is composed of multiple cell types and undergoes profound morphological regeneration and remodeling in response to cyclic hormonal changes [32,33] (Fig. 1B). Over the past two decades, investigations on the transcriptional signature of endometrial biopsies (bulk RNA-sequencing) have provided insights into the repertoire of molecules expressed throughout the menstrual cycle, in both physiological and pathological conditions [34–47]. Nevertheless, reproducibility has proven challenging, leading to discrepancies in gene expression profiles amongst studies [48,49]. Indeed, in bulk RNA

Table 1
In vitro platforms for studying human implantation.

Compartment	Human Embryo	Human Embryo Proxy	Implantation Process
Human primary endometrial epithelial cells	Lindenberg et al. 1985 Simon et al. 1999 Gonzalez et al. 2000 Galan et al. 2000 Meseguer et al. 2001 Cabelloeri-Campo et al. 2002 Dominguez et al. 2003 Horcajadas et al. 2005 Berger et al. 2015 Boggavarapu et al. 2016 Le Saint et al. 2019	Wang et al. 2012 Wang et al. 2015	**Apposition and Attachment**
Human endometrial proxy	Kang et al. 2014 Aberkane at el. 2018 Ruane et al. 2020	Hohn et al. 2000 Heneweer et al. 2003, 2005 Uchida et al. 2007 Harduf et al. 2009 Aboussahoud et al. 2010 Liu et al. 2011 Ho et al. 2012 Holmberg et al. 2012 Wei et al. 2012 Bhagwat et al. 2014 Xie et al. 2014, 2015 Buck et al. 2015 Cheng et al. 2017 Huang et al. 2017 Jiang et al. 2014, 2017 Yang et al. 2017 Yu et al. 2017 Chen et al. 2018 Miyazaki et al. 2018 Kakar-Bhanot et al. 2019 Vergaro et al. 2019, 2021	
Human primary endometrial stromal cells	Carver et al. 2003 Grewal et al. 2008 Teklenburg et al. 2010 Weimar et al. 2012 Brosens et al. 2014	Harun et al. 2006 Gonzalez et al. 2011 Estella et al. 2012 Holmberg et al. 2012 Gellersen et al. 2010, 2013 Schwenke et al. 2013 Tapia-Pizarro et al. 2013 Wang et al. 2015 Cheng et al. 2017	**Invasion**

(*continued on next page*)

Table 1 (*continued*)

Compartment	Human Embryo	Human Embryo Proxy	Implantation Process
3D model, endometrial epithelial & stromal cells	Bentin-Ley et al. 2000 Berger et al. 2015 You et al. 2019	Evron et al. 2011 Wang et al. 2012 Nishiguchi et al. 2018	

studies, the proportions of each cell type in the endometrium biopsy inherently influence transcriptomics data.

More recently, single-cell analyses have endeavored to identify transcriptional signatures and different cell states in the human endometrium. Using single cell RNA-sequencing (scRNAseq), Krjutškov et al. identified differences in gene expression relating to cell cycle, translational and metabolic processes between *in vitro* cultured and uncultured endometrial biopsies [50]. In a landmark study, Wang et al. used endometrial biopsies from 29 healthy women to map cell-specific and time-dependent transcriptomic signatures across the menstrual cycle, providing novel insights into cell transitions and interactions [33]. The authors identified a less abundant transcriptionally-distinct ciliated epithelial cell population in the luminal and glandular endometrial compartments. The study also revealed crosstalk between stromal fibroblasts and lymphocytes in the decidualizing endometrium, suggesting direct interplay between these two cell types, just before the window of implantation. The authors suggest that the abrupt and discontinuous transcriptional activation of the endometrial epithelia, followed by widespread decidualization in the stromal fibroblasts may be used to predict the window of implantation [33].

Identifying potential biomarkers of the receptive endometrium is currently an intense area of research. Given that endometrial receptivity is critical for the establishment of pregnancy, substantial efforts have been directed towards developing a clinical diagnostic assay to better synchronize the embryo and the uterus [51–59]. While compelling, such approaches remain controversial, as the dynamic nature of the endometrium, and considerable individual and menstrual inter-cycle variability present an inherent limitation [49,60–62]. Nevertheless, more refined knowledge of the genetic and molecular transitions of the endometrium may determine more precise indicators of endometrial receptivity for the prediction of successful pregnancy.

Understanding the cyclic regeneration of the endometrium will also deliver important opportunities for elucidating normal endometrial physiology, as well as subfertility disorders characterized by dysregulated endometrial repair, including endometriosis, adenomyosis and Asherman's syndrome. While several stem/progenitor cells have been suggested to drive the regenerative process, their exact identity and location remain unclear [63–65]. Using scRNAseq on endometrial biopsies in the proliferative stage (between the menstrual and secretory stage), Queckbörner et al. identified multiple stromal populations suggestive of specific stromal niches with the ability to regulate inflammation and ECM composition. They further characterized vascular smooth muscle cells, pericytes, endothelial and immune cells [66].

Single cell transcriptomic studies have undoubtedly provided important insights into the genetic and functional complexities of the human endometrium. However, accurate interpretation of scRNAseq data remains a significant challenge. As findings are fundamentally restricted to the material that can be assessed, biological interpretations are ultimately hindered by low capture efficiency and poor-quality data following tissue dissociation. As such, lowly abundant cell populations and weakly expressed genes are often missed (reviewed in [67]). Furthermore, difficulties in obtaining endometrial material ultimately limit the statistical power of transcriptomic datasets, while logistical challenges in sample acquisition may introduce further technical variability due to batch effects. Moving forward, the unique nature of the endometrium may require additional efforts to standardize dissociation methods, harmonize cell ontology nomenclature and optimize computational algorithms to ensure greater reproducibility.

Novel technologies allowing localization of gene expression of many genes in a small tissue area (spatial transcriptomics) have also been applied to endometrial biopsies to investigate the molecular mechanisms driving epithelial differentiation in the luminal and glandular microenvironments [68]. Notably, this led to the discovery of a novel population of fibroblasts (fibroblasts C7) restricted to the endometrium basal layer, as well as a pre-ciliated population that appears in response to estrogen and is dependent on WNT signalling. By mapping cell transcriptional signatures into tissues, the authors were also able to allocate epithelial cells into the main endometrial layers: functional and basal [68]. While future innovation will rely on novel computational tools and mathematical modeling, as the resolution and sensitivity of spatial transcriptomic technologies improves, the systematic analysis of larger tissue areas will certainly drive progress in our understanding of endometrial physiology (reviewed in [69]). Interestingly, recent 3D imaging studies have revealed complex gland branching networks spanning the basal and functional layer of the endometrium [70,71]. This approach further enabled lineage tracing of glandular cell types [70]. 3D imaging technologies undoubtedly offer valuable opportunities for understanding the anatomical and histological features of the human uterus. However, large uterine tissue samples (including the endometrium and myometrium) are required to capture the morphological complexities of the uterus. As this material can only be obtained from hysterectomies, sampling bias remains inevitable, as histological samples from young women are incredibly scarce [71].

4. Omics technologies to study the embryo-maternal interface during implantation

The analysis of the composition of uterine secretions may be key in resolving embryo-maternal interactions. Proteomic analyses of uterine fluid aimed at identifying signatures of endometrial receptivity [72–76], have provided valuable insights into the composition of secretions promoting implantation and placental development (reviewed in ([77]). Accordingly, essential ions, lipids, glycoproteins (glycodelin-A, osteopontin, uteroglobin), glycogen and growth factors (leukaemia inhibitory factor, epidermal growth factor and vascular endothelial growth factor) may have multiple potential effects at the maternal-placental interface (reviewed in [19]).

Interestingly, new forms of embryo-maternal communication via the exchange of extracellular vesicles (EVs) have been identified in the uterine fluid [78–81]. EVs regulate the microenvironment by selectively packaging molecules and transferring them between cells. A recent study performed quantitative mass spectrometry-based proteomic profiling of EVs obtained from uterine fluid and showed that antioxidant activity was enriched in uterine EVs from fertile women [82]. Indeed, exogenous supplementation of culture medium with antioxidants (acetyl-L-carnitine, N-acetyl-L-cysteine and alpha-lipoic acid) has been shown to significantly improve the development of mouse preimplantation embryos *in vitro* [83]. While currently being trialed for the *in vitro* culture of human embryos (NCT02999958), these findings may also have important implications for improving current *in vitro* models of implantation. Interestingly, EVs may also mediate communication within the blastocyst itself. Embryonic stem cells derived from the blastocyst ICM have been shown to shed EVs, which can interact directly with the TE, activating pathways that promote trophoblast migration and support implantation [84]. Moreover, Evans et al. revealed that uptake of endometrial EVs enhance adhesion and invasion of human TE-spheroids [80]. While the low number of EVs in individual uterine fluid samples remains a major limitation for robust proteomic profiling, further studies may lead to interesting discoveries in understanding protein expression regulating human embryo-endometrial interactions.

5. Modelling the human endometrium in 2D and 3D

Traditionally, *in vitro* studies of the human endometrium have largely involved two-dimensional (2D) cell culture approaches. These have included primary endometrial epithelial and stromal cells, as well as immortalized cell lines derived from endometrial adenocarcinoma, such as Ishikawa [85], HEC-1-A [86] and RL95-2 [87]. Compared to immortalized cells, primary cell lines are thought to be physiologically more similar to endometrial cells *in vivo* and have been used for studying molecules associated with endometrial receptivity and recurrent miscarriage [20]. Nevertheless, *in vitro* models employing primary cell lines have largely focused on the stromal compartment, as stromal cells can be maintained in culture long-term. Conversely, endometrial epithelial cells are generally limited in their yield and do not grow well in culture. Moreover, the use of primary endometrial cells can introduce experimental variability, due to patient heterogeneity and culture artefacts [50]. The Ishikawa immortalized epithelial cell line has been useful for modelling the endometrial epithelium. It responds to estrogen and progesterone [88,89] and expresses known endometrial markers ([90,91], reviewed in [72]). As such, it has been used to study endocrine signalling in the endometrium, in addition to trophoblast adhesion and attachment *in vitro* (Table 1). RL95-2 and HEC-1A model high and low endometrial receptivity, respectively. These lines have been used for characterizing cell-surface proteins relevant for embryo adhesion [92] (Table 1). Immortalized human endometrial stromal cell lines have also been used to model the endometrium, to study genetic and chemical modulators of the human decidual response [93], as well as processes involved in trophoblast invasion [94] (Table 1). Established cell lines certainly offer flexibility and high throughput, delivering convenient and practical systems for straightforward hypothesis driven research (reviewed in [95]). However, 2D models are limited in capturing the full complexity of the *in vivo* endometrial microenvironment, restricting appropriate cell-cell interactions, whilst also lacking cellular heterogeneity. To this end, the establishment of tissue-derived three-dimensional (3D) culture systems, including endometrial organoids and organ-on-chip technologies, constitutes a promising way forward.

