REVIEW

Organoids to model the endometrium: implantation and beyond

Thomas M Rawlings1, Komal Makwana1, Maria Tryfonos1 and Emma S Lucas1,2

1Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, UK
2Centre for Early Life, Warwick Medical School, University of Warwick, Coventry, UK

Correspondence should be addressed to E S Lucas: E.S.Lucas@warwick.ac.uk

Abstract

Despite advances in assisted reproductive techniques in the 4 decades since the first human birth after in vitro fertilisation, 1–2% of couples experience recurrent implantation failure, and some will never achieve a successful pregnancy even in the absence of a confirmed dysfunction. Furthermore, 1–2% of couples who do conceive, either naturally or with assistance, will experience recurrent early loss of karyotypically normal pregnancies. In both cases, embryo-endometrial interaction is a clear candidate for exploration. The impossibility of studying implantation processes within the human body has necessitated the use of animal models and cell culture approaches. Recent advances in 3-dimensional modelling techniques, namely the advent of organoids, present an exciting opportunity to elucidate the unanswerable within human reproduction. In this review, we will explore the ontogeny of implantation modelling and propose a roadmap to application and discovery.

Lay summary

A significant number of couples experience either recurrent implantation failure or recurrent pregnancy loss. Often, no underlying disorder can be identified. In both cases, the interaction of the embryo and maternal tissues is key. The lining of the womb, the endometrium, becomes receptive to embryo implantation during each menstrual cycle and provides a nourishing and supportive environment to support ongoing pregnancy. It is not possible to study early pregnancy directly, therefore, modelling embryo-endometrial interactions in the laboratory is essential if we wish to understand where this goes wrong. Advances in the lab have resulted in the development of organoids in culture: 3D cellular structures that represent the characteristics of a particular tissue or organ. We describe past and present models of the endometrium and propose a roadmap for future work with organoid models, from fundamental understanding of the endometrial function and implantation processes to the development of therapeutics to improve pregnancy outcomes and gynaecological health.

Key Words: ► embryo implantation ► endometrium ► organoid ► assembloid

Introduction

Although reported success rates in assisted reproduction approach or even surpass natural conceptions, 1–2% of couples will experience recurrent implantation failure, defined as the absence of a positive pregnancy test after three transfers of high-quality embryos (Mascarenhas et al. 2021). Some will never achieve a successful pregnancy even in the absence of a confirmed dysfunction. Furthermore, 1–2% of couples who do conceive, either naturally or
with assistance, will experience recurrent early loss of karyotypically normal pregnancies with no identifiable cause even after extensive clinical investigation (Bender Atik et al. 2018). In both cases, the loss of overtly ‘normal’ embryos suggests that defects of embryo-endometrial interaction might be a plausible explanation.

The purpose of any model system is to generate a physical, theoretical, or mathematical representation of a real phenomenon that is difficult or impractical to observe directly (Rogers 2012), a situation entirely descriptive of the study of human implantation. The ethical and practical impossibility of studying implantation processes within the human body has necessitated the use of animal models and cell culture approaches. These approaches have revealed considerable detail about the requirements for successful implantation but remain far from ideal. Recent advances in 3-dimensional (3D) modelling techniques, namely the advent of organoids, present an exciting opportunity to elucidate the unanswerable within human reproduction. In this review, we will explore the ontogeny of implantation modelling and propose a roadmap to application and discovery.

The endometrium and implantation

The mucosal lining of the uterus, the endometrium, is a complex tissue comprising luminal and glandular epithelium, as well as stromal, endothelial, and immune cells and provides a nourishing, immune-privileged environment to support successful embryo implantation (Gellersen & Brosens 2014). Formed of two distinct layers, the endometrium undergoes hormone-dependent cyclic renewal with proliferation, differentiation, shedding (menstruation), and regeneration of the upper functional layer in each cycle. Spontaneous, embryo-independent transformation of the tissue, termed decidualisation, occurs in the midluteal phase of each cycle facilitating the development of the decidual of pregnancy to support placentaion and foetal development until parturition. Humans are one of only a handful of species in which spontaneous decidualisation has evolved. This is proposed to reflect the need for maternal control over the challenge posed by genetically diverse and mosaic embryos in species where deep haemochorial placentation requires complete maternal investment in the pregnancy (Gellersen & Brosens 2014).

Functional defects in the endometrium, and particularly in the decidualisation process, have been associated with a spectrum of reproductive disorders, ranging from implantation failure and miscarriages to major obstetrical syndromes such as preeclampsia, foetal growth restriction, and preterm birth (reviewed by Lucas et al. 2013). However, other than histological descriptions of embryo implantation in hysterectomy samples from the early and mid-20th century (Hertig et al. 1956), we know very little about the earliest stages of implantation of the human blastocyst and the pivotal interactions taking place between the midluteal endometrium and the embryo. In vivo studies of dynamic implantation processes have been dependent on the use of animal models, while the generation of in vitro systems has facilitated the study of human embryo development and endometrial physiology and pathology, albeit largely in isolation. The development of embryo-endometrium co-cultures that faithfully mimic in vivo processes will truly unlock the black box of human reproduction.

Modelling implantation

Animal models

In the absence of human in vivo studies, murine, ovine, and bovine models, among others, have contributed much to the study of endometrial biology and pregnancy. However, the extent to which animal models represent human physiology is limited due to marked interspecies differences in reproductive strategy, implantation, and placentation. These have been reviewed extensively elsewhere (Lee & DeMayo 2004, Carter 2007, 2020, Morrison et al. 2018).

Livestock models have been utilised to pioneer many techniques used in modern assisted reproductive technologies (ART) (reviewed by Morrison et al. 2018). Sheep and cattle have been used to model human pregnancy while also advancing their commercial productivity with similar goals to human ART: to improve conception and live birth rates and reduce the incidence of pregnancy loss. Considerable differences in maternal recognition of pregnancy, implantation timing, and placental morphology make direct inference to human implantation difficult, nonetheless, conserved mechanisms are present (Tinning et al. 2020). Notwithstanding the value of sheep to model human foetal development (Morrison et al. 2018), the utility of large domestic animals to model early human pregnancy is limited (Carter 2020). Mouse models have been used to explore all aspects of reproduction, including implantation and the establishment of pregnancy. The mouse has been a preferred model for researchers for a number of reasons including litter-bearing status, rapid conception and gestation, haemochorial placentation, and genetic tractability; all of which provide an abundance
of material for study and the ability to target specific pathways of interest to determine the impact on fecundity and fertility (Carter 2020). However, progesterone receptor expression dynamics vary greatly between humans and mice (Teilmann et al. 2006), and decidualisation in mouse is dependent on an embryonic stimulus (Lopes et al. 2004) rather than a spontaneous cyclic process. Overall, despite the many advances in our understanding of early pregnancy from animal models, interspecies differences have hindered the translation of understanding to human embryo implantation research.

**In vitro models**

To circumvent interspecies variability at implantation, in vitro models have been developed using primary endometrial epithelial or stromal cell cultures as well as endometrium-derived cell lines. In vitro endometrial models aim to represent at least certain aspects of the human implantation environment while retaining levels of experimental control and manipulability (Fig. 1 and Table 1). In combination with a range of molecular techniques and other assays, these models present effective tools to close the knowledge gap regarding human embryo implantation and answer a diverse panel of questions.

**Monolayer cultures**

Two-dimensional (2D) cultures of endometrial stromal and epithelial cells have both been utilised to investigate implantation processes (Fig. 1). Since the first report using monolayer epithelial cells to describe interactions between the luminal epithelium and an implanting embryo (Lindenberg et al. 1985), many studies have used this approach to study early feto-maternal interactions (Weimar et al. 2013, Aplin & Ruane 2017). In response to embryonic signals, co-cultured epithelial cells upregulate expression of the receptivity genes CXC chemokine receptor type 1 (CXCR1), CXCR4, and CXCR5, and the chemokine interleukin (IL)-8 (Caballero-Campo et al. 2002, Dominguez et al. 2003), encoded by CXCL8, as well as epithelial cell surface molecules implicated in embryo implantation, such as mucin 1 (MUC1) (Simón et al. 1997, Meseguer et al. 2001) and osteopontin (OPN) (von Wolff et al. 2001, Erikson et al. 2009, Berneau et al. 2019) (Table 1). Co-culture of in vitro fertilised (IVF) embryos with an autologous epithelial monolayer prior to embryo transfer increased the frequency of high-quality blastocyst formation and implantation rates (Simón et al. 1999, Le Saint et al. 2019). However, a significant benefit of the co-culture to live birth rates remains to be confirmed (Le Saint et al. 2019). Trophoblast spheroids, as a surrogate for human blastocysts, have also been used successfully in co-culture with epithelial monolayers to investigate embryo attachment to the luminal epithelium (Weimar et al. 2013, Lee et al. 2015, Huang et al. 2017, Evans et al. 2020, Ruane et al. 2020) (Table 1). These studies demonstrate the reciprocal relationship between luminal epithelium and preimplantation embryos in preparation for human implantation. However, endometrial epithelial cells are technically challenging to propagate as monolayers and cannot be cultured long-term (Varma et al. 1982, Fitzgerald et al. 2021). They also do not represent the normal physiology and architecture of glands in vivo (Grav et al. 2001).

