Placenta and Reproductive Medicine

REVIEW ARTICLE

# Endometrial organoids and trophoblast organoids: Novel models for investigation of maternal-fetal interactions

Lijin Peng[1,2], Tingxuan Yin[1,2], Weijie Zhao[1,2], Chunfang Xu[1,2], Meirong Du[1,2]

[1]NHC Key Lab of Reproduction Regulation (Shanghai Institute of Planned Parenthood Research), Hospital of Obstetrics and Gynecology, Fudan University Shanghai Medical College, Shanghai 200090, China;

[2]Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Shanghai 200011, China

**Abstract**

A coordinated crosstalk between the maternal body and embryo is indispensable in a successful pregnancy. However, reliable models to study the potential cellular and molecular mechanisms of maternal-fetal interaction have not been established. The widely used research models including animal models and cell