Early efforts to generate a 3D model of the human endometrium involved embedding endometrial tissues and glands in Matrigel to form spheroid structures [96,97]. The spheroids displayed similar properties to endometrial glands, and contained polarized columnar epithelium surrounding a lumen. Adopting a similar approach, two groups recently generated endometrial epithelial cell organoids using chemically-defined medium [98,99]. Glandular epithelial fragments were cultured in Matrigel droplets, in medium containing growth factors and activators of the WNT and MAPK signalling pathways to promote proliferation, while inhibitors of TGFβ and BMP signaling were used to prevent differentiation. Endometrial organoids were composed of a central lumen surrounded by a single layer of polarized epithelial cells. They could be cultured long-term and responded to hormonal stimulation, recapitulating changes of the luminal epithelium across the menstrual cycle [68]. Notably, endometrial organoids have also recently been derived from gland fragments recovered from menstrual fluid [100], post-menopausal women, as well as a broad spectrum of diseased endometrial tissues, including endometriosis, endometrial carcinomas, endometrial hyperplasia and Lynch syndrome [98]. While organoids do provide a more standardized approach to modeling endometrial physiology and disease, they lack the complete complement of endometrial cell types. Current endometrial organoids do not contain stromal or immune cells, and thus cannot be used for studying cellular interactions underlying proliferation, differentiation, and decidualization. Interestingly, hydrogels generated from decellularized pig endometrium have been suggested to provide a more favourable system for culturing endometrial organoids, as they allow interactions between the endometrial epithelial cells and soluble ECM molecules [101].

6. Bioengineering approaches to mimic the human endometrium

Generating a more complex multicellular model of the endometrium, Bentin-Ley et al. combined endometrial epithelial and stromal cells by embedding stromal cells in a collagen matrix, separated from the epithelial cells by Matrigel [102] (Table 1). Notably, this model was applied for testing a wide variety of contraceptives on implantation [103,104], highlighting the translational potential of such *in vitro* endometrial systems. Nevertheless, the 3D model could not be maintained in culture long-term. To this end, Wiwatpanit et al. recently constructed human endometrial organoids using primary endometrial epithelial and stromal cells from endometrial biopsies. This model was used to investigate the effects of polycystic ovary syndrome on androgen levels [105]. One unique feature of this endometrial organoid system is that no exogenous basement membrane matrix was used. The epithelial-stromal cell suspension was seeded into agarose and the organoids organized into distinct layers by responding to cues from each cell type. This promoted polarization of the epithelial cells with the basolateral side in contact with the stromal cells [105]. Abbas et al. adopted an alternative approach, by developing 3D porous collagen scaffolds tailored for seeding stromal cells and endometrial epithelial organoid fragments. The authors confirmed apical polarization of the epithelial cells to the outside surface, with their basal surface attached to the scaffold [106].

Interestingly, Cheung et al., generated endometrial epithelial-stromal cell organoids by combining human pluripotent stem cell-derived endometrial stromal fibroblasts with endometrial epithelial cells derived from the placenta [107]. The co-cultures displayed specific endometrial markers, appropriate organization and cell polarity, as well as hormone responsiveness of both cell types. Such models may be particularly valuable for studying stromal-epithelial interactions, and for mechanistic studies of cyclic endometrial responses. In a further report, Rawlings et al., modified the gland-like organoid model by combining purified endometrial stromal cells with organoid glandular cells in a hydrogel matrix [108]. When treated with hormones, the so-called “assembloids” mimicked gene expression patterns of endometrial cells during implantation. Interestingly, this model was further used to establish co-cultures with human blastocysts to model different pathological states associated with implantation failure and early pregnancy loss.

While these studies constitute an important step towards bioengineering the human endometrium, they also highlight the limitations of organoids for capturing the complex nature of distinct endometrial environments during human implantation. In particular, the absence of key cellular constituents, including innate immune cells and vascular endothelial cells, as well as the lack of surface epithelium, limit the use of these platforms for studying implantation. Pregnancy-related complications and endometrial pathologies are closely related to immune cell and vasculature dysfunctions (reviewed in [109,110]). Incorporating these cell types will thus be critical for capturing the endometrial histoarchitecture more closely, in both healthy and diseased states. Future efforts to fine tune the development and vascularization of multilineage human endometrial organoids, may benefit from ongoing optimization of organoids derived from other organs, such as kidney and liver [111,112]. These approaches may provide valuable clues for improving endometrial co-culture efficiency and long-term expansion.

Bioengineering approaches promise to capture the full structural and functional properties of the *in vivo* endometrium. A bioengineered endometrium may provide important opportunities for investigating implantation, placentation and embryo-maternal interactions during early human development. Such a model should capture mechanical cues of the ECM and integrate epithelial, stromal and immune cells in order to recapitulate tissue interfaces and appropriate signalling gradients. One of the most explored bioengineering strategies has been the generation of ECM scaffolds. The ECM preserves the tissue architecture

and maintains signaling responsible for proliferation, differentiation, and cell migration [113]. ECM scaffolds can be obtained through the process of decellularization or using (synthetic or natural) hydrogels, a more flexible culture matrix. Nevertheless, both approaches require extensive optimization and more importantly uterine decellularized material has largely been obtained from animal models [114–118]. Olalekan et al. generated a 3D *in vitro* model of the endometrium from decellularized human endometrial tissue repopulated with primary endometrial cells. After 28 days of hormonal treatment, the cells within the recellularized scaffolds expressed both estrogen and progesterone receptors, providing a promising experimental strategy for studying endometrial biology and clinical drug testing [119].

A further approach involves the use of biomaterials, such as collagen-based or collagen-containing compounds. Several studies have provided encouraging results for uterine regeneration using this approach [120–125]. In some cases, mesenchymal stem cells have been used to repopulation the scaffold [121,125]. Notably, two clinical studies explored the regeneration of endometrium in patients with Asherman's syndrome and recurrent intrauterine adhesions. Zhao et al. successfully treated five patients with Asherman's syndrome by transplanting a collagen scaffold seeded with autologous bone marrow and mononuclear cells [126]. Similarly, Cao et al. used umbilical cord-derived mesenchymal stromal cells loaded onto a collagen scaffold to treat 26 patients with recurrent intrauterine adhesions, leading to 8 live births [127].

Future research will certainly aid in determining the most optimal biomaterials and cells for bioengineering uterine tissues. The *in vivo* characteristics of both biomaterials and decellularized scaffolds will be critical for future clinical applications (reviewed in [128]). At present, the low efficiency of recellularization of the scaffolds remains a significant challenge. Future applications of 3D bioprinting techniques for uterine bioengineering may provide a promising strategy for capturing the spatial architecture of uterine tissues more precisely. Nevertheless, progress will ultimately rely on a greater understanding of uterine tissues.

To this end, organ-on-chip strategies and microfluidics provide a promising way forward. Notably, Gnecco et al. developed a human "endometrium-on-chip" model to study interactions between human perivascular stroma and endothelial cells [129]. The authors were able to capture several physiological changes associated with the *in vivo* reproductive cycle within their microengineered compartmentalized chip. Notably, microfluidics allowed the recreation of shear stress able to induce endothelial cell polarization. Xiao et al. used an integrated organ-on-chip platform (termed EVATAR) to model the *in vivo* female reproductive tract [130]. Using different microengineered units with sustained circulating flow, the authors simulated an endocrine loop between the ovary, fallopian tube, uterus, cervix and liver. While both mouse and human tissues were used, replicating these hormonal events *in vitro*, highlights the potential of organ-on-chip technologies for studying organ–organ integrations and endocrine signalling pathways. More recently, Ahn et al. micro-engineered a 3D vascularized endometrium-on-chip. The device captured the endometrial environment including the three distinct layers of epithelium, stroma and blood vessels. Moreover, the authors demonstrated appropriate responsiveness to pro-angiogenic factors and hormonal stimulation [131]. Further research will be required to measure the efficiency of such models to recapitulate the *in vivo* environment, as well as their variability depending on tissue and patient characteristics. The complexity of the endometrium remains a major limitation for developing an integrated model. It is still not possible to mimic the multifaceted interactions between various cell types that occur *in vivo*. Nevertheless, organ-on-chip microfluidic approaches hold great promise for personalized treatment of infertility, including disease modeling, toxicological studies and drug discovery.

7. Modelling the embryo-maternal interface during implantation

In an effort to overcome the inaccessibility of the human implantation environment *in vivo*, several approaches have been developed to model human embryo-endometrial interactions *in vitro* (Table 1). These combine human embryos and endometrial cells or their proxies, to recapitulate apposition, attachment and invasion. While no single system captures the entire process of implantation, these platforms have been valuable for studying the endometrial environment (as aforementioned) and identifying molecular cues that govern different stages of implantation. Models combining human embryos and primary endometrial epithelial cells [30,132–141] or Ishikawa cells [29,142,143] have been used to study embryo-endometrial interactions. Such models have provided evidence for the embryonic regulation of endometrial epithelial cell surface molecules and signalling pathways required for successful apposition and attachment. Interestingly, co-culture of endometrial cells with human embryos has also been applied in a clinical context. Le Saint et al. recently demonstrated that the culture of preimplantation embryos with autologous endometrial cells improved the number of good-quality blastocysts, compared to embryos cultured in conventional culture medium [141]. Similarly, Ruane and colleagues co-cultured human blastocysts with Ishikawa cells to evaluate the effects of EmbryoGlue, a hyaluronate-containing transfer medium, widely offered to patients undergoing IVF treatment [143].

TE-spheroids have extensively been used as an embryo proxy, employed to study the first steps of trophoblast adhesion and attachment [92,144–152]. TE-spheroids and immortalized cell lines (such as JEG-3) are amenable to gene manipulation techniques, providing a valuable platform for mechanistic studies, overexpression assays and fluorescence microscopy [153,154]. Using TE-spheroids, Vergaro et al. recently showed that TE attachment induced a wave of transcriptional changes in the endometrial epithelium [155].

To recapitulate embryo invasion events, *in vitro* models using stromal cells and human embryos and respective proxies have been used (Table 1). For example, transwell culture wells have been used to model paracrine signalling between the endometrial stromal cells and the trophoblast, while co-cultured separately, but remaining in the same medium [156–158] (Table 1). Transwell culture has provided direct evidence of early embryo-maternal interactions, allowing the identification of several molecules involved in this process.