Primary human endometrial stromal cells have been used extensively to study human decidualisation and the peri-implantation endometrial environment. Studies of primary stromal monolayers have demonstrated endometrium-stroma interactions and the retention of patient phenotypes by these cells. Carver and colleagues (2003) co-cultured stromal cell monolayers with hatched day five human blastocysts for 3 days and demonstrated that blastocysts could attach to and invade a decidualised stromal monolayer but did not interact with undifferentiated cells. In midluteal endometrium, decidualising stromal cells transiently attain a myo-fibroblastic phenotype, in which they become

---

**Figure 1 In vitro endometrial models to study implantation. Schematic representations of existing models for modelling endometrium-embryo interactions. The main benefits (+) and shortfalls (-) of each system are indicated.**
| Model format/cell types | Embryo/spheroid | Measures of interaction | References |
|-------------------------|-----------------|-------------------------|------------|
| Monolayer               |                 |                         |            |
| Primary human           |                 |                         |            |
| endometrial stromal     | Hatched human   | Attachment (apposition  | Carver et al. (2003), |
| cells                   | blastocysts,    | and anchoring),         | Grewal et al. (2008, |
|                         | high- and       | trophoblast outgrowth    | 2010), Teklenburg  |
|                         | low-quality     | and invasion, hCG        | et al. (2010),     |
|                         | human embryos,  | secretion                | Weimar et al. (2012), |
|                         | human          |                         | Brosens et al. (2014), |
|                         | embryo-        |                         | Lee et al. (2015), |
|                         | conditioned     |                         | Berkhout et al. ( |
|                         | media, mouse    |                         | 2018)           |
|                         | blastocysts,    |                         |            |
|                         | human          |                         |            |
|                         | trophoblast     |                         |            |
|                         | spheroids,      |                         |            |
|                         | embryonic       |                         |            |
|                         | stem cell-      |                         |            |
|                         | derived         |                         |            |
|                         | trophoblast     |                         |            |
|                         | spheroids       |                         |            |
| Human endometrial       | Hatched and     | Attachment and          | Lindeberg et al. ( |
| stromal cell lines      | unhatched       | outgrowth/invasion      | 1985), Simón et al. ( |
| (T-HESC;                | human           |                         | 1997, 1999), Meseguer et al. ( |
| hTERT-immortalised      | blastocysts,    |                         | 2001), Caballero- |
| endometrial stromal    | human           |                         | Campo et al. (2002), |
| cells)                 | embryo-         |                         | Dominguez et al. (2003), |
|                         | conditioned      |                         | Lee et al. (2015), |
|                         | media, mouse    |                         | Le Saint et al. ( |
|                         | blastocysts,    |                         | 2019), Evans et al. ( |
|                         | human           |                         | 2020), Lee et al. ( |
|                         | embryonic       |                         | 2015), Huang et al. ( |
|                         | stem cell-      |                         | 2017), Berneau et al. ( |
|                         | derived         |                         | 2019), Evans et al. ( |
|                         | trophoblast      |                         | 2020), Ruane et al. ( |
|                         | spheroids       |                         | 2020).            |
|                         | (choriocarcinoma- |                         |            |
|                         | derived)        |                         |            |
| Human endometrial       | Hatched/hatching | Attachment and           | Bentin-Ley et al. ( |
| epithelial cell lines   | human           | trophoblast invasion    | 2000), Wang et al. ( |
| (Ishikawa, RL95-2)     | blastocysts,    | and syncytium formation | 2012).          |
|                         | trophoblast     |                         |            |
|                         | spheroids       |                         |            |
|                         | (choriocarcinoma-|                         |            |
|                         | derived)        |                         |            |
| Layered culture         | Expanded/hatching| Embryo attachment,      |            |
| Primary human           | human           | trophoblast invasion    |            |
| endometrial epithelial  | blastocysts,    | and syncytium formation |            |
| and stromal cells       | trophoblast      |                         |            |
|                         | spheroids (      |                         |            |
|                         | choriocarcinoma-|                         |            |
|                         | derived)        |                         |            |
migratory and can produce extracellular matrix (ECM)-degrading matrix metalloproteinases (MMP) (Anacker et al. 2011), a process which is essential to allow trophoblast outgrowth (Grewal et al. 2008, 2010). Decidualised trophoblast cells are sensitive to embryo quality, selectively migrating towards high-quality human embryos but downregulating implantation-associated genes and the migratory phenotype in the presence of poor-quality or arresting embryos or conditioned media (Teklenburg et al. 2010, Weimar et al. 2012, Brosens et al. 2014, Berkhout et al. 2018) (Table 1).

Monolayer stromal co-cultures with blastocysts have also been used to investigate endometrial function and dysfunction in reproductive failure. Decidualising stromal cells of recurrent pregnancy loss (RPL) patients fail to inhibit implantation genes when co-cultured with poor-quality embryos (Weimar et al. 2012). This suggests a defect in embryo quality ‘biosensing’ in the endometrium of these women, manifesting as a loss of embryo selectivity. Also referred to as the ‘selection failure hypothesis, this failure to prevent implantation of poor-quality embryos is predicted to lead to subsequent miscarriage (Aplin et al. 1996, Macklon & Brosens 2014).

Overall, monolayer endometrial cell models provide a simple and robust model for studying early embryo implantation and embryo-endometrial interactions in humans. A major disadvantage of monolayer models is the restriction to a single endometrial cell type, disregarding stromal-epithelial interactions within the in vivo environment, as well as the roles of resident immune and endothelial cell populations. Additionally, although these models have been utilised to study the initial attachment and invasion of embryos, they lack the 3D architecture essential to study trophoblast invasion and the foundations of placentation.

**Layered co-cultures**

Glandular development and differentiation are dependent on stromal secretions (reviewed by Fitzgerald et al. 2021), exemplifying the dependence of the peri-implantation endometrial environment on synchronous paracrine signals, cell–cell, and cell-matrix interactions between multiple cell types of the decidualising endometrium. To study these interactions, more complex co-culture models have been developed (Fig. 1 and Table 1) and these will be explored briefly here.

To establish 3D cultures, cells are seeded into or on top of a scaffold to mimic their spatial environment within the structure of the ECM. The most common scaffold is the hydrogel, a hydrophilic polymer chain network that can be easily fine-tuned to mimic the structural
properties of a native tissue ECM. Layered co-cultures of endometrial epithelial cells grown over a stromal cell-containing collagen hydrogel resemble a simplified human endometrium and aim to replicate the complexity of the in vivo endometrium more closely (Bentin-Ley et al. 1994, Evron et al. 2011, Wang et al. 2012). Luminal epithelium polarisation and embryo attachment within 48 h were revealed in one such model by scanning electron microscopy, with apparent penetration of syncytiotrophoblast through the epithelium into the underlying stromal layer (Bentin-Ley et al. 2000). In place of embryos, trophoblast cell line spheroids, such as the JAr cell line (John et al. 1993), have also been utilised to study implantation in layered co-culture models (Evron et al. 2011, Wang et al. 2012). The attachment of trophoblast spheroids to epithelial cell lines was improved by co-culturing with stromal cells (Wang et al. 2012) and dependent on the receptivity status of the stromal cells (Evron et al. 2011). Occasional spontaneous gland-like structures were observed in these cultures (Wang et al. 2012) although layered models generally fail to form glandular structures reproducibly and thus while useful for studying very early implantation processes, are not truly representative of the tissue at implantation.

Transwell co-culture models enhanced the ability to study trophoblast invasion: using dual-chambered systems containing an extracellular matrix (Matrigel®)-coated porous filter insert, adherent trophoblast cells can invade the matrix and migrate through the filter (Aplin et al. 2006, Lash et al. 2007) (Fig. 1). By seeding endometrial epithelial cells on top of the Matrigel® in the transwell insert and culturing stromal cells in the well beneath the filter (Arnold et al. 2001, Pierro et al. 2001, Bläuer et al. 2005), the transwell approach provides an easily manipulatable assay to assess trophoblast invasion in the presence of endometrial cells. The presence of trophoblasts also improves the invasiveness of deciduaedal stromal cells in transwell systems (Gellersen et al. 2010, 2013). However, cell–cell contact between epithelial and stromal cells is not established in these models and so critical interactions to orchestrate trophoblast invasion and endometrial tissue remodelling are likely to be missing.

Clearly, layered and transwell co-culture approaches improve upon monolayer cultures by incorporating both stromal and epithelial cells into a 3D space, providing the architecture for a more physiological model to study implantation. However, these models conspicuously lack glandular structures, a functional unit of the endometrium essential for embryo implantation.

Modelling endometrial glands: the missing link?

Secretory transformation of endometrial glands results in the production of histotroph, or ‘uterine milk’. Histotroph composition has been studied extensively during the menstrual cycle, consisting largely of glycogen and glycoproteins, as well as other components such as amino acids and lipid droplets (Burton et al. 2002). This rich secreted product provides nutrition for the embryo until the onset of placental perfusion (Burton et al. 2007, 2010). Studies using animal models, such as the uterine gland knockout sheep, produced by a progestin-induced gland knock out, demonstrate that without endometrial glands, embryo implantation is inhibited (Gray et al. 2001). Progestin uterine gland knockout mice also exhibit implantation failure, with reduced expression of key implantation genes (Kelleher et al. 2016).

Besides providing nutritional support to the implanting embryo, endometrial glands upregulate genes required for embryo receptivity and implantation, including numerous secreted factors essential for implantation (Fig. 2). For example, osteopontin (OPN), encoded by phosphoprotein 1 (SPP1), contains the integrin-binding motif RGD, a tripeptide of amino acids arginine, glycine, and aspartate, which facilitates embryo adhesion to the luminal epithelium (Singh & Aplin 2009, Berneau et al. 2019). Leukaemia inhibitory factor, encoded by LIF, is a protein related to blastocyst adhesion, as well as having roles in embryonic development and trophoblast differentiation (Salleh & Giribabu 2014). Glycolisin, a dimeric glycoprotein, encoded by progestogen-associated endometrial protein (PAEP), also participates in interactions between the implanting blastocyst and luminal epithelium. In vitro, induction of glycolisin secretion improves trophoblast spheroid attachment while silencing PAEP inhibits attachment (Uchida et al. 2007, So et al. 2012).