Multilayered co-culture models including both primary endometrial epithelial and stromal cells or proxies have also been used to study trophoblast invasion. Wang et al. used a fibrin-based scaffold to layer epithelial endometrial cells on top of stromal cells, embedded into a hydrogel. The endometrial epithelial cells polarized on top of the stromal layer, resulting in the spontaneous formation of epithelial glands. Time-dependent experiments demonstrated a high rate of attachment of TE-spheroids to the epithelium [159] (Table 1). A similar system was employed by You et al. to determine the impact of endometrial factors on trophoblast cell function. Endometrial stromal cells, covered in a layer of Matrigel, promoted the migration and invasion of TE-spheroids [160]. Such models will serve as useful tools for studying the molecular mechanism of embryo-maternal interactions. Nevertheless, their reproducibility and flexibility to integrate additional components, such as immune cells, will be an essential factor for further applications.

Models incorporating an endometrial perivascular niche for studying blastocyst adhesion and invasion are still profoundly lacking. To examine trophoblast interactions with maternal vasculature, Cartwright et al. developed a 3D *in vitro* model using spiral artery explants, extravillous trophoblast cell lines and primary cytotrophoblasts [161]. The platform demonstrated that trophoblasts are capable of interacting with maternal blood vessels, as cells attached to the vessel surface and invaded into the vessel wall. While a variety of *in vitro* models using explant cultures have been developed to study cellular interactions at the embryo-maternal interface [162–164], tissue degradation generally

limits the utility of these platforms, rendering them unsuitable for long-term studies. Moreover, evaluation of individual cell components remains a challenge.

In an effort to define interactions between the endometrial perivascular niche, endometrial stromal cells and trophoblasts, several groups have generated "placenta-on-a-chip" models of chorionic villus to study cell-cell interaction at the fetal-maternal interface [165–172]. Nishiguchi et al. recapitulated the complexity of the placental barrier by culturing TE cells with an underlying network of fibroblast stroma and endothelial cells. While previous studies generally used immortalized cell lines, Nishiguchi et al. incorporated primary cytotrophoblasts in their model. Overall, placenta-on-chip technology promises to overcome the drawbacks of conventional *in vitro* platforms, with the potential to deliver more physiologically relevant insights. Nevertheless, capturing the full dynamics of the placental barrier remains a challenge. Moving forward, compatibility with high-throughput analysis platforms will be important for ensuring greater characterization and standardization of bioengineered models.

8. Conclusion

Emerging technologies have unlocked important opportunities for addressing essential research questions surrounding the early embryo-maternal interface (Fig. 1C). Technologies at the crossroads of bioengineering, developmental and stem cell biology provide greater control of culture conditions, delivering platforms for studying specific stages of human implantation. Boosted by cutting-edge omics technologies, the field is certainly gaining momentum. These robust high-throughput approaches have revealed important insights into early developmental programs, as well as the endometrial milieu. Omics studies have played a critical role in identifying new cell types, specific marker genes and exploring novel molecular mechanisms regulating the embryo-maternal crosstalk. Moreover, 3D high-resolution imaging techniques have enhanced morphological studies, providing valuable insights into the spatial architecture of endometrial cell types. Such insights have laid important groundwork for further fine-tuning of the *in vitro* culture environment. Accordingly, novel *in vitro* platforms using human cells and embryos are becoming increasingly more complex, enhancing our ability to capture specific stages of implantation. While certainly a challenging field of study, future efforts shaped to include an interdisciplinary approach will certainly drive significant change in understanding implantation and the early embryo-maternal microenvironment. We are likely to encounter great advances in this field of research, ultimately supporting the quest towards personalized medicine approaches for the treatment of infertility.

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References

[1] T.Y. Khong, F. De Wolf, W.B. Robertson, I. Brosens, Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants, BJOG: An Int. J. Obstet. Gynaecol. 93 (1986) 1049–1059, https://doi.org/10.1111/j.1471-0528.1986.tb07830.x.

[2] N.S. Macklon, J.P.M. Geraedts, B.C.J.M. Fauser, Conception to ongoing pregnancy: the "black box" of early pregnancy loss, Hum. Reprod. Update 8 (2002) 333–343, https://doi.org/10.1093/humupd/8.4.333.

[3] X. Wang, C. Chen, L. Wang, D. Chen, W. Guang, J. French, Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study, Fertil. Steril. 79 (2003) 577–584, https://doi.org/10.1016/s0015-0282(02)04694-0.

[4] V.A. Kushnir, D.H. Barad, D.F. Albertini, S.K. Darmon, N. Gleicher, Systematic review of worldwide trends in assisted reproductive technology 2004–2013, Reprod. Biol. Endocrinol. 15 (2017) 6, https://doi.org/10.1186/s12958-016-0225-2.

[5] T. Philipp, K. Philipp, A. Reiner, F. Beer, D.K. Kalousek, Embryoscopic and cytogenetic analysis of 233 missed abortions: factors involved in the pathogenesis of developmental defects of early failed pregnancies, Hum. Reprod. 18 (2003) 1724–1732, https://doi.org/10.1093/humrep/deg309.

[6] E.J. Margalioth, A. Ben-Chetrit, M. Gal, T. Eldar-Geva, Investigation and treatment of repeated implantation failure following IVF-ET, Hum. Reprod. 21 (2006) 3036–3043, https://doi.org/10.1093/humrep/del305.

[7] L.T. Polanski, M.N. Baumgarten, S. Quenby, J. Brosens, B.K. Campbell, N. J. Raine-Fenning, What exactly do we mean by 'recurrent implantation failure'? A systematic review and opinion, Reprod. Biomed. Online 28 (2014) 409–423, https://doi.org/10.1016/j.rbmo.2013.12.006.

[8] N. Macklon, Recurrent implantation failure is a pathology with a specific transcriptomic signature, Fertil. Steril. 108 (2017) 9–14, https://doi.org/10.1016/j.fertnstert.2017.05.028.

[9] K.Y. Lee, F.J. DeMayo, Animal models of implantation, Reproduction 128 (2004) 679–695, https://doi.org/10.1530/rep.1.00340.

[10] R.L. Stouffer, T.K. Woodruff, Nonhuman primates: a vital model for basic and applied research on female reproduction, prenatal development, and women's health, ILAR J. 58 (2017) 281–294, https://doi.org/10.1093/ilar/ilx027.

[11] E.W. Kuijk, L.T. van Tol, H. Van de Velde, R. Wubbolts, M. Welling, N. Geijsen, B. A. Roelen, The roles of FGF and MAP kinase signaling in the segregation of the epiblast and hypoblast cell lineages in bovine and human embryos, Development 139 (2012) 871–882, https://doi.org/10.1242/dev.071688.

[12] M. Roode, K. Blair, P. Snell, K. Elder, S. Marchant, A. Smith, J. Nichols, Human hypoblast formation is not dependent on FGF signalling, Dev. Biol. 361 (2012) 358–363, https://doi.org/10.1016/j.ydbio.2011.10.030.

[13] R. John, M. Hemberger, A placenta for life, Reprod. Biomed. Online 25 (2012) 5–11, https://doi.org/10.1016/j.rbmo.2012.03.018.

[14] J.C. Cross, Z. Werb, S.J. Fisher, Implantation and the placenta: key pieces of the development puzzle, Science 266 (1994) 1508–1518, https://doi.org/10.1126/science.7985020.

[15] A. Psychoyos, Uterine receptivity for nidation, Ann. N. Y. Acad. Sci. 476 (1986) 36–42, https://doi.org/10.1111/j.1749-6632.1986.tb20920.x.

[16] D. Navot, R.T. Scott, K. Droesch, L.L. Veeck, H.C. Liu, Z. Rosenwaks, The window of embryo transfer and the efficiency of human conception in vitro, Fertil. Steril. 55 (1991) 114–118, https://doi.org/10.1016/s0015-0282(16)54069-2.

[17] J.J. Brosens, N. Hayashi, J.O. White, Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal Cells 1, Endocrinology 140 (1999) 4809–4820, https://doi.org/10.1210/endo.140.10.7070.

[18] B. Gellersen, I.A. Brosens, J.J. Brosens, Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives, Semin. Reprod. Med. 25 (2007) 445–453, https://doi.org/10.1055/s-2007-991042.

[19] G.J. Burton, T. Cindrova-Davies, M.Y. Turco, Review: histotrophic nutrition and the placental-endometrial dialogue during human early pregnancy, Placenta 102 (2020) 21–26, https://doi.org/10.1016/j.placenta.2020.02.008.

[20] J.J. Brosens, M.S. Salker, G. Teklenburg, J. Nautiyal, S. Salter, E.S. Lucas, J. H. Steel, M. Christian, Y.-W. Chan, C.M. Boomsma, J.D. Moore, G.M. Hartshorne, S. Šućurović, B. Mulac-Jericevic, C.J. Heijnen, S. Quenby, M.J. Groot Koerkamp, F.C.P. Holstege, A. Shmygol, N.S. Macklon, Uterine selection of human embryos at implantation, Sci. Rep. 4 (2014) 3894, https://doi.org/10.1038/srep03894.

[21] G. Teklenburg, M. Salker, C. Heijnen, N.S. Macklon, J.J. Brosens, The molecular basis of recurrent pregnancy loss: impaired natural embryo selection, Mol. Hum. Reprod. 16 (2010) 886–895, https://doi.org/10.1093/MOLEHR/GAQ079.

[22] C.H.E. Weimar, A. Kavelaars, J.J. Brosens, B. Gellersen, J.M.T. de Vreeden-Elbertse, C.J. Heijnen, N.S. Macklon, Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos, PLoS One 7 (2012), e41424, https://doi.org/10.1371/journal.pone.0041424.

[23] L. Loeb, The Production of Deciduomata and the Relation between the Ovaries and the Formation of the Decidua/Leo Loeb, JAMA - Journal of the American Medical Association, 1908.

[24] J.A. Sakoff, R.N. Murdoch, Alterations in uterine calcium ions during induction of the decidual cell reaction in pseudopregnant mice, J. Reprod. Fertil. 101 (1994) 97–102, https://doi.org/10.1530/jrf.0.1010097.

[25] B.C. Paria, W. Ma, J. Tan, S. Raja, S.K. Das, S.K. Dey, B.L. Hogan, Cellular and molecular responses of the uterus to embryo implantation can be elicited by locally applied growth factors, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1047–1052, https://doi.org/10.1073/pnas.98.3.1047.

[26] A.T. Hertig, J. Rock, E.C. Adams, A description of 34 human ova within the first 17 days of development, Am. J. Anat. 98 (1956) 435–493, https://doi.org/10.1002/aja.1000980306.

[27] H. Wang, S.K. Dey, Roadmap to embryo implantation: clues from mouse models, Nat. Rev. Genet. 7 (2006) 185–199, https://doi.org/10.1038/nrg1808.

[28] O.D. Genbacev, A. Prakobphol, R.A. Foulk, A.R. Krtolica, D. Ilic, M.S. Singer, Z.-Q. Yang, L.L. Kiessling, S.D. Rosen, S.J. Fisher, Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface, Science 299 (2003) 405–408, https://doi.org/10.1126/science.1079546.

[29] Y.-J. Kang, K. Forbes, J. Carver, J.D. Aplin, The role of the osteopontin-integrin αvβ3 interaction at implantation: functional analysis using three different in vitro models, Hum. Reprod. 29 (2014) 739–749, https://doi.org/10.1093/humrep/det433.

[30] M. Meseguer, J.D. Aplin, P. Caballero-Campo, J.E. O'Connor, J.C. Martín, J. Remohí, A. Pellicer, C. Simón, Human endometrial mucin MUC1 is up-

regulated by progesterone and down-regulated in vitro by the human blastocyst, Biol. Reprod. 64 (2001) 590–601, https://doi.org/10.1095/biolreprod64.2.590.
[31] J. Evans, L.A. Salamonsen, A. Winship, E. Menkhorst, G. Nie, C.E. Gargett, E. Dimitriadis, Fertile ground: human endometrial programming and lessons in health and disease, Nat. Rev. Endocrinol. 12 (2016) 654–667, https://doi.org/10.1038/nrendo.2016.116.
[32] R.W. Noyes, A.T. Hertig, J. Rock, Dating the endometrial biopsy, Fertil. Steril. 1 (1950) 3–25, https://doi.org/10.1016/S0015-0282(16)30062-0.
[33] W. Wang, F. Vilella, P. Alama, I. Moreno, M. Mignardi, A. Isakova, W. Pan, C. Simon, S.R. Quake, Single-cell transcriptomic atlas of the human endometrium during the menstrual cycle, Nat. Med. 26 (2020) 1644–1653, https://doi.org/10.1038/s41591-020-1040-z.
[34] D.D. Carson, E. Lagow, A. Thathiah, R. Al-Shami, M.C. Farach-Carson, M. Vernon, L. Yuan, M.A. Fritz, B. Lessey, Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening, Mol. Hum. Reprod. 8 (2002) 871–879, https://doi.org/10.1093/molehr/8.9.871.
[35] L.C. Kao, S. Tulac, S. Lobo, B. Imani, J.P. Yang, A. Germeyer, K. Osteen, R. N. Taylor, B.A. Lessey, L.C. Giudice, Global gene profiling in human endometrium during the window of implantation, Endocrinology 143 (2002) 2119–2138, https://doi.org/10.1210/endo.143.6.8885.
[36] J.M. Borthwick, D.S. Charnock-Jones, B.D. Tom, M.L. Hull, R. Teirney, S. C. Phillips, S.K. Smith, Determination of the transcript profile of human endometrium, Mol. Hum. Reprod. 9 (2003) 19–33, https://doi.org/10.1093/molehr/gag004.
[37] A. Riesewijk, J. Martín, R. van Os, J.A. Horcajadas, J. Polman, A. Pellicer, S. Mosselman, C. Simón, Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology, Mol. Hum. Reprod. 9 (2003) 253–264, https://doi.org/10.1093/molehr/gag037.
[38] A.P. Ponnampalam, G.C. Weston, A.C. Trajstman, B. Susil, P.A.W. Rogers, Molecular classification of human endometrial cycle stages by transcriptional profiling, Mol. Hum. Reprod. 10 (2004) 879–893, https://doi.org/10.1093/molehr/gah121.
[39] S. Talbi, A.E. Hamilton, K.C. Vo, S. Tulac, M.T. Overgaard, C. Dosiou, N. Le Shay, C.N. Nezhat, R. Kempson, B.A. Lessey, N.R. Nayak, L.C. Giudice, Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women, Endocrinology 147 (2006) 1097–1121, https://doi.org/10.1210/en.2005-1076.
[40] R.O. Burney, S. Talbi, A.E. Hamilton, K.C. Vo, M. Nyegaard, C.R. Nezhat, B. A. Lessey, L.C. Giudice, Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis, Endocrinology 148 (2007) 3814–3826, https://doi.org/10.1210/en.2006-1692.
[41] S. Altmäe, J.A. Martínez-Conejero, A. Salumets, C. Simón, J.A. Horcajadas, A. Stavreus-Evers, Endometrial gene expression analysis at the time of embryo implantation in women with unexplained infertility, Mol. Hum. Reprod. 16 (2010) 178–187, https://doi.org/10.1093/molehr/gap102.
[42] S. Altmäe, M. Koel, U. Võsa, P. Adler, M. Suhorutšenko, T. Laisk-Podar, V. Kukushkina, M. Saare, A. Velthut-Meikas, K. Krjutškov, L. Aghajanova, P. G. Lalitkumar, K. Gemzell-Danielsson, L. Giudice, C. Simón, A. Salumets, Meta-signature of human endometrial receptivity: a meta-analysis and validation study of transcriptomic biomarkers, Sci. Rep. 7 (2017) 10077, https://doi.org/10.1038/s41598-017-10098-3.
[43] D. Haouzi, H. Dechaud, S. Assou, J. De Vos, S. Hamamah, Insights into human endometrial receptivity from transcriptomic and proteomic data, Reprod. Biomed. Online 24 (2012) 23–34, https://doi.org/10.1016/j.rbmo.2011.09.009.
[44] R. Othman, M.H. Omar, L.P. Shan, M.N. Shafiee, R. Jamal, N.M. Mokhtar, Microarray profiling of secretory-phase endometrium from patients with recurrent miscarriage, Reprod. Biol. 12 (2012) 183–199, https://doi.org/10.1016/s1642-431x(12)60085-0.
[45] S. Hu, G. Yao, Y. Wang, H. Xu, X. Ji, Y. He, Q. Zhu, Z. Chen, Y. Sun, Transcriptomic changes during the pre-receptive to receptive transition in human endometrium detected by RNA-Seq, J. Clin. Endocrinol. Metab. 99 (2014) E2744–E2753, https://doi.org/10.1210/jc.2014-2155.
[46] B. Sigurgeirsson, H. Åmark, A. Jemt, D. Ujvari, M. Westgren, J. Lundeberg, S. Gidlöf, Comprehensive RNA sequencing of healthy human endometrium at two time points of the menstrual cycle, Biol. Reprod. 96 (2017) 24–33, https://doi.org/10.1095/biolreprod.116.142547.
[47] S. Messaoudi, I. El Kasmi, A. Bourdiec, K. Crespo, L. Bissonnette, C. Le Saint, F. Bissonnette, I.-J. Kadoch, 15 years of transcriptomic analysis on endometrial receptivity: what have we learnt? Fertil. Res. Pract. 5 (2019) 9, https://doi.org/10.1186/s40738-019-0059-7.
[48] M. Suhorutshenko, V. Kukushkina, A. Velthut-Meikas, S. Altmäe, M. Peters, R. Mägi, K. Krjutškov, M. Koel, F.M. Codoñer, J.F. Martinez-Blanch, F. Vilella, C. Simón, A. Salumets, T. Laisk, Endometrial receptivity revisited: endometrial transcriptome adjusted for tissue cellular heterogeneity, Hum. Reprod. 33 (2018) 2074–2086, https://doi.org/10.1093/humrep/dey301.
[49] A. Devesa-Peiro, P. Sebastian-Leon, F. Garcia-Garcia, V. Arnau, A. Aleman, A. Pellicer, P. Diaz-Gimeno, Uterine disorders affecting female fertility: what are the molecular functions altered in endometrium? Fertil. Steril. 113 (2020) 1261–1274, https://doi.org/10.1016/j.fertnstert.2020.01.025.