Human endometrial glands also secrete the factors EGF, vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGFβ), the cognate receptors for which are expressed by the trophectoderm of the implanting blastocyst. EGF stimulates proliferation of cytrophoblast and secretion of human chorionic gonadotropin (hCG) and human placental lactogen (hPL) by the syncytiotrophoblast (Ladines-Llave et al. 1991, Maruo et al. 1997) (Fig. 2). VEGF enhances the adhesion of the trophoblast to the luminal epithelium (Binder et al. 2014), while TGFβ increases ECM remodelling, via the secretion of fibronectin, for example, thereby facilitating

https://raf.bioscientifica.com
https://doi.org/10.1530/RAF-21-0023
© 2021 The authors
Published by Bioscientifica Ltd

This work is licensed under a Creative Commons Attribution 4.0 International License.
http://creativecommons.org/licenses/by/4.0/
endometrial adhesion for invading trophoblasts (Feinberg et al. 1994).

Overall, the glands and their secretions are essential for implantation, and therefore, being able to model the glands in vitro is an essential step towards studying and understanding human embryo implantation and early pregnancy events. Recent development of glandular organoid models may have brought us closer than ever to establish a physiological model of the human endometrium.

Organoids of the reproductive system

‘Organoid’ describes 3D structures that resemble organs or tissues ex vivo in a supportive hydrogel droplet, such as Matrigel”, replacing the ECM of the originating tissue. Organoids arise from single cells or small clusters of cells with stem or progenitor properties in cultures with complex growth media designed to mimic organ- or tissue-specific signalling pathways and recapitulate the niche environment (Kim et al. 2020b). Organoids differ from previous in vitro model systems in that they spontaneously organise into architectures that resemble their corresponding in vivo tissue or organ in a culture plate. Additionally, organoid models functionally mimic their tissue of origin and can recapitulate developmental processes that take place in vivo, thus allowing the study of developing and differentiating cells in real time. Growth factor and inhibitor combinations in these complex media support proliferation and renewal as well as differentiation and substitute for the absence of the tissue complexity present in vivo. Most reported models employ a consistent core set of factors that are reviewed elsewhere (Kretzschmar & Clevers 2016, Alzamil et al. 2021). Organoids have been generated from the vast majority of endoderm-derived tissues including the colon (Sato et al. 2011), intestine (Fujii et al. 2018), stomach (Bartfeld et al. 2015, Schlaermann et al. 2016), liver (Huch et al. 2015, Hu et al. 2018), pancreas (Loomans et al. 2018), lung/airway (Sachs et al. 2019) and bladder (Lee et al. 2018).

Several recent reports have demonstrated progress in modelling human reproductive epithelia using organoid systems. In the female reproductive system, endometrium (Boretto et al. 2017, Turco et al. 2017), fallopian tube (Kessler et al. 2015), cervix (Chumduri et al. 2021, Maru et al. 2020, Lohmuusaar et al. 2021), and ovarian cancer (Kopper et al. 2019, Maenhoudt et al. 2020) models have been described (Fig. 3), as well as mammary gland (Linnemann et al. 2015). These models facilitate the study of the general biology and histology of the reproductive tissues since they recapitulate epithelial cell morphology, function, and cellular heterogeneity while being genetically stable. These organoids are hormone-responsive and can be differentiated under specific conditions (reviewed by Alzamil et al. 2021). In the male reproductive tract, organoid systems are described for both testis (Alves-Lopes et al. 2017, Baert et al. 2017, Pendergraft et al. 2017) and prostate (Chua et al. 2014, Karthaus et al. 2014). Trophoblast organoids to model placental development (Haider et al. 2018, Turco et al. 2018), as well as embryo-like organoids, known as blastoids have also been described (Zheng et al. 2019, Liu et al. 2021,
Yu et al. 2021). Human blastoids recapitulate the key morphology of preimplantation blastocysts, with cell-lineage composition and allocation, with transcriptomic similarities but lack important structures like the zona pellucida (Liu et al. 2021, Yu et al. 2021). Blastoids and trophoblast organoids both offer the opportunity to study implantation and early developmental processes at a greater scale than possible with research embryos, and in cases where embryos are unavailable for research use or such applications are prohibited.

The emergence of these organoid models for human reproduction and fertility represents a huge leap forward for the field, presenting a considerable opportunity to advance our knowledge of many ‘hidden’ processes within these systems. Limitations remain, for example, ease of accessibility means comparisons with healthy tissue are limited in some cases, while optimisation of other models is still required.

Endometrial gland organoids

Organoid-like structures established from endometrial epithelial cells were first reported in 1988, derived from primary gland fragments seeded into Matrigel™ (Rinehart et al. 1988). Glandular structures were retained after tissue digest collapsed and monolayer colony outgrowth was observed within 7 to 10 days. Following several weeks of culture, these colonies formed large cystic organoid structures of polarised columnar epithelial cells with luminal microvilli and could be maintained in culture for at least 6 months. Endometrial gland organoids were also produced in Matrigel™ and grown in a transwell system, in co-culture with stromal cells grown in the lower chamber (Bläuer et al. 2005). In this system, the organoids were responsive to oestradiol treatment in the presence of the stromal cell layer. These reports failed to recapitulate the gland phenotype fully, and genetic stability was not confirmed, nor were the culture conditions chemically defined. Renewed interest in endometrial gland organoids was stimulated recently by parallel reports delivering a step-change in our ability to study the function of these glands.

Figure 3 Organoid models of the female reproductive tract. In the human female reproductive tract, organoid models have been derived from the endometrium, fallopian tube, ovary, and cervix tissues and recapitulate the epithelial structure of the tissue of origin. All systems have been cultured in basal medium including Advanced DMEM/F12, B-27, and N-2 supplements, antibiotics, and L-Glutamine but also include a cocktail of growth factors and inhibitors to promote proliferation and maintain undifferentiated or progenitor cell-like conditions. Common to all models is the growth factor EGF and the WNT signalling activator R-Spondin-1, as well as the ROCK inhibitor Y-27632 and BMP pathway inhibitor Noggin, and Nicotinamide, a survival factor, and ROCK inhibitor. Other factors which are often added include the TGFB receptor inhibitor A83-01, the activin/ BMP/TGFB inhibitor, SB431542, and the p38 MAPK inhibitor SB202190; Growth factors FGF-10 (fibroblast growth factor) and WNT3A as well as other factors such as N-acetyl-L-cysteine (antioxidant), β-estradiol (mitogen), heregulin-β (growth factor), forskolin (adenyl cyclase activator), and hydrocortisone (glucocorticoid).

endometrial gland organoids are cultured in Matrigel™ and are dependent on a chemically defined complex ‘expansion medium’ containing a variety of growth factors required to promote proliferation and inhibit differentiation, including fibroblast growth factor 10 (FGF10), EGF, Wingless and Int1 (WNT) signalling activator R-spondin 1 (RSPON1), bone morphogenetic protein (BMP) inhibitor Noggin, and the TGFB antagonist, A83-01. Unlike the initial report (Rinehart et al. 1988), these recent models see rapid organoid formation from gland fragments within 7–10 days. Oestrogen treatment induces proliferation of the cells with an increase in Ki67+ cells (Borett et al. 2017) as well as stimulating ciliogenesis (Haider et al. 2019) and, when combined with progesterone, secretion of glycolenin-A corresponds to tissue profiles in mid-secretory endometrium (Ludii et al. 2020). Glycolenin
profiles were further modulated after incubation of organoids with embryo-conditioned medium (Luddi et al. 2021), confirming embryo-endometrial crosstalk is possible within the model (Table 1). Metabolomic analysis of the secretome of endometrial organoids also demonstrates unique characteristics of the apical (inter-organoid) and basolateral (extra-organoid) secretory profiles, characteristic of that predicted in vivo (Simintiras et al. 2021). Through exposure to pregnancy signals of the stroma (cyclic AMP and prolactin (PRL)) and trophoblast (hCG and hPRL), the organoids acquired a decidual-like phenotype akin to early pregnancy (Turco et al. 2017).

Thus far, endometrial gland organoid models are still in their infancy, albeit one that surpasses prior systems physiologically. The opportunity to address questions of normal endometrial physiology and implantation processes, therefore, lies along our path.

**Endometrial gland organoids and the future: a roadmap to utility**

**Endometrial gland organoids, assembloids, and modelling embryo implantation**

Despite their structural and functional recapitulation of endometrial gland phenotypes, endometrial organoid models do not faithfully represent the midluteal endometrium, due to being grown in isolation without the influence of other endometrial cell types present in the tissue. Endometrial stromal cells are essential for glandular regeneration, expansion, and differentiation throughout the menstrual cycle. For example, through the secretion of PRL, decidualising stroma regulates gland differentiation via the PRL receptor (PRLR), whose expression peaks in the endometrial glands during the mid-secretory phase (Jones et al. 1998). PRL induces glandular differentiation by stimulating the Janus kinase (JAK)/STAT5 and mitogen-activated protein kinase (MAPK) pathways. In parallel, apoptosis in the glandular epithelium is inhibited by activation of the phosphatidylinositol 3 kinase (PI3K) pathway (Jabbour et al. 2002).