[50] K. Krjutškov, S. Katayama, M. Saare, M. Vera-Rodriguez, D. Lubenets, K. Samuel, T. Laisk-Podar, H. Teder, E. Einarsdottir, A. Salumets, J. Kere, Single-cell transcriptome analysis of endometrial tissue, Hum. Reprod. 31 (2016) 844–853, https://doi.org/10.1093/humrep/dew008.
[51] D. Haouzi, K. Mahmoud, M. Fourar, K. Bendhaou, H. Dechaud, J. De Vos, T. Rème, D. Dewailly, S. Hamamah, Identification of new biomarkers of human endometrial receptivity in the natural cycle, Hum. Reprod. 24 (2009) 198–205, https://doi.org/10.1093/humrep/den360.
[52] P. Díaz-Gimeno, J.A. Horcajadas, J.A. Martínez-Conejero, F.J. Esteban, P. Alamá, A. Pellicer, C. Simón, A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature, Fertil. Steril. 95 (2011) 50–60, https://doi.org/10.1016/j.fertnstert.2010.04.063, e15.
[53] M. Ruiz-Alonso, D. Blesa, P. Díaz-Gimeno, E. Gómez, M. Fernández-Sánchez, F. Carranza, J. Carrera, F. Vilella, A. Pellicer, C. Simón, The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure, Fertil. Steril. 100 (2013) 818–824, https://doi.org/10.1016/j.fertnstert.2013.05.004.
[54] M. Ruiz-Alonso, N. Galindo, A. Pellicer, C. Simón, What a difference two days make: "personalized" embryo transfer (pET) paradigm: a case report and pilot study, Hum. Reprod. 29 (2014) 1244–1247, https://doi.org/10.1093/humrep/deu070.
[55] J.A. Garcia-Velasco, A. Fassbender, M. Ruiz-Alonso, D. Blesa, T. D'Hooghe, C. Simon, Is endometrial receptivity transcriptomics affected in women with endometriosis? A pilot study, Reprod. Biomed. Online 31 (2015) 647–654, https://doi.org/10.1016/j.rbmo.2015.07.014.
[56] N. Mahajan, Endometrial receptivity array: clinical application, J. Hum. Reprod. Sci. 8 (2015) 121–129, https://doi.org/10.4103/0974-1208.165153.
[57] M. Enciso, J.P. Carrascosa, J. Sarasa, P.A. Martínez-Ortiz, S. Munné, J. A. Horcajadas, J. Aizpurua, Development of a new comprehensive and reliable endometrial receptivity map (ER Map/ER Grade) based on RT-qPCR gene expression analysis, Hum. Reprod. 33 (2018) 220–228, https://doi.org/10.1093/humrep/dex370.
[58] M. Enciso, J. Aizpurua, B. Rodríguez-Estrada, I. Jurado, M. Ferrández-Rives, E. Rodríguez, E. Pérez-Larrea, A.B. Climent, K. Marron, J. Sarasa, The precise determination of the window of implantation significantly improves ART outcomes, Sci. Rep. 11 (2021) 13420, https://doi.org/10.1038/s41598-021-92955-w.
[59] F. Meng, G. Zapantis, S.Z. Williams, H.J. Lieman, E. Buyuk, U.T. Meier, Status of nucleolar channel systems in uterine secretions accurately reflects their prevalence—a marker for the window of implantation—in simultaneously obtained endometrial biopsies, Fertil. Steril. 109 (2018) 165–171, https://doi.org/10.1016/j.fertnstert.2017.10.005.
[60] L. Craciunas, I. Gallos, J. Chu, T. Bourne, S. Quenby, J.J. Brosens, A. Coomarasamy, Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis, Hum. Reprod. Update 25 (2019) 202–223, https://doi.org/10.1093/humupd/dmy044.
[61] Z. Ben Rafael, Endometrial Receptivity Analysis (ERA) test: an unproven technology, Hum. Reprod. Open (2021) 2021, https://doi.org/10.1093/hropen/hoab010.
[62] A.M. Quaas, R.J. Paulson, Is the endometrial receptivity analysis batting high enough to warrant widespread-or at least selective-use? Fertil. Steril. 116 (2021) 341–342, https://doi.org/10.1016/j.fertnstert.2021.05.112.
[63] C.E. Gargett, S. Gurung, Endometrial mesenchymal stem/stromal cells, their fibroblast progeny in endometriosis, and more, Biol. Reprod. 94 (2016) 129, https://doi.org/10.1095/biolreprod.116.141325.
[64] H.P.T. Nguyen, L. Xiao, J.A. Deane, K.-S. Tan, F.L. Cousins, H. Masuda, C. N. Sprung, A. Rosamilia, C.E. Gargett, N-cadherin identifies human endometrial epithelial progenitor cells by in vitro stem cell assays, Hum. Reprod. 32 (2017) 2254–2268, https://doi.org/10.1093/humrep/dex289.
[65] D.K. Hapangama, J. Drury, L. Da Silva, H. Al-Lamee, A. Earp, A.J. Valentijn, D. P. Edirisinghe, P.A. Murray, A.T. Fazleabas, C.E. Gargett, Abnormally located SSEA1+/SOX9+ endometrial epithelial cells with a basalis-like phenotype in the eutopic functionalise layer may play a role in the pathogenesis of endometriosis, Hum. Reprod. 34 (2019) 56–68, https://doi.org/10.1093/humrep/dey336.
[66] S. Queckbörner, C. von Grothusen, N.R. Boggavarapu, R.M. Francis, L.C. Davies, K. Gemzell-Danielsson, Stromal heterogeneity in the human proliferative endometrium—a single-cell RNA sequencing study, J. Personalized Med. 11 (2021) 448, https://doi.org/10.3390/jpm11060448.
[67] O. Stegle, S.A. Teichmann, J.C. Marioni, Computational and analytical challenges in single-cell transcriptomics, Nat. Rev. Genet. 16 (2015) 133–145, https://doi.org/10.1038/nrg3833.
[68] L. Garcia-Alonso, L.-F. Handfield, K. Roberts, K. Nikolakopoulou, R.C. Fernando, L. Gardner, B. Woodhams, A. Arutyunyan, K. Polanski, R. Hoo, C. Sancho-Serra, T. Li, K. Kwakwa, E. Tuck, V. Kleshchevnikov, A. Tarkowska, T. Porter, C. I. Mazzeo, S. van Dongen, M. Dabrowska, V. Vaskivskyi, K.T. Mahbubani, J. Park, M. Jimenez-Linan, L. Campos, V. Kiselev, C. Lindskog, P. Ayuk, E. Prigmore, M. R. Stratton, K. Saeb-Parsy, A. Moffett, L. Moore, O.A. Bayraktar, S.A. Teichmann, M.Y. Turco, R. Vento-Tormo, Mapping the Temporal and Spatial Dynamics of the Human Endometrium in Vivo and in Vitro, 2021, https://doi.org/10.1101/2021.01.02.425073.
[69] A. Rao, D. Barkley, G.S. França, I. Yanai, Exploring tissue architecture using spatial transcriptomics, Nature 596 (2021) 211–220, https://doi.org/10.1038/s41586-021-03634-9.
[70] N. Tempest, M. Jansen, A.-M. Baker, C.J. Hill, M. Hale, D. Magee, D. Treanor, N. A. Wright, D.K. Hapangama, Histological 3D reconstruction and in vivo lineage tracing of the human endometrium, J. Pathol. 251 (2020) 440–451, https://doi.org/10.1002/path.5478.
[71] M. Yamaguchi, K. Yoshihara, K. Suda, H. Nakaoka, N. Yachida, H. Ueda, K. Sugino, Y. Mori, K. Yamawaki, R. Tamura, T. Ishiguro, T. Motoyama, Y. Watanabe, S. Okuda, K. Tainaka, T. Enomoto, Three-dimensional

understanding of the morphological complexity of the human uterine endometrium, iScience 24 (2021) 102258, https://doi.org/10.1016/j.isci.2021.102258.
[72] N.J. Hannan, P. Paiva, E. Dimitriadis, L.A. Salamonsen, Models for study of human embryo implantation: choice of cell lines? Biol. Reprod. 82 (2010) 235–245, https://doi.org/10.1095/biolreprod.109.077800.
[73] L.A. Salamonsen, T. Edgell, L.J.F. Rombauts, A.N. Stephens, D.M. Robertson, A. Rainczuk, G. Nie, N.J. Hannan, Proteomics of the human endometrium and uterine fluid: a pathway to biomarker discovery, Fertil. Steril. 99 (2013) 1086–1092, https://doi.org/10.1016/j.fertnstert.2012.09.013.
[74] F. Vilella, L.B. Ramirez, C. Simón, Lipidomics as an emerging tool to predict endometrial receptivity, Fertil. Steril. 99 (2013) 1100–1106, https://doi.org/10.1016/j.fertnstert.2012.12.026.
[75] T. Garrido-Gómez, A. Quiñonero, O. Antúnez, P. Díaz-Gimeno, J. Bellver, C. Simón, F. Domínguez, Deciphering the proteomic signature of human endometrial receptivity, Hum. Reprod. 29 (2014) 1957–1967, https://doi.org/10.1093/humrep/deu171.
[76] A. Canha-Gouveia, A. Paradela, A. Ramos-Fernández, M.T. Prieto-Sánchez, M. L. Sánchez-Ferrer, F. Corrales, P. Coy, Which low-abundance proteins are present in the human milieu of gamete/embryo maternal interaction? Int. J. Mol. Sci. 20 (2019) 5305, https://doi.org/10.3390/ijms20215305.
[77] Y. Zhang, Q. Wang, H. Wang, E. Duan, Uterine fluid in pregnancy: a biological and clinical outlook, Trends Mol. Med. 23 (2017) 604–614, https://doi.org/10.1016/j.molmed.2017.05.002.
[78] Y.H. Ng, S. Rome, A. Jalabert, A. Forterre, H. Singh, C.L. Hincks, L.A. Salamonsen, Endometrial exosomes/microvesicles in the uterine microenvironment: a new paradigm for embryo-endometrial cross talk at implantation, PLoS One 8 (2013), e58502, https://doi.org/10.1371/journal.pone.0058502.
[79] D. Tannetta, R. Dragovic, Z. Alyahyaei, J. Southcombe, Extracellular vesicles and reproduction–promotion of successful pregnancy, Cell. Mol. Immunol. 11 (2014) 548–563, https://doi.org/10.1038/cmi.2014.42.
[80] J. Evans, A. Rai, H.P.T. Nguyen, Q.H. Poh, K. Elglass, R.J. Simpson, L. A. Salamonsen, D.W. Greening, Human endometrial extracellular vesicles functionally prepare human trophectoderm model for implantation: understanding bidirectional maternal-embryo communication, Proteomics 19 (2019), e1800423, https://doi.org/10.1002/pmic.201800423.
[81] A. Bridi, F. Perecin, J.C. da Silveira, Extracellular vesicles mediated early embryo–maternal interactions, Int. J. Mol. Sci. 21 (2020) 1163, https://doi.org/10.3390/ijms21031163.
[82] A. Rai, Q.H. Poh, M. Fatmous, H. Fang, S. Gurung, B. Vollenhoven, L. A. Salamonsen, D.W. Greening, Proteomic profiling of human uterine extracellular vesicles reveal dynamic regulation of key players of embryo implantation and fertility during menstrual cycle, Proteomics 21 (2021), e2000211, https://doi.org/10.1002/pmic.202000211.
[83] T.T. Truong, Y.M. Soh, D.K. Gardner, Antioxidants improve mouse preimplantation embryo development and viability, Hum. Reprod. 31 (2016) 1445–1454, https://doi.org/10.1093/humrep/dew098.
[84] L.M. Desrochers, F. Bordeleau, C.A. Reinhart-King, R.A. Cerione, M.A. Antonyak, Microvesicles provide a mechanism for intercellular communication by embryonic stem cells during embryo implantation, Nat. Commun. 7 (2016) 11958, https://doi.org/10.1038/ncomms11958.
[85] M. Nishida, K. Kasahara, M. Kaneko, H. Iwasaki, K. Hayashi, [Establishment of a new human endometrial adenocarcinoma cell line, Ishikawa cells, containing estrogen and progesterone receptors], Nippon. Sanka Fujinka Gakkai Zasshi 37 (1985) 1103–1111.
[86] H. Kuramoto, S. Tamura, Y. Notake, Establishment of a cell line of human endometrial adenocarcinoma in vitro, Am. J. Obstet. Gynecol. 114 (1972) 1012–1019, https://doi.org/10.1016/0002-9378(72)90861-7.
[87] D.L. Way, D.S. Grosso, J.R. Davis, E.A. Surwit, C.D. Christian, Characterization of a new human endometrial carcinoma (RL95-2) established in tissue culture, In Vitro 19 (1983) 147–158, https://doi.org/10.1007/BF02618053.
[88] H. Hata, H. Kuramoto, Immunocytochemical determination of estrogen and progesterone receptors in human endometrial adenocarcinoma cells (Ishikawa cells), J. Steroid Biochem. Mol. Biol. 42 (1992) 201–210, https://doi.org/10.1016/0960-0760(92)90029-i.
[89] K. Tamm-Rosenstein, J. Simm, M. Suhorutshenko, A. Salumets, M. Metsis, Changes in the transcriptome of the human endometrial Ishikawa cancer cell line induced by estrogen, progesterone, tamoxifen, and mifepristone (RU486) as detected by RNA-sequencing, PLoS One 8 (2013), e68907, https://doi.org/10.1371/journal.pone.0068907.
[90] E. Chatzaki, C.J. Gallagher, R.K. Iles, T.E. Ind, A.M. Nouri, C.M. Bax, J. G. Grudzinskas, Characterisation of the differential expression of marker antigens by normal and malignant endometrial epithelium, Br. J. Cancer 69 (1994) 1010–1014, https://doi.org/10.1038/bjc.1994.198.
[91] A.J. Castelbaum, L. Ying, S.G. Somkuti, J. Sun, A.O. Ilesanmi, B.A. Lessey, Characterization of integrin expression in a well differentiated endometrial adenocarcinoma cell line (Ishikawa), J. Clin. Endocrinol. Metab. 82 (1997) 136–142, https://doi.org/10.1210/jcem.82.1.3658.
[92] S.R. Bhagwat, T. Redij, K. Phalnikar, S. Nayak, S. Iyer, S. Gadkar, U. Chaudhari, S. D. Kholkute, G. Sachdeva, Cell surfactomes of two endometrial epithelial cell lines that differ in their adhesiveness to embryonic cells, Mol. Reprod. Dev. 81 (2014) 326–340, https://doi.org/10.1002/mrd.22301.
[93] M. Haller, Y. Yin, L. Ma, Development and utilization of human decidualization reporter cell line uncovers new modulators of female fertility, Proc. Natl. Acad. Sci. Unit. States Am. 116 (2019) 19541–19551, https://doi.org/10.1073/pnas.1907652116.
[94] B. Gellersen, A. Wolf, M. Kruse, M. Schwenke, A.-M. Bamberger, Human endometrial stromal cell-trophoblast interactions: mutual stimulation of chemotactic migration and promigratory roles of cell surface molecules CD82 and CEACAM1, Biol. Reprod. 88 (2013) 80, https://doi.org/10.1095/biolreprod.112.106724.
[95] S. Ojosnegros, A. Seriola, A.L. Godeau, A. Veiga, Embryo implantation in the laboratory: an update on current techniques, Hum. Reprod. Update 27 (2021) 501–530, https://doi.org/10.1093/humupd/dmaa054.
[96] C.A. Rinehart, B.D. Lyn-Cook, D.G. Kaufman, Gland formation from human endometrial epithelial cells in vitro, Vitro Cell Dev. Biol. 24 (1988) 1037–1041, https://doi.org/10.1007/BF02620878.
[97] M. Bläuer, P.K. Heinonen, P.M. Martikainen, E. Tomás, T. Ylikomi, A novel organotypic culture model for normal human endometrium: regulation of epithelial cell proliferation by estradiol and medroxyprogesterone acetate, Hum. Reprod. 20 (2005) 864–871, https://doi.org/10.1093/humrep/deh722.
[98] M. Boretto, B. Cox, M. Noben, N. Hendriks, A. Fassbender, H. Roose, F. Amant, D. Timmerman, C. Tomassetti, A. Vanhie, C. Meuleman, M. Ferrante, H. Vankelecom, Development of organoids from mouse and human endometrium showing endometrial epithelium physiology and long-term expandability, Development 144 (2017) 1775–1786, https://doi.org/10.1242/dev.148478.
[99] M.Y. Turco, L. Gardner, J. Hughes, T. Cindrova-Davies, M.J. Gomez, L. Farrell, M. Hollinshead, S.G.E. Marsh, J.J. Brosens, H.O. Critchley, B.D. Simons, M. Hemberger, B.-K. Koo, A. Moffett, G.J. Burton, Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium, Nat. Cell Biol. 19 (2017) 568–577, https://doi.org/10.1038/ncb3516.
[100] T. Cindrova-Davies, X. Zhao, K. Elder, C.J.P. Jones, A. Moffett, G.J. Burton, M. Y. Turco, Menstrual flow as a non-invasive source of endometrial organoids, Commun Biol 4 (2021) 1–8, https://doi.org/10.1038/s42003-021-02194-y.
[101] E. Francés-Herrero, E. Juárez-Barber, H. Campo, S. López-Martínez, L. de Miguel-Gómez, A. Faus, A. Pellicer, H. Ferrero, I. Cervelló, Improved models of human endometrial organoids based on hydrogels from decellularized endometrium, J. Personalized Med. 11 (2021) 504, https://doi.org/10.3390/jpm11060504.
[102] U. Bentin-Ley, B. Pedersen, S. Lindenberg, J.F. Larsen, L. Hamberger, T. Horn, Isolation and culture of human endometrial cells in a three-dimensional culture system, Reproduction 101 (1994) 327–332, https://doi.org/10.1530/jrf.0.1010327.
[103] P.G.L. Lalitkumar, S. Lalitkumar, C.X. Meng, A. Stavreus-Evers, F. Hambiliki, U. Bentin-Ley, K. Gemzell-Danielsson, Mifepristone, but not levonorgestrel, inhibits human blastocyst attachment to an in vitro endometrial three-dimensional cell culture model, Hum. Reprod. 22 (2007) 3031–3037, https://doi.org/10.1093/humrep/dem297.
[104] C.-X. Meng, K.L. Andersson, U. Bentin-Ley, K. Gemzell-Danielsson, P.G. L. Lalitkumar, Effect of levonorgestrel and mifepristone on endometrial receptivity markers in a three-dimensional human endometrial cell culture model, Fertil. Steril. 91 (2009) 256–264, https://doi.org/10.1016/j.fertnstert.2007.11.007.
[105] T. Wiwatpanit, A.R. Murphy, Z. Lu, M. Urbanek, J.E. Burdette, T.K. Woodruff, J. J. Kim, Scaffold-free endometrial organoids respond to excess androgens associated with polycystic ovarian syndrome, J. Clin. Endocrinol. Metab. 105 (2020), https://doi.org/10.1210/clinem/dgz100 dgz100.
[106] Y. Abbas, L.G. Brunel, M.S. Hollinshead, R.C. Fernando, L. Gardner, I. Duncan, A. Moffett, S. Best, M.Y. Turco, G.J. Burton, R.E. Cameron, Generation of a three-dimensional collagen scaffold-based model of the human endometrium, Interface Focus 10 (2020) 20190079, https://doi.org/10.1098/rsfs.2019.0079.
[107] V.C. Cheung, C.-Y. Peng, M. Marinić, N.J. Sakabe, I. Aneas, V.J. Lynch, C. Ober, M.A. Nobrega, J.A. Kessler, Pluripotent stem cell-derived endometrial stromal fibroblasts in a cyclic, hormone-responsive, coculture model of human decidua, Cell Rep. 35 (2021) 109138, https://doi.org/10.1016/j.celrep.2021.109138.
[108] T.M. Rawlings, K. Makwana, D.M. Taylor, M.A. Molè, K.J. Fishwick, M. Tryfonos, J. Odendaal, A. Hawkes, M. Zernicka-Goetz, G.M. Hartshorne, J.J. Brosens, E. S. Lucas, Modelling the impact of decidual senescence on embryo implantation in human endometrial assembloids, Elife 10 (2021), e69603, https://doi.org/10.7554/eLife.69603.
[109] E. Dimitriadis, E. Menkhorst, S. Saito, W.H. Kutteh, J.J. Brosens, Recurrent pregnancy loss, Nat. Rev. Dis. Prim. 6 (2020) 1–19, https://doi.org/10.1038/s41572-020-00228-z.
[110] J.M. Franasiak, D. Alecsandru, E.J. Forman, L.C. Gemmell, J.M. Goldberg, N. Llarena, C. Margolis, J. Laven, S. Schoenmakers, E. Seli, A review of the pathophysiology of recurrent implantation failure, Fertil. Steril. 116 (2021) 1436–1448, https://doi.org/10.1016/j.fertnstert.2021.09.014.
[111] E. Garreta, P. Prado, C. Tarantino, R. Oria, L. Fanlo, E. Martí, D. Zalvidea, X. Trepat, P. Roca-Cusachs, A. Gavaldà-Navarro, L. Cozzuto, J.M. Campistol, J. C. Izpisúa Belmonte, C. Hurtado Del Pozo, N. Montserrat, Fine tuning the extracellular environment accelerates the derivation of kidney organoids from human pluripotent stem cells, Nat. Mater. 18 (2019) 397–405, https://doi.org/10.1038/s41563-019-0287-6.
[112] J.J. Velazquez, R. LeGraw, F. Moghadam, Y. Tan, J. Kilbourne, J.C. Maggiore, J. Hislop, S. Liu, D. Cats, S.M. Chuva de Sousa Lopes, C. Plaisier, P. Cahan, S. Kiani, M.R. Ebrahimkhani, Gene regulatory network analysis and engineering directs development and vascularization of multilineage human liver organoids, Cell Systems 12 (2021) 41–55, https://doi.org/10.1016/j.cels.2020.11.002, e11.
[113] G.H.D.R. Almeida, R.P. Iglesia, M.S. Araújo, A.C.O. Carreira, E.X. Dos Santos, C.V. A.Q. Calomeno, M.A. Miglino, Uterine Tissue Engineering: where We Stand and the Challenges Ahead, Tissue Eng Part B Rev, 2021, https://doi.org/10.1089/ten.TEB.2021.0062.