Recently, several protocols have described stromal-epithelial co-cultures retaining physiological structure. Slices of full thickness endometrial tissue cultured in collagen gel maintain true histoarchitecture of the endometrium, and a decidual response similar to the in vivo profile can be induced following differentiation with oestradiol (E2) and progesterone (P4) (Muruganandan et al. 2020). Others have reported the use of porous 3D scaffolds to establish co-cultures. Using a collagen-based scaffold, Abbas and colleagues demonstrated that stromal cells were able to proliferate within the scaffold pores and deposit their own ECM, while fragments of endometrial gland organoids seeded on top of the scaffolds formed a luminal epithelium reminiscent of the tissue structure in vivo (Abbas et al. 2020). As well as solid scaffolds, synthetic hydrogels, such as polyethylene glycol (PEG), functionalised with ECM- and integrin-binding peptides have been utilised as a replacement for animal-derived hydrogels. When cultured in PEG hydrogels, endometrial stromal cells proliferate and decidualise in response to hormonal stimulation (Cook et al. 2017). Modulating the PEG hydrogel altered cell behaviour, highlighting the importance of matrix conditions when developing a representative model of the endometrium (Cook et al. 2017). Finally, another recent protocol reported self-aggregation of epithelial and stromal cells in a scaffold-free environment to form structures containing a stromal cell centre and an outer layer of epithelial cells within an agarose mould (Wiwatpanit et al. 2020).

An alternative approach to produce a structurally and functionally physiological model of the endometrium is to incorporate endometrial stromal cells into endometrial organoid cultures. Co-culturing of organoids with stromal cells has been reported in other organoid systems, such as the bladder and brain, and these models are designated as ‘assembloids’ (Kim et al. 2020a, Andersen et al. 2020, Miura et al. 2020). In the case of bladder, the applicability of organoids to in vivo pathology and disease is uncertain because, in isolation, they do not recapitulate the native tissue architecture and microenvironment. To overcome these limitations, bladder organoids were mixed with components of the bladder stroma, such as stromal, endothelial, and immune cells, and a muscle layer to form bladder assembloids (Kim et al. 2020). The assembloids represent a more physiological model of the native organ or tissue, and therefore, should provide a more faithful model for testing drug responses and mimicking disease phenotypes.

Recently, we have reported the establishment of endometrial assembloids whereby the gland organoid model was modified to incorporate stromal cells (Rawlings et al. 2021). Gland organoids were expanded from primary epithelial cells while in parallel the stromal fraction was propagated by standard monolayer culture (Fig. 4). Single-cell stromal suspensions were combined with manually digested organoids, seeded into a collagen hydrogel, and cultured in an expansion medium supplemented with E2. In this model, gland organoid formation was unperturbed by stromal co-culture, and assembloids...
resemble the architecture of native endometrium more closely than the gland organoids alone. The addition of stromal cells should abrogate the need for many components of the complex medium of growth factors and inhibitors to maintain secretory gland organoids, and indeed a minimal differentiation medium supported robust glandular differentiation in the assembloid model (Rawlings et al. 2021). Based on single-cell transcriptomic analysis, decidualised assemboids closely resemble the mid-secretory endometrium, containing several subpopulations of both stromal and epithelial cells, including senescent stromal and epithelial cells, which secrete many canonical implantation factors. Co-culturing human blastocysts with decidualised assemboids demonstrated that in the presence of senescent decidual cells, the assemboids engender a dynamic implantation environment, enabling embryo expansion and attachment, although persistent endometrial senescence led to the gradual disintegration of the assembloid matrix, likely through the actions of MMPs (Freitas-Rodriguez et al. 2017). Pharmacological inhibition of stress responses in pre-decidual cells by a tyrosine kinase inhibitor inhibited the propagation of decidual senescence, impeding assembloid breakdown. However, the lack of senescent cells resulted in the entrapment of the blastocysts in the largely static assembloid matrix. This study not only demonstrated that the endometrial assembloid model could be used as a novel embryo implantation model but also confirms previous reports that decidual senescence controls endometrial fate decisions during implantation and early pregnancy (Brighton et al. 2017, Lucas et al. 2020). Challenges for the endometrial assembloid model are to introduce a luminal epithelium, in order to study sequential blastocyst attachment and invasion, as well as the incorporation of immune cells such as uterine natural killer cells to mimic tissue remodelling processes predicted to take place during implantation (Brighton et al. 2017, Kong et al. 2021). Moreover, long-term maintenance and propagation of endometrial assemboids to mimic the cycling tissue have yet to be developed.

The major challenge in increasing complexity for a physiological model of the endometrium suitable for use in implantation studies is being able to balance the needs of different cell types within the model. For example, the basement membrane preparation Matrigel™, used frequently for organoid culture, is unsuitable as an extracellular matrix for stromal cells (Arnold et al. 2001). Beyond the co-culture of stromal cells with epithelial organoids, endothelial cells may be required to directly invade trophoblasts for placentation (Weiss et al. 2016). Additionally, immune cells, such as resident uterine natural killer cells, have been demonstrated to be essential for providing a robust decidual matrix suitable for successful embryo implantation (Kong et al. 2021). A microfluidic approach may be the solution to this potential dilemma.

**Endometrial pathologies, genetic manipulation, and biomarker discovery**

Beyond understanding the fundamentals of embryo implantation, endometrial pathologies that impede fertility must also be studied to advance the provision of therapeutics (Fig. 5). The development of medical treatments for many human diseases is limited by patient variation, difficulties in predicting outcomes, and lengthy preclinical drug testing. This is restricted further for applications in human reproduction by our limited ability to study in vivo processes in normal tissues. Therefore, organoid and assembloid cultures based on specific
pathologies and even on individual patients are expected to develop into powerful tools for precision therapy, permitting a personalised approach to drug discovery, development, and screening. Primary cancers, infectious diseases, and developmental diseases can be replicated with ex vivo biopsy samples from patients. Testing prospective pharmacological treatments on human organoids could facilitate the identification of patient group-specific responses and lead to targeted therapeutic approaches. Indeed, initial explorations into the utility of gland organoids in studying endometrial pathology have been reported, demonstrating the value of patient-specific cultures to study pathologies such as endometriosis and cancer (Boretto et al. 2017, 2019).

Endometriosis is an endometrial disorder characterised by the ectopic growth of endometrial tissue. Endometriosis is associated with infertility and affects 10% of women of reproductive age. The aetiology and pathogenesis of this disease remain unclear, and therefore, effective treatments are limited. Mouse models of endometriosis, while useful, do not model the complexity of the disease fully (Greaves et al. 2017). Gland organoids established from endometriotic lesions have been shown to differ from organoids derived in parallel from patient-matched eutopic endometrium, maintaining a heterogeneous disease profile, including aberrant signalling in integrin, P13K-AKT, and WNT pathways, and thus identifying potential drug targets (Boretto et al. 2019). Furthermore, differences in glycodelin secretion mirror those in patients and controls (Luddi et al. 2020). However, this model is limited to only glandular epithelial cells. Sampson’s theory of retrograde menstruation proposes that endometriotic lesions derive from endometrial cells seeding the peritoneum at menstruation (Filby et al. 2020). Incorporating decidualised stromal cells from endometriotic lesions to generate endometriosis assembloids could provide a more encompassing model for lesion establishment and behaviour, while crossover experiments with healthy and pathological cell subpopulations could also aid in pinpointing the exact mechanisms for endometrial diseases.

Endometrial cancer is another potential candidate for organoid modelling since the pathology of endometrial cancer is largely unknown, and treatment is limited, mainly due to a lack of reliable preclinical models. Tumour-derived cell lines do not recapitulate clinical cancer heterogeneity, and genetic mouse models show aberrations inconsistent with clinical endometrial cancer (Boretto et al. 2019). Tumour-derived organoids from endometrial cancers have been shown to maintain clinical heterogeneity under long-term expansion but required medium optimisation to recapitulate the tumour niche, owing to non-cancerous organoids outcompeting cancerous organoids in the standard protocol (Boretto et al. 2019). This key observation highlights the necessity to consider the nuances of the local environment when developing future endometrial organoid-based disease models. Pre-cancerous organoid models have also been developed, such as organoids derived from hyperplastic endometrium, which faithfully reproduced the disease genotype. These organoids may provide a model that can be used to identify patient-specific biomarkers for early detection and intervention (Boretto et al. 2019).

The development of pathological endometrial organoid and assembloid models, in concert with healthy controls, provides promising tools to interrogate endometrial biology and pathology. In models that are physiologically or pathologically relevant, single-cell omics and functional assays may provide initial targets for gene editing. Owing to their clonal capacity, organoids provide an excellent candidate model for gene editing through CRISPR/Cas9 (Jinek et al. 2012, Cong et al. 2013, Mali et al. 2013) and this has been achieved in numerous organoid systems already, including the brain (Wang et al. 2015, Ogawa et al. 2018), liver (Artegiani et al. 2019), kidney (Freedman et al. 2015), and intestine (Matano et al. 2015, Roper et al. 2018). To our knowledge, only one study has demonstrated CRISPR/Cas9 in endometrial organoids (Chen et al. 2021), derived from mouse tissue, and therefore, efforts to advance the manipulation of human endometrial organoids will be
welcomed. Genetic and gene regulatory manipulations will be essential for underpinning the key mechanisms and aberrations that lead to disease states, and as well as to open new avenues for biomarker investigation and drug discovery.