[114] M. Hellström, R.R. El-Akouri, C. Sihlbom, B.M. Olsson, J. Lengqvist, H. Bäckdahl, B.R. Johansson, M. Olausson, S. Sumitran-Holgersson, M. Brännström, Towards the development of a bioengineered uterus: comparison of different protocols for rat uterus decellularization, Acta Biomater. 10 (2014) 5034–5042, https://doi.org/10.1016/j.actbio.2014.08.018.
[115] E.G. Santoso, K. Yoshida, Y. Hirota, M. Aizawa, O. Yoshino, A. Kishida, Y. Osuga, S. Saito, T. Ushida, K.S. Furukawa, Application of detergents or high hydrostatic pressure as decellularization processes in uterine tissues and their subsequent effects on in vivo uterine regeneration in murine models, PLoS One 9 (2014), e103201, https://doi.org/10.1371/journal.pone.0103201.
[116] H. Campo, X. García-Domínguez, S. López-Martínez, A. Faus, J.S. Vicente Antón, F. Marco-Jiménez, I. Cervelló, Tissue-specific decellularized endometrial substratum mimicking different physiological conditions influences in vitro embryo development in a rabbit model, Acta Biomater. 89 (2019) 126–138, https://doi.org/10.1016/j.actbio.2019.03.004.
[117] F. Miki, T. Maruyama, K. Miyazaki, T. Takao, Y. Yoshimasa, S. Katakura, H. Hihara, S. Uchida, H. Masuda, H. Uchida, T. Nagai, S. Shibata, M. Tanaka, The orientation of a decellularized uterine scaffold determines the tissue topology and architecture of the regenerated uterus in rats, Biol. Reprod. 100 (2019) 1215–1227, https://doi.org/10.1093/biolre/ioz004.
[118] T.T. Tiemann, A.M. Padma, E. Sehic, H. Bäckdahl, M. Oltean, M.J. Song, M. Brännström, M. Hellström, Towards uterus tissue engineering: a comparative study of sheep uterus decellularisation, Mol. Hum. Reprod. 26 (2020) 167–178, https://doi.org/10.1093/molehr/gaaa009.
[119] S.A. Olalekan, J.E. Burdette, S. Getsios, T.K. Woodruff, J.J. Kim, Development of a novel human recellularized endometrium that responds to a 28-day hormone treatment, Biol. Reprod. 96 (2017) 971–981, https://doi.org/10.1093/biolre/iox039.
[120] R.C. Young, R. Schumann, P. Zhang, Three-dimensional culture of human uterine smooth muscle myocytes on a resorbable scaffolding, Tissue Eng. 9 (2003) 451–459, https://doi.org/10.1089/107632703322066633.
[121] K. Su, S.L. Edwards, K.S. Tan, J.F. White, S. Kandel, J.A.M. Ramshaw, C. E. Gargett, J.A. Werkmeister, Induction of endometrial mesenchymal stem cells into tissue-forming cells suitable for fascial repair, Acta Biomater. 10 (2014) 5012–5020, https://doi.org/10.1016/j.actbio.2014.08.031.
[122] J.C. Pence, K.B.H. Clancy, B.A.C. Harley, The induction of pro-angiogenic processes within a collagen scaffold via exogenous estradiol and endometrial epithelial cells, Biotechnol. Bioeng. 112 (2015) 2185–2194, https://doi.org/10.1002/bit.25622.
[123] A.M. Eissa, F.S.V. Barros, P. Vrljicak, J.J. Brosens, N.R. Cameron, Enhanced differentiation potential of primary human endometrial cells cultured on 3D scaffolds, Biomacromolecules 19 (2018) 3343–3350, https://doi.org/10.1021/acs.biomac.8b00635.
[124] S.A. Richardson, T.M. Rawlings, J. Muter, M. Walker, J.J. Brosens, N.R. Cameron, A.M. Eissa, Covalent attachment of fibronectin onto emulsion-templated porous polymer scaffolds enhances human endometrial stromal cell adhesion, infiltration, and function, Macromol. Biosci. 19 (2019) 1800351, https://doi.org/10.1002/mabi.201800351.
[125] L. Xin, X. Lin, Y. Pan, X. Zheng, L. Shi, Y. Zhang, L. Ma, C. Gao, S. Zhang, A collagen scaffold loaded with human umbilical cord-derived mesenchymal stem cells facilitates endometrial regeneration and restores fertility, Acta Biomater. 92 (2019) 160–171, https://doi.org/10.1016/j.actbio.2019.05.012.
[126] G. Zhao, Y. Cao, X. Zhu, X. Tang, L. Ding, H. Sun, J. Li, X. Li, C. Dai, T. Ru, H. Zhu, J. Lu, C. Lin, J. Wang, G. Yan, H. Wang, L. Wang, Y. Dai, B. Wang, R. Li, J. Dai, Y. Zhou, Y. Hu, Transplantation of collagen scaffold with autologous bone marrow mononuclear cells promotes functional endometrium reconstruction via downregulating ΔNp63 expression in Asherman's syndrome, Sci. China Life Sci. 60 (2017) 404–416, https://doi.org/10.1007/s11427-016-0328-y.
[127] Y. Cao, H. Sun, H. Zhu, X. Zhu, X. Tang, G. Yan, J. Wang, D. Bai, J. Wang, L. Wang, Q. Zhou, H. Wang, C. Dai, L. Ding, B. Xu, Y. Zhou, J. Hao, J. Dai, Y. Hu, Allogeneic cell therapy using umbilical cord MSCs on collagen scaffolds for patients with recurrent uterine adhesion: a phase I clinical trial, Stem Cell Res. Ther. 9 (2018) 192, https://doi.org/10.1186/s13287-018-0904-3.
[128] M. Hellström, S. Bandstein, M. Brännström, Uterine tissue engineering and the future of uterus transplantation, Ann. Biomed. Eng. 45 (2017) 1718–1730, https://doi.org/10.1007/s10439-016-1776-2.
[129] J.S. Gnecco, V. Pensabene, D.J. Li, T. Ding, E.E. Hui, K.L. Bruner-Tran, K. G. Osteen, Compartmentalized culture of perivascular stroma and endothelial cells in a microfluidic model of the human endometrium, Ann. Biomed. Eng. 45 (2017) 1758–1769, https://doi.org/10.1007/s10439-017-1797-5.
[130] S. Xiao, J.R. Coppeta, H.B. Rogers, B.C. Isenberg, J. Zhu, S.A. Olalekan, K. E. McKinnon, D. Dokic, A.S. Rashedi, D.J. Haisenleder, S.S. Malpani, C.A. Arnold-Murray, K. Chen, M. Jiang, L. Bai, C.T. Nguyen, J. Zhang, M.M. Laronda, T. J. Hope, K.P. Maniar, M.E. Pavone, M.J. Avram, E.C. Sefton, S. Getsios, J. E. Burdette, J.J. Kim, J.T. Borenstein, T.K. Woodruff, A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle, Nat. Commun. 8 (2017) 14584, https://doi.org/10.1038/ncomms14584.
[131] J. Ahn, M.-J. Yoon, S.-H. Hong, H. Cha, D. Lee, H.S. Koo, J.-E. Ko, J. Lee, S. Oh, N. L. Jeon, Y.-J. Kang, Three-dimensional microengineered vascularised endometrium-on-a-chip, Hum. Reprod. 36 (2021) 2720–2731, https://doi.org/10.1093/humrep/deab186.
[132] S. Lindenberg, M.H. Nielsen, S. Lenz, In vitro studies of human blastocyst implantationa, Ann. N. Y. Acad. Sci. 442 (1985) 368–374, https://doi.org/10.1111/j.1749-6632.1985.tb37541.x.
[133] C. Simón, A. Mercader, J. Garcia-Velasco, G. Nikas, C. Moreno, J. Remohí, A. Pellicer, Coculture of human embryos with autologous human endometrial epithelial cells in patients with implantation Failure 1, J. Clin. Endocrinol. Metabol. 84 (1999) 2638–2646, https://doi.org/10.1210/jcem.84.8.5873.
[134] R.R. González, P. Caballero-Campo, M. Jasper, A. Mercader, L. Devoto, A. Pellicer, C. Simon, Leptin and leptin receptor are expressed in the human endometrium and endometrial leptin secretion is regulated by the human Blastocyst 1, J. Clin. Endocrinol. Metabol. 85 (2000) 4883–4888, https://doi.org/10.1210/jcem.85.12.7060.
[135] A. Galán, J.E. O'Connor, D. Valbuena, R. Herrer, J. Remohí, S. Pampfer, A. Pellicer, C. Simón, The human blastocyst regulates endometrial epithelial apoptosis in embryonic Adhesion 1, Biol. Reprod. 63 (2000) 430–439, https://doi.org/10.1093/biolreprod/63.2.430.
[136] P. Caballero-Campo, F. Domínguez, J. Coloma, M. Meseguer, J. Remohí, A. Pellicer, C. Simón, Hormonal and embryonic regulation of chemokines IL-8, MCP-1 and RANTES in the human endometrium during the window of implantation, Mol. Hum. Reprod. 8 (2002) 375–384, https://doi.org/10.1093/molehr/8.4.375.
[137] F. Dominguez, A. Galan, J.J.L. Martin, J. Remohi, A. Pellicer, C. Simón, Hormonal and embryonic regulation of chemokine receptors CXCR1, CXCR4, CCR5 and CCR2B in the human endometrium and the human blastocyst, Mol. Hum. Reprod. 9 (2003) 189–198, https://doi.org/10.1093/molehr/gag024.
[138] J.A. Horcajadas, R. Catalano, B. Gadea, A. Sharkey, A. Pellicer, C. Simon, The human embryo-endometrial dialogue: impact of a single blastocyst in the gene expression pattern of endometrial epithelial cells, Fertil. Steril. 84 (2005) S60–S61, https://doi.org/10.1016/j.fertnstert.2005.07.145.
[139] C. Berger, N.R. Boggavarapu, J. Menezes, P.G.L. Lalitkumar, K. Gemzell-Danielsson, Effects of ulipristal acetate on human embryo attachment and endometrial cell gene expression in an in vitro co-culture system, Hum. Reprod. 30 (2015) 800–811, https://doi.org/10.1093/humrep/dev030.
[140] N.R. Boggavarapu, S. Lalitkumar, V. Joshua, S. Kasvandik, A. Salumets, P. G. Lalitkumar, K. Gemzell-Danielsson, Compartmentalized gene expression profiling of receptive endometrium reveals progesterone regulated ENPP3 is differentially expressed and secreted in glycosylated form, Sci. Rep. 6 (2016) 33811, https://doi.org/10.1038/srep33811.
[141] C. Le Saint, K. Crespo, A. Bourdiec, F. Bissonnette, K. Buzaglo, B. Couturier, S. Bisotto, S.J. Phillips, M. Stutz, J.-N. Gouze, J.S. Sampalis, S. Hamamah, I. J. Kadoch, Autologous endometrial cell co-culture improves human embryo development to high-quality blastocysts: a randomized controlled trial, Reprod. Biomed. Online 38 (2019) 321–329, https://doi.org/10.1016/j.rbmo.2018.12.039.
[142] A. Aberkane, W. Essahib, C. Spits, C. De Paepe, K. Sermon, T. Adriaenssens, S. Mackens, H. Tournaye, J.J. Brosens, H. Van de Velde, Expression of adhesion and extracellular matrix genes in human blastocysts upon attachment in a 2D co-culture system, Mol. Hum. Reprod. 24 (2018) 375–387, https://doi.org/10.1093/molehr/gay024.
[143] P.T. Ruane, C.J. Buck, P.A. Babbington, W. Aboussahoud, S.C. Berneau, M. Westwood, S.J. Kimber, J.D. Aplin, D.R. Brison, The effects of hyaluronate-containing medium on human embryo attachment to endometrial epithelial cells in vitro, Human Reproduction Open (2020) 2020, https://doi.org/10.1093/hropen/hoz033.
[144] X. Cheng, J. Liu, H. Shan, L. Sun, C. Huang, Q. Yan, R. Jiang, L. Ding, Y. Jiang, J. Zhou, G. Yan, H. Sun, Activating transcription factor 3 promotes embryo attachment via up-regulation of leukemia inhibitory factor in vitro, Reprod. Biol. Endocrinol. 15 (2017) 42, https://doi.org/10.1186/s12958-017-0260-7.
[145] X. Huang, H. Liu, R. Li, Prostaglandin E2 promotes BeWo spheroids implantation in RL95-2 cell monolayers, Gynecol. Endocrinol. 33 (2017) 548–552, https://doi.org/10.1080/09513590.2017.1296125.
[146] R. Jiang, L. Ding, J. Zhou, C. Huang, Q. Zhang, Y. Jiang, J. Liu, Q. Yan, X. Zhen, J. Sun, G. Yan, H. Sun, Enhanced HOXA10 sumoylation inhibits embryo implantation in women with recurrent implantation failure, Cell Death Dis. 3 (2017) 1–8, https://doi.org/10.1038/cddiscovery.2017.57.
[147] Y. Jiang, G. Yan, H. Zhang, H. Shan, C. Kong, Q. Yan, B. Xue, Z. Diao, Y. Hu, H. Sun, Activation of matrix metalloproteinase-26 by HOXA10 promotes embryo adhesion in vitro, Biochem. Biophys. Res. Commun. 445 (2014) 622–628, https://doi.org/10.1016/j.bbrc.2014.02.057.
[148] S. Liu, X. Yang, J. Wang, J. Wei, D. Zhang, X. Wang, Q. Yan, Differential expression of LeY and fucosyltransferase IV correlates with the receptivity of RL95-2 and HEC-1A human uterine epithelial cells, Cell Biol. Int. 36 (2012) 469–474, https://doi.org/10.1042/CBI20100644.
[149] Y. Miyazaki, A. Horie, H. Tani, M. Ueda, A. Okunomiya, K. Suginami, E. Kondoh, T. Baba, I. Konishi, T. Shinomura, Y. Sato, Versican V1 in human endometrial epithelial cells promotes BeWo spheroid adhesion in vitro, Reproduction 157 (2019) 53–64, https://doi.org/10.1530/REP-18-0333.
[150] X. Wei, S. Liu, X. Wang, Q. Yan, CD82 expression alters with human endometrial cycles and affects the uterine endometrial receptivity in vitro, Exp. Biol. Med. 237 (2012) 254–262, https://doi.org/10.1258/ebm.2011.011309.
[151] Y. Yang, Y. Sun, L. Cheng, A. Li, Y. Shen, L. Jiang, X. Deng, L. Chao, GRIM-19, a gene associated with retinoid-interferon-induced mortality, affects endometrial receptivity and embryo implantation, Reprod. Fertil. Dev. 29 (2017) 1447–1455, https://doi.org/10.1071/RD16104.
[152] M. Yu, J. Wang, S. Liu, X. Wang, Q. Yan, Novel function of pregnancy-associated plasma protein A: promotes endometrium receptivity by up-regulating N-fucosylation, Sci. Rep. 7 (2017) 5315, https://doi.org/10.1038/s41598-017-04735-0.
[153] Y. Xie, D. Cui, L. Sui, Y. Xu, N. Zhang, Y. Ma, Y. Li, Y. Kong, Induction of forkhead box M1 (FoxM1) by EGF through ERK signaling pathway promotes trophoblast

cell invasion, Cell Tissue Res. 362 (2015) 421–430, https://doi.org/10.1007/s00441-015-2211-y.
[154] Y. Xie, D. Cui, Y. Kong, FoxM1 influences embryo implantation and is regulated by 17 beta-estradiol and progesterone in mouse uteri and endometrium cells, Int. J. Clin. Exp. Pathol. 7 (2014) 6585–6595.
[155] P. Vergaro, G. Tiscornia, F. Zambelli, A. Rodríguez, J. Santaló, R. Vassena, Trophoblast attachment to the endometrial epithelium elicits compartment-specific transcriptional waves in an in-vitro model, Reprod. Biomed. Online 42 (2021) 26–38, https://doi.org/10.1016/j.rbmo.2020.08.037.
[156] B. Gellersen, K. Reimann, A. Samalecos, S. Aupers, A.-M. Bamberger, Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals, Hum. Reprod. 25 (2010) 862–873, https://doi.org/10.1093/humrep/dep468.
[157] A. Tapia-Pizarro, F. Argandoña, W.A. Palomino, L. Devoto, Human chorionic gonadotropin (hCG) modulation of TIMP1 secretion by human endometrial stromal cells facilitates extravillous trophoblast invasion in vitro, Hum. Reprod. 28 (2013) 2215–2227, https://doi.org/10.1093/humrep/det136.
[158] Z.-Y. Wang, J. Lu, Y.-Z. Zhang, M. Zhang, T. Liu, X.-L. Qu, Effect of Bisphenol A on invasion ability of human trophoblastic cell line BeWo, Int. J. Clin. Exp. Pathol. 8 (2015) 14355–14364.
[159] H. Wang, F. Pilla, S. Anderson, S. Martínez-Escribano, I. Herrer, J.M. Moreno-Moya, S. Musti, S. Bocca, S. Oehninger, J.A. Horcajadas, A novel model of human implantation: 3D endometrium-like culture system to study attachment of human trophoblast (Jar) cell spheroids, Mol. Hum. Reprod. 18 (2012) 33–43, https://doi.org/10.1093/molehr/gar064.
[160] Y. You, P. Stelzl, Y. Zhang, J. Porter, H. Liu, A.-H. Liao, P.B. Aldo, G. Mor, Novel 3D in vitro models to evaluate trophoblast migration and invasion, Am. J. Reprod. Immunol. 81 (2019), e13076, https://doi.org/10.1111/aji.13076.
[161] J.E. Cartwright, L.C. Kenny, P.R. Dash, I.P. Crocker, J.D. Aplin, P.N. Baker, G.S. J. Whitley, Trophoblast invasion of spiral arteries: a novel in vitro model, Placenta 23 (2002) 232–235, https://doi.org/10.1053/plac.2001.0760.
[162] D. Newby, L. Marks, F. Cousins, E. Duffie, F. Lyall, Villous explant culture: characterization and evaluation of a model to study trophoblast invasion, Hypertens. Pregnancy 24 (2005) 75–91, https://doi.org/10.1081/PRG-45785.
[163] M. Siwetz, A. Blaschitz, A. El-Heliebi, U. Hiden, G. Desoye, B. Huppertz, M. Gauster, TNF-α alters the inflammatory secretion profile of human first trimester placenta, Lab. Invest. 96 (2016) 428–438, https://doi.org/10.1038/labinvest.2015.159.
[164] D. Forstner, S. Maninger, O. Nonn, J. Guettler, G. Moser, G. Leitinger, E. Pritz, D. Strunk, K. Schallmoser, G. Marsche, A. Heinemann, B. Huppertz, M. Gauster, Platelet-derived factors impair placental chorionic gonadotropin beta-subunit synthesis, J. Mol. Med. (Berl.) 98 (2020) 193–207, https://doi.org/10.1007/s00109-019-01866-x.
[165] J.S. Lee, R. Romero, Y.M. Han, H.C. Kim, C.J. Kim, J.-S. Hong, D. Huh, Placenta-on-a-chip: a novel platform to study the biology of the human placenta, J. Matern. Fetal Neonatal Med. 29 (2016) 1046–1054, https://doi.org/10.3109/14767058.2015.1038518.
[166] N. Arumugasaamy, L.E. Ettehadieh, C.-Y. Kuo, D. Paquin-Proulx, S.M. Kitchen, M. Santoro, J.K. Placone, P.P. Silveira, R.S. Aguiar, D.F. Nixon, J.P. Fisher, P.C. W. Kim, Biomimetic placenta-fetus model demonstrating maternal–fetal transmission and fetal neural toxicity of zika virus, Ann. Biomed. Eng. 46 (2018) 1963–1974, https://doi.org/10.1007/s10439-018-2090-y.
[167] C. Blundell, Y.-S. Yi, L. Ma, E.R. Tess, M.J. Farrell, A. Georgescu, L.M. Aleksunes, D. Huh, Placental drug transport-on-a-chip: a microengineered in vitro model of transporter-mediated drug efflux in the human placental barrier, Adv Healthc Mater 7 (2018), https://doi.org/10.1002/adhm.201700786.
[168] Y. Zhu, F. Yin, H. Wang, L. Wang, J. Yuan, J. Qin, Placental barrier-on-a-chip: modeling placental inflammatory responses to bacterial infection, ACS Biomater. Sci. Eng. 4 (2018) 3356–3363, https://doi.org/10.1021/acsbiomaterials.8b00653.
[169] A. Nishiguchi, C. Gilmore, A. Sood, M. Matsusaki, G. Collett, D. Tannetta, I. L. Sargent, J. McGarvey, N.D. Halemani, J. Hanley, F. Day, S. Grant, C. Murdoch-Davis, H. Kemp, P. Verkade, J.D. Aplin, M. Akashi, C.P. Case, In vitro placenta barrier model using primary human trophoblasts, underlying connective tissue and vascular endothelium, Biomaterials 192 (2019) 140–148, https://doi.org/10.1016/j.biomaterials.2018.08.025.
[170] R.L. Pemathilaka, J.D. Caplin, S.S. Aykar, R. Montazami, N.N. Hashemi, Placenta-on-a-Chip: in vitro study of caffeine transport across placental barrier using liquid chromatography mass spectrometry, Global Chall. 3 (2019) 1800112, https://doi.org/10.1002/gch2.201800112.
[171] F. Yin, Y. Zhu, M. Zhang, H. Yu, W. Chen, J. Qin, A 3D human placenta-on-a-chip model to probe nanoparticle exposure at the placental barrier, Toxicol. Vitro 54 (2019) 105–113, https://doi.org/10.1016/j.tiv.2018.08.014.
[172] Y. Pu, J. Gingrich, A. Veiga-Lopez, A 3-dimensional microfluidic platform for modeling human extravillous trophoblast invasion and toxicological screening, Lab Chip 21 (2021) 546–557, https://doi.org/10.1039/D0LC01013H.