**Endometrial precision medicine, clinical trials, and therapeutic potential**

The clearest benefit of patient-derived organoids and assembloids is to provide a novel opportunity to discover personalised treatments. Unlike previous models, patient-derived organoids have been demonstrated to maintain the heterogeneity and complexity of their clinical derivatives, allowing for the study of patient-specific phenotypes. Therefore, the next step in the development of pathological endometrial organoid models is to utilise them in preclinical drug screening tools. Organoids from other systems have already been used for drug screening. For example, cystic fibrosis (CF) is a genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and causes particularly severe damage to the pulmonary and digestive systems (Dekkers et al. 2013). Clinical trials with CFTR-targeting drugs have shown variable efficacy among individuals. Organoids can be harnessed to target different mutations in the CFTR protein with the intention to discover personalised and effective treatments for CF (Dekkers et al. 2013, 2016, Berkers et al. 2019, Ramalho et al. 2021).

Endometrial organoids have already been used in pre-clinical drug screening studies for endometrial cancer (Chen et al. 2021). Unbiased drug screening of tumour organoids from mouse endometrial cancer identified MI-136 as a potential inhibitor of endometrial cancer through regulation of the HIF pathway, a novel mechanism distinct from those in acute myeloid leukaemia and prostate cancer (Chen et al. 2021). Additionally, the study reported that MI-136 also inhibited growth significantly in primary cancer organoids derived from patients (Chen et al. 2021). This provides evidence that endometrial organoids could be used as in pre-clinical drug screening studies.

To maximise the effective use of therapeutics, stratification of subjects in clinical trials is essential, especially in the case of a heterogeneous disease. Stratification and personalised medicine could be extrapolated to endometrial organoids and assembloids and the treatment of infertility. Our recent research has demonstrated that excessive decidual cellular senescence is associated with recurrent pregnancy loss (Lucas et al. 2020). Biomarkers for excessive decidual senescence could be utilised for screening for organoid- and assembloid-based pre-clinical drug interventions to reduce senescence and possibly reduce the burden of miscarriage. Only patients experiencing cycles with excessive senescence would likely see a benefit in reducing senescence, and therefore, it is essential to target this subset of patients. Primary endometrial stromal cells in vitro retain phenotypic characteristics of the donating patient. For example, endometrial stromal cells from women with recurrent pregnancy loss exhibit an imbalance of mature decidual and senescent decidual cells and an inability to recognise poor-quality embryos (Weimar et al. 2012, Lucas et al. 2020). Therefore, endometrial assembloid models should be able to mimic the behaviour of the tissue in vivo and provide a first step approach to patient-specific testing and stratification for treatment. Indeed, since organoid cultures can be bio-banked for future drug testing (Boretto et al. 2019), reducing the requirement for repeated biopsies, directed testing should be possible on specified cohorts of bio-banked samples.

Overall, organoids appear to be a promising model for drug discovery, pre-clinical evaluation, and stratification of subject groups for clinical trials and our challenge lie in driving the models from the fundamental study into the translational application to realise their full potential (Fig. 5).

**Conclusions**

Organoid and assembloid models of the endometrium offer the opportunity to study embryo-endometrium interactions at the earliest timepoints in implantation, with increased physiological relevance, and thus present a promising tool for future research. Advancing our knowledge of the mechanisms and requirements for successful implantation also offers the prospect of identifying the routes through which the process fails, thus indicating possible therapeutic avenues. Through the ongoing development and characterisation of patient-specific organoid and assembloid models, targeted pre-conception therapies can be developed and tested extensively in pre-clinical studies. These exciting possibilities mean much work ahead: the full potential of organoid and assembloid models has not yet been realised, and the challenge now is to ensure that we drive the models forward to translational outcomes.

**Declaration of interest**

E S L is an ordinary member of SRF council. The authors have no financial interests to declare.
Funding

T M R is supported by a fellowship from the Warwick-Wellcome Trust Translational Partnership initiative. K M is funded by a Warwick Medical School scholarship. T M is funded by a EUROPIC Co-tutelle PhD Scholarship between the University of Warwick and Vrije Universiteit Brussel (VUB). E S L is funded through a Wellcome Trust Investigator Award to Professor Jan Brossen (212233/2/18/2).

Author contribution statement

T M R and E S L conceptualised the article. T M R, K M, M T, and E S L drafted the article. K M, T M R, and E S L prepared the figures. E S L edited the article. All authors approved the final version.

References

Abbas Y, Brunel LG, Hollinshead MS, Fernando RG, Gardner L, Duncan I, Moffett A, Best S, Turco MY, Burton GJ, et al. 2020 Generation of a three-dimensional collagen scaffold-based model of the human endometrium. Interface Focus 10 20190079. (https://doi.org/10.1098/rsfs.2019.0079)

Alves-Lopes JP, Söder O & Stukenborg JB 2017 Testicular organoid generation by a novel in vitro three-layer gradient system. Biomaterials 130 76–89. (https://doi.org/10.1016/j.biomaterials.2017.03.025)

Alzamir I, Nikolakopoulos K & Turco MY 2021 Organoid systems to study the human female reproductive tract and pregnancy. Cell Death and Differentiation 28 35–51. (https://doi.org/10.1038/s41418-020-0565-5)

Anacker J, Segerer SE, Hagemann C, Feix S, Kapp M, Bausch R & Kämmerer U 2011 Human decidual and invasive trophoblasts are rich sources of nearly all human matrix metalloproteinases. Molecular Human Reproduction 17 637–652. (https://doi.org/10.1093/molehr/gar033)

Andersen J, Revah O, Miura Y, Thom N, Amin ND, Kelley KW, Singh M, Chen X, Thete MV, Walczak EM, et al. 2020 Generation of functional human 3D cortico-motor assembloids. Journal of Cell Science 133 e193. e26–1929.e26. (https://doi.org/10.1242/jcs.2020.11.017)

Aplin JD 2006 In vitro analysis of trophoblast invasion. In Placenta and Trophoblast: Methods and Protocols. Totowa, NJ: Humana Press.

Aplin JD & Ruane PT 2017 Embryo-epithelium interactions during implantation at a glance. Journal of Cell Science 130 15–22. (https://doi.org/10.1242/jcs.175943)

Aplin JD, Hey NA & Li TC 1996 MUC1 as a cell surface and secretory component of endometrial epithelium: reduced levels in recurrent miscarriage. American Journal of Reproductive Immunology 35 261–266. (https://doi.org/10.1111/j.1600-0897.1996.tb0042.2)

Arnold JT, Kaufman DG, Seppälä M & Lessey BA 2001 Endometrial stromal cells regulate epithelial cell growth in vitro: a new co-culture model. Human Reproduction 16 836–845. (https://doi.org/10.1093/humrep/16.5.836)

Artegiani B, Van Voorthuizen L, Lindeboom RGH, Seinstra D, Heo I, Tapia P, López-Iglesias C, Postrach D, Dayton T, Oka R, et al. 2019 Probing the tumor suppressor function of BAP1 in CRISPR-engineered human liver organoids. Stem Cell Reports 24 927.e6–943.e6. (https://doi.org/10.1016/j.stemcr.2019.04.017)

Baert Y, De Kock J, Alves-Lopes JP, Söder O, Stukenborg JB & Goossens E 2017 Primary human testicular cells self-organize into organoids with testicular properties. Stem Cell Reports 8 30–38. (https://doi.org/10.1016/j.stemcr.2016.11.012)

Bartfeld S, Bayram T, Van De Wetering M, Huch M, Begthel H, Kujala P, Vries R, Peters PJ & Clevers H 2015 In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology 148 126.e6–136.e6. (https://doi.org/10.1016/j.gastro.2014.09.042)

Bentin-Ley U, Pedersen B, Lindenberg S, Larsen JF, Hamberger L & Hornt J 1994 Isolation and culture of human endometrial cells in a three-dimensional culture system. Human Reproduction and Fertility 107 237–332. (https://doi.org/10.1530/JPT.0.1010327)

Bentin-Ley U, Horn T, Sjögren A, Sorensen F, Falck Larsen J & Hamberger L 2000 Ultrastructure of human blastocyst-endometrial interactions in vitro. Journal of Reproduction and Fertility 120 337–350. (https://doi.org/10.1530/jrp.1.1200337)

Berkers G, Van Mourik P, Von AM, Kruijssenbrink E, Dekkers JF, De Winter-De Groot KM, Argets HGM, Mark-Van Der Will RP, Dijkema JS, Vanderschuren MM, et al. 2019 Rectal organoids enable personalized treatment of cystic fibrosis. Cell Reports 26 1701, e3–1708.e3. (https://doi.org/10.1016/j.celrep.2019.01.068)

Berkhout RP, Lambalk CB, Huirne J, Mijatovic V, Repping S, Hamer G & Mastenbroek S 2018 High-quality human preimplantation embryos actively influence endometrial stromal migration. Journal of Assisted Reproduction and Genetics 35 659–667. (https://doi.org/10.1007/s10815-017-1107-z)

Berneau SC, Ruane PT, Brison DR, Kimber SJ, Westwood M & Aplin JD 2019 Characterisation of osteopontin in an in vitro model of embryo implantation. Cells 8 432. (https://doi.org/10.3390/cells8050432)

Binder NK, Evans J, Gardner DK, Salamonsen LA & Hannan NJ 2014 Endometrial signals improve embryo outcome: functional role of vascular endothelial growth factor isoforms on embryo development and implantation in mice. Human Reproduction 29 2278–2286. (https://doi.org/10.1093/humrep/deu211)

Bläuer M, Heinonen PK, Martikainen PM, Tomáš E & Ylikomi T 2000 A novel organotypic culture model for normal human endometrium: regulation of epithelial cell proliferation by estradiol and medroxyprogesterone acetate. Human Reproduction 20 864–871. (https://doi.org/10.1093/humrep/deh722)

Boretto M, Cox B, Noben M, Hendriks N, Fassbender A, Roose H, Amant F, Timmerman D, Tomassetti C, VanHie A, et al. 2017 Development of organoids from mouse and human endometrium showing endometrial epithelial physiology and long-term expandability. Development 144 1775–1786. (https://doi.org/10.1242/dev.148478)

Boretto M, Maenhoudt N, Luo X, Hennes A, Roeckx B, Bui B, Heremans R, Pernee L, Kobayashi H, Van Zundert I, et al. 2019 Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. Nature Cell Biology 21 1041–1051. (https://doi.org/10.1038/s41556-019-0360-z)

Brighton PJ, Maruyama Y, Fishwick K, Vrijlak P, Tewary S, Fujihara R, Muter J, Lucas ES, Yamada T, Woods L, et al. 2017 Clearance of senescent decidual cells by uterine natural killer cells in cycling human endometrium. eLife 6 e31274. (https://doi.org/10.7554/eLife.31274)

Brosens JJ, Salker MS, Teklenburg G, Nautiyal J, Salter S, Brighton PJ, Watson AL, Hempstock J, Skepper JN & Jauniaux E 2002 Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. Journal of Clinical Endocrinology and Metabolism 87 2954–2959. (https://doi.org/10.1210/jcem.87.6.8563)

Burtin CJ, Jauniaux E & Charnock-Jones DS 2007 Human early placentation development: potential roles of the endometrial glands.
ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, Nelsen W, Peramo B, Quenby S, et al. 2018 ESHRE guideline: recurrent pregnancy loss. Human Reproduction Open 2018 hoy004. (https://doi.org/10.1093/hroopen/hoy004)

Evans J, Walker KJ, Bilandzic M, Kinnear S & Salamonsen LA 2020 A novel ‘embryo-endometrial’ adhesion model can potentially predict ‘receptive’ or ‘non-receptive’ endometrium. Journal of Assisted Reproduction and Genetics 37 5–16. (https://doi.org/10.1007/s11680-019-01629-0)

Evron A, Goldman S & Shaley E 2011 Effect of primary human endometrial stromal cells on epithelial cell receptivity and protein expression is dependent on menstrual cycle stage. Human Reproduction 26 176–190. (https://doi.org/10.1093/humrep/dep296)

Feinberg RF, Kliman HJ & Wang CL 1994 Transforming growth factor-beta stimulates trophoblast oncofetal fibronectin synthesis in vitro: implications for trophoblast implantation in vivo. Journal of Clinical Endocrinology and Metabolism 78 1241–1248. (https://doi.org/10.1210/jcem.78.5.8175984)

Filby CE, Rombauds L, Montgomery GW, Giudice LC & Gargett CE 2020 Cellular origins of endometriosis: towards novel diagnostics and therapeutics. Seminars in Reproductive Medicine 38 201–215. (https://doi.org/10.1055/s-0040-1713429)

Fitzgerald HC, Dhakal P, Behura SK, Schust DJ & Spencer TE 2019 Self-renewing endometrial epithelial organoids of the human uterus. PNAS 116 23132–23142. (https://doi.org/10.1073/pnas.1915389116)

Fitzgerald HC, Schust DJ & Spencer TE 2021 In vitro models of the human endometrium: evolution and application for women’s health. Biology of Reproduction 104 282–293. (https://doi.org/10.1093/bioreprod/oia183)

Freedman BS, Brooks CR, Lam AQ, Fu H, Morizane R, Agrawal V, Saad AF, Li MK, Hughes MR, Werff RV, et al. 2015 Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nature Communications 6 8715. (https://doi.org/10.1038/ncomms9715)

Freitas-Rodriguez S, Folgueras AR & Lopez-Otin C 2017 The role of matrix metalloproteinases in aging: tissue remodeling and beyond. Biochimica et Biophysica Acta: Molecular Cell Research 1864 2015–2025. (https://doi.org/10.1016/j.bbamcr.2017.05.007)

Fujii M, Matano M, Toshimitsu K, Takano A, Mikami Y, Nishikori S, Sugimoto S & Sato T 2018 Human intestinal organoids maintain self-renewal capacity and cellular diversity in niche-inspired culture condition. Cell Stem Cell 23 787.e6–793.e6. (https://doi.org/10.1016/j.stem.2018.11.016)

Gellersen B & Brosens JJ 2014 Cyclic decidualization of the human endometrium in reproductive health and failure. Endocrine Reviews 35 851–905. (https://doi.org/10.1210/er/2014-1045)

Gellersen B, Reimann K, Samalecos A, Aupers S & Bamberger AM 2010 Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. Human Reproduction 25 862–873. (https://doi.org/10.1093/humrep/dep468)

Gellersen B, Wolf A, Kruse M, Schwenke M & Bamberger AM 2013 Human endometrial stromal cell-trophoblast interactions: mutual stimulation of chemotactic migration and promigratory roles of cell surface molecules CD82 and CEACAM1. Journal of Assisted Reproduction and Genetics 30 839–850. (https://doi.org/10.1007/s11680-013-9682-8)

Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW & Spencer TE 2001 Developmental biology of uterine glands. Biology of Reproduction 65 1311–1323. (https://doi.org/10.1095/biolreprod.65.5.1311)

Greaves E, Critchley HOD, Horne AW & Saunders PTK 2017 Relevant human tissue resources and laboratory models for use in endometriosis research. Acta Obstetrica et Gynecologica Scandinavica 96 644–658. (https://doi.org/10.1080/00016349.2016.1206865)

Grewal S, Carver JG, Ridley AJ & Mardon HJ 2008 Implantation of the human embryo requires Rac1-dependent endometrial stromal...
cell migration. PNAS 105 16189–16194. (https://doi.org/10.1073/pnas.0806219105)

Grewal S, Carver J, Ridley AJ & Mardon HJ 2010 Human endometrial stromal cell rho GTPlases have opposing roles in regulating local adhesion turnover and embryo invasion in vitro. Biology of Reproduction 83 75–82. (https://doi.org/10.1098/biolreprod.2009.0630)

Haider S, Meinhardt G, Saleh L, Kunihis V, Gamper M, Kaindl U, Ellinger A, Burkard TR, Fiala C, Pollemier J, et al. 2018 Self-renewing trophoblast organoids recapitulate the developmental program of the early human placenta. Stem Cell Reports 11 537–551. (https://doi.org/10.1016/j.stemcr.2018.07.004)

Haider S, Gamper M, Burkard TR, Kunihis V, Kaindl U, Junttila S, Fiala C, Schmidt K, Mendjan S, Knöffler M, et al. 2019 Estrogen signaling drives ciliogenesis in human endometrial organoids. Endocrinology 160 2282–2297. (https://doi.org/10.1210/endo-2019-00314)

Hertig AT, Rock J & Adams EC 1956 A description of 34 human ova within the first 17 days of development. American Journal of Anatomy 96 435–493. (https://doi.org/10.1002/aja.1000980306)

Holmberg JC, Haddad S, Wünsche S, Yang Y, Aldo PB, Gnaiinsky Y, Granot I, Dekel N & Mor G 2012 An in vitro model for the study of human implantation. American Journal of Reproductive Immunology 67 169–178. (https://doi.org/10.1111/j.1600-0897.2011.01095.x)

Hu H, Gehart H, Artigiani B, López-Iglesias C, Dekkers F, Basak O, van Es J, Chua de Sousa Lopes SM, Bortolin M, et al. 2018 Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Nature 550 228–232. (https://doi.org/10.1038/nature24440)

Huang X, Liu H & Li R 2017 Prolactin in human endometrium: role in implantation and the stem cell niche in a dish. Developmental Cell 40 255–268. (https://doi.org/10.1016/j.devcel.2016.08.014)

Kessler M, Hoffmann K, Brinkmann V, Thieck O, Jackisch S, Toelle B, Berger H, Mollenkopf HJ, Mangler M, Sehoul j, et al. 2015 The Notch and Wnt pathways regulate stemness and differentiation in human Fallopian tube organoids. Nature Communications 6 8999. (https://doi.org/10.1038/ncomms9999)

Kim E, Choi S, Kang B, Kong J, Kim Y, Yoon WH, Lee HR, Kim S, Kim HM, Lee H, et al. 2020a Creation of blastoderm assembloids mimicking tissue regeneration and cancer. Nature 588 664–669. (https://doi.org/10.1038/s41586-020-3034-x)

Kim J, Koo BK & Kohchib J 2020b Human organoids: model systems for human biology and medicine. Nature Reviews: Molecular Cell Biology 21 571–584. (https://doi.org/10.1038/s41580-020-0259-3)

Kong G, Ordóñez AA, Turner S, Tremaine T, Muter J, Lucas ES, Salisbury E, Vassena R, Tiscornia G, Fouladi-Nashta AA, et al. 2021 Embryo biosensing by uterine natural killer cells determines endometrial fate decisions at implantation. FASEB Journal 35 e21336. (https://doi.org/10.1096/fj.2020022178)

Kopper O, De Witte CJ, Löhmußar A, Valle-Inclan JE, Hani M, Kester L, Balogbond AV, Korving J, Proost N, Begthel H, et al. 2019 An ovarian platform for ovarian cancer captures intra-and interpatient heterogeneity. Nature Medicine 25 838–849. (https://doi.org/10.1038/s41591-019-0422-6)

Kretzschmar K & Clevers H 2016 Organoids: modeling development and the stem cell niche in a dish. Developmental Cell 38 590–600. (https://doi.org/10.1016/j.devcel.2016.08.014)

Ladines-Illave CA, Manuel AB & Mochizuki M 1991 Cytologic localization of epidermal growth factor and its receptor in developing human placenta varies over the course of pregnancy. American Journal of Obstetrics and Gynecology 165 1377–1382. (https://doi.org/10.1016/S0002-9378(91)90372-x)

Lash GE, Hornbuckle J, Brunat A, Kirkley M, Searle RF, Robson SC & Bulmer JN 2007 Effect of low oxygen concentrations on trophoblast-like cell line invasion. Placenta 28 390–396. (https://doi.org/10.1016/j.placenta.2006.06.001)

Le Saint C, Crespo K, Bourdiec A, Bissonnette F, Buzaglo K, Coutardier B, Bisotto S, Phillips SJ, Stutz M, Gouze JN, et al. 2019 Autologous endometrial cell culture improves human embryo development to high-quality blastocysts: a randomized controlled trial. Reproductive BioMedicine Online 38 321–329. (https://doi.org/10.1016/j.rbmo.2018.12.039)

Lee KY & DeMayo FJ 2004 Animal models of implantation. Reproduction 128 679–695. (https://doi.org/10.1530/rep.1.00340)

Lee YL, Fong SW, Chen ACH, Li T, Yue C, Lee CL, Ng EHY, Yeung WSB & Lee KF 2015 Establishment of a novel human embryonic stem cell-derived trophoblastic spheroid implantation model. Human Reproduction 30 2621–2626. (https://doi.org/10.1093/humrep/dev223)

Lee SH, Hu W, Matalay JT, Silva MV, Owczarek TB, Kim K, Chu CW, Barlow LJ, Kandooth C, Williams AB, et al. 2018 Tumor evolution and drug response in patient-derived organoid models of bladder cancer. Cell 173 515.e17–528.e17. (https://doi.org/10.1016/j.cell.2018.03.017)

Lindenberg S, Nielsen MH & Lenz S 1985 In vitro studies of human blastocyst implantation. Annals of the New York Academy of Sciences 442 368–374. (https://doi.org/10.1111/j.1749-6632.1985.tb37541.x)

Linnemann JR, Miura H, Meixner LK, Irmler M, Kloos UJ, Obara H, Schiepers C, Wetterau J, et al. 2017 Establishment of a novel human embryonic stem cell-derived trophoblastic spheroid implantation model. Human Reproduction 32 2621–2626. (https://doi.org/10.1093/humrep/dev223)

Liu X, Tan JP, Schröder J, Aberkan e A, Ouyang JF, Mohenska M, Lim SM, Sun YBY, Chen J, Sun G, et al. 2021 Modelling human blastocysts by reprogramming fibroblasts into iPS cells. Nature 591 627–632. (https://doi.org/10.1038/s41467-021-01372-y)

Lohmußar A, Oka R, Espejo Valle-Inclan J, Smits MHH, Wardak H, Korving J, Begthel H, Proost N, Van De Ven M, Kranenburg OW, et al. 2021 Patient-derived organoids model cervical tissue dynamics and viral oncogenesis in cervical cancer. Cell Stem Cell 28 1380.e6–1386.e6. (https://doi.org/10.1016/j.stem.2021.03.012)

Loomans CJM, Giuliani NW, Balak J, Ringnalda F, van Gurp L, Buzaglo K, Bissonnette F, Buzaglo K, Coutardier B, Bisotto S, Phillips SJ, Stutz M, Gouze JN, et al. 2019 Autologous endometrial cell culture improves human embryo development to high-quality blastocysts: a randomized controlled trial. Reproductive BioMedicine Online 38 321–329. (https://doi.org/10.1016/j.rbmo.2018.12.039)

Lindenberg S, Nielsen MH & Lenz S 1985 In vitro studies of human blastocyst implantation. Annals of the New York Academy of Sciences 442 368–374. (https://doi.org/10.1111/j.1749-6632.1985.tb37541.x)

Hirnschi B, Bartsch HS, Sassi S, Beckers J, Theis FJ, et al. 2015 Quantification of regenerative potential in primary human mammary epithelial cells. Development 142 3239–3251. (https://doi.org/10.1242/dev.123554)

Jahanpour B, Oka R, Espejo Valle-Inclan J, Smits MHH, Wardak H, Korving J, Begthel H, Proost N, Van De Ven M, Kranenburg OW, et al. 2021 Patient-derived organoids model cervical tissue dynamics and viral oncogenesis in cervical cancer. Cell Stem Cell 28 1380.e6–1386.e6. (https://doi.org/10.1016/j.stem.2021.03.012
et al. 2018 Expansion of adult human pancreatic tissue yields organoids harboring progenitor cells with endocrine differentiation potential. Stem Cell Reports 10 712–724. (https://doi.org/10.1016/j.stemcr.2018.02.005)

Lopes FL, Desmarais JA & Murphy BD 2004 Embryonic diapause and its regulation. Reproduction 128 669–678. (https://doi.org/10.1530/rep.1.00444)

Lucas ES, Salker MS & Brosens JJ 2013 Uterine plasticity and reproductive fitness. Reproductive BioMedicine Online 27 506–514. (https://doi.org/10.1016/j.rbmo.2013.06.012)

Lucas ES, Vrijkapec P, Mutter J, Dinziz-Da Costa MM, Brighton PJ, Kong CS, Lipecki J, Fishwick KJ, Odendaal J, Ewington LJ, et al. 2020 Recurrent pregnancy loss is associated with a pro-senescent decidual response during the peri-implantation window. Communications Biology 3 37. (https://doi.org/10.1038/s42003-020-0763-1)

Luddi A, Pavone V, Semplici B, Governini L, Criscuoli M, Paccagnini E, Gentile M, Morgante G, Leo V, Belmonte G, et al. 2020 Organoids of human endometrium: a powerful in vitro model for the endometrium-embryo cross-talk at the implantation site. Cells 9 1211. (https://doi.org/10.3390/cells9051211)

Luddi A, Pavone V, Governini L, Capaldo A, Landi C, Jetta F, Paccagnini E, Morgante G, De Leo V & Piomboni P 2021 Emerging role of embryo secretome in the paracrine communication at the implantation site: a proof of concept. Fertility and Sterility 115 1054–1062. (https://doi.org/10.1016/j.fertnstert.2020.10.058)

Macklon NS & Brosens JJ 2014 The human endometrium as a sensor of embryo quality. Biology of Reproduction 91 98. (https://doi.org/10.1095/biolreprod.114.122846)

Maenhoudt N, Defraye C, Boretto M, Janz Z, Heremans R, Mali P, Ruane PT, Fishwick KJ, Todd DM, Molé MA, Fishwick KJ, et al. 2021 Management of recurrent implantation failure: a scientific commentary on the current understanding and treatment options. Fertility and Sterility 115 116–129. (https://doi.org/10.1016/j.fertnstert.2020.03.004)

Mali P, Yang L, Esvelt KM, Aach J, Guell M, Miccoli D, Norville JE & Church GM 2013 RNA-guided human genome engineering via Cas9. Science 339 823–826. (https://doi.org/10.1126/science.1232033)

Maru Y, Kawata A, Taguchi A, Ishii Y, Baba S, Mori M, Nagamatsu T, Oda K, Kukimoto I, Osuga Y, et al. 2020 Establishment and molecular phenotyping of organoids from the squamo-carcinoma junction region of the uterine cervix. Cancers 12 2384. (https://doi.org/10.3390/cancers12092384)

Maruo T, Matsuo H, Otani T & Mochizuki M 1997 Epidermal growth factor (EGF) regulates trophoblast proliferation and endocrine function in synergy with thyroid hormone. Placenta 18 27–39. (https://doi.org/10.1016/S0143-4004(05)80158-4)

Mascarenhas M, Jeve Y, Polanski L, Sharpe A, Yasin E & Bhandari HM 2021 Management of recurrent implantation failure: British Fertility Society Policy and Practice Guideline. Human Fertility 1–25. (https://doi.org/10.1046/j.14647273.2021.1905886)

Meseguer A, Aplin JD, Caballero-Campo P, O’Connor JE, Martín JC, Remohí J, Pellicer A & Simón C 2001 Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. Biology of Reproduction 64 590–601. (https://doi.org/10.1095/biolreprod.64.2.590)

Miura Y, Li MY, Birey F, Ikeda K, Revah O, Thete MV, Park JY, Puno A, Lee SH, Porteus MH, et al. 2020 Generation of human striatal organoids and cortico-striatal assembloids from human pluripotent stem cells. Nature Biotechnology 38 1421–1430. (https://doi.org/10.1038/s41587-020-00763-w)

Morrison JL, Berry MJ, Bottig KJ, Darby JRT, Frasch MG, Gatford KG, Giusani DA, Gray CL, Harding R, Herrera EA, et al. 2018 Improving pregnancy outcomes in humans through studies in sheep. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 315 R1123–R1153. (https://doi.org/10.1152/ajpregu.00391.2017)

Muruganandan S, Fan X, Dhal S & Nayak NR 2020 Development of a 3D tissue slice culture model for the study of human endometrial repair and regeneration. Biomolecules 10 136. (https://doi.org/10.3390/biom10010136)

Ogawa J, Pao GM, Shokhirev MN & Verma IM 2018 Glioblastoma model using human cerebral organoids. Cell Reports 23 1220–1229. (https://doi.org/10.1016/j.celrep.2018.03.109)

Pendergast SS, Sadri-Ardekani H, Atala A & Bishop CE 2017 Three-dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity in vitro. Biology of Reproduction 96 720–732. (https://doi.org/10.1095/biolreprod.116.143446)

Pierro E, Minici F, Alessiani O, Miceli F, Proto C, Scepani T, Manson C & Lanzone A 2001 Stromal-epithelial interactions modulate estrogen responsiveness in normal human endometrium. Biology of Reproduction 64 831–838. (https://doi.org/10.1095/biolreprod64.3.831)

Ramlalho AS, Fürstová E, Vonk AM, Ferrante M, Verfaillie C, Dupont L, Boon M, Proomsmans M, Beekman JM, Sarouk I, et al. 2021 Correction of CTFR function in intestinal organoids to guide treatment of cystic fibrosis. European Respiratory Journal 57 1902426. (https://doi.org/10.1183/13993003.02426-2019)

Rawlings TM, Makwana K, Taylor DM, Moli MA, Fishwick KJ, Tryfonos M, Odendaal J, Hawkes A, Zernicka-Goetz M, Harthorne GM, et al. 2021 Modelling the impact of decidual senescence on embryo implantation in human endometrial assembloids. bioRxiv 2021.03.02.433560. (https://doi.org/10.1101/2021.03.02.433560)

Rinehart CA, Lyn-Cook BD & Kaufman DG 1988 Gland formation from human endometrial epithelial cells in vitro. In Vitro Cellular and Developmental Biology 24 1037–1041. (https://doi.org/10.1007/BF02620878)

Rogers K 2012 Scientific Modeling [Online]. Encyclopedia Britannica. (available at: https://www.britannica.com/science/scientific-modeling). Accessed on 21 February 2021.

Ruane PT, Buck CJ, Babbington PA, Aboussahoud W, Berneau SC, Westwood M, Kimber SJ, Aplin JD & Brison DR 2020 The effects of hyaluronate-containing medium on human embryo attachment to endometrial epithelial cells in vitro. Human Reproduction Open 2020 hzo033. (https://doi.org/10.1093/hrodev/hzo033)

Sachs N, Papaspyropoulos A, Zomer-Van Ommen DD, Heo J, Lottinger L, Klay D, Weeber I, Huelse-Prince G, Iakobachvili N, Amatgiamal GD, et al. 2019 Long-term expanding human airway organoids for disease modeling. EMBO Journal 38 e100300. (https://doi.org/10.15252/embr.2018100300)

Salleh N & Girabu N 2014 Leukemia inhibitory factor: roles in embryo implantation and in nonhomonal contraception. ScientificWorldJournal 2014 201514. (https://doi.org/10.1155/2014/201514)

Schlaermann P, Toelle B, Berger H, Schmidt SC, Glanemann M, Ordemann J, Bartfeld S, Mollenkopf HJ & Meyer TF 2016 A novel human gastric primary cell culture system for modelling Helicobacter pylori infection in vitro. Gut 65 202–213. (https://doi.org/10.1136/gutjnl-2014-307949)

Simintiras CA, Dhakal P, Ranjit C, Fitzgerald HC, Balboula AZ & Spencer TE 2021 Capture and metabolomic analysis of the human endometrial epithelial organoid secretome. PNAS 118 e2026804118. (https://doi.org/10.1073/pnas.2026804118)

Simón C, Gimeno MJ, Mercader A, O’Connor JE, Remohí J, Polan ML & Pellicer A 1997 Embryonic regulation of integrins β3, α4, and α1 in human endometrial epithelial cells in vitro. Journal of Clinical Endocrinology and Metabolism 82 2607–2616. (https://doi.org/10.1210/jcem.82.8.4153)

Simón C, Mercader A, Garcia-Velasco J, Nikas G, Moreno C, Remohí J & Pellicer A 1999 Coculture of human embryos with autologous human endometrial epithelial cells in patients with...
implantation failure. *Journal of Clinical Endocrinology and Metabolism* 84:2638–2646. (https://doi.org/10.1210/jcem.84.8.5873)

**Singh H & Aplin JD** 2009 Adhesion molecules in endometrial epithelium: tissue integrity and embryo implantation. *Journal of Anatomy* 215:3–13. (https://doi.org/10.1111/j.1469-7580.2008.01034.x)

**So KH, Lee CL, Yeung WS & Lee KF** 2012 Glycodelin suppresses endometrial cell migration and invasion but stimulates spheroid attachment. *Reproductive Biomedicine Online* 24:639–645. (https://doi.org/10.1016/j.rbmo.2012.03.004)

**Teilmann SC, Clement CA, Thorrup J, Byskov AG & Christensen ST** 2006 Expression and localization of the progesterone receptor in mouse and human reproductive organs. *Journal of Endocrinology* 191:525–535. (https://doi.org/10.1677/joe.1.06565)

**Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, et al.** 2010 Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS ONE* 5:e10258. (https://doi.org/10.1371/journal. pone.0010258)

**Tinning H, Taylor A, Wang D, Constantiniades B, Sutton R, Oikonomon G, Velazquez MA, Thompson P, Treumann A, O’Connell MJ, et al.** 2020 The role of CAPG in PACG as a mediator of the communication between the embryo and the uterine endometrium: is its function conserved in species with different implantation strategies? *FASEB Journal* 34:11015–11029. (https://doi.org/10.1096/ fj.202000882RR)

**Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, Hollinshead M, Marsh SGE, Brosens JJ, Critchley HO, et al.** 2017 Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Reproductive Biomedicine Online* 27:461–476. (https://doi.org/10.1016/j.rbmo.2013.08.002)

**Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, McWhinnie A, Esposito L, Fernando R, Skelton H, et al.** 2018 Trophoblast organoids as a model for maternal-fetal interactions during human placentaation. *Nature* 564:263–267. (https://doi.org/10.1038/s41586-018-0753-3)

**Uchida H, Maruyama T, Ono M, Ohta K, Kajitani T, Masuda H, Nagashima T, Arase T, Asada H & Yoshimura Y** 2007 Histone deacetylase inhibitors stimulate cell migration in human endometrial adenocarcinoma cells through up-regulation of glycodelin. *Endocrinology* 148:896–902. (https://doi.org/10.1210/en.2006-0896)

**Varma VA, Melin SA, Adamec TA, Dorman BH, Siegfried JM, Walton LA, Carney CN, Norton CR & Kaufman DG** 1982 Monolayer culture of human endometrium: methods of culture and identification of cell types. *In Vitro* 18:911–918. (https://doi.org/10.1007/BF02796347)

**von Wolff M, Strowitzki T, Becker V, Zepf C, Tabibzadeh S & Thaler CJ** 2001 Endometrial osteopontin, a ligand of beta3-integrin, is maximally expressed around the time of the ‘implantation window’. *Fertility and Sterility* 76:775–781. (https://doi.org/10.1016/S0015-0282(01)00215-5)

**Wang H, Pilla F, Anderson S, Martínez-Escribano S, Herrr I, Moreno-Moya JM, Musti S, Bocca S, Oehninger S & Horcajadas JA** 2012 A novel model of human implantation: 3D endometrium-like culture system to study attachment of human trophoblast (Jar) cell spheroids. *Molecular Human Reproduction* 18:33–43. (https://doi.org/10.1093/molehr/gar064)

**Wang P, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W, Lachman HM & Zheng D** 2015 CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. *Molecular Autism* 6:55. (https://doi.org/10.1186/s13229-015-0048-6)

**Weimar CH, Kavelaars A, Brosens JJ, Gellersen B, De Vreeden-Elbertse JM, Heijnen CJ & Macklon NS** 2012 Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. *PLoS ONE* 7:e41424. (https://doi.org/10.1371/journal.pone.0041424)

**Weimar CH, Post Uiterweerd ED, Teklenburg G, Heijnen CJ & Macklon NS** 2013 In-vitro model systems for the study of human embryo-endometrium interactions. *Reproductive Biomedicine Online* 27:639–645. (https://doi.org/10.1016/j.rbmo.2013.12.010)

**Wiwatpanit T, Murphy AR, Lu Z, Urbanek M, Burdette JE, Woodruff TK & Kim JJ** 2020 Scaffold-free endometrial organoids respond to excess androgens associated with polycystic ovarian syndrome. *Journal of Clinical Endocrinology and Metabolism* 105:769–780. (https://doi.org/10.1210/clinem/dgz100)

**Yu L, Wei Y, Duan J, Schmitz DA, Sakurai M, Wang L, Wang K, Zhao S, Hon GC & Wu J** 2021 Blastocyst-like structures generated from human pluripotent stem cells. *Nature* 591:620–626. (https://doi.org/10.1038/s41586-021-03356-y)

**Zheng Y, Xue X, Shao Y, Wang S, Esfahani SN, Li Z, Muncie JM, Lachman HM & Zheng D** 2015 CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. *Molecular Autism* 6:55. (https://doi.org/10.1186/s13229-015-0048-6)

**Zheng Y, Xue X, Shao Y, Wang S, Esfahani SN, Li Z, Muncie JM, Lachman HM & Zheng D** 2015 CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. *Molecular Autism* 6:55. (https://doi.org/10.1186/s13229-015-0048-6)

**Zhu S, Huang Y, Zhu Y, Kao KW, Mahowald AL, Ma Y, Lui GP, de Souza PM, Wang Z, et al.** 2015 A novel model of human implantation: 3D endometrium-like culture system to study attachment of human trophoblast (Jar) cell spheroids. *Molecular Human Reproduction* 18:33–43. (https://doi.org/10.1093/molehr/gar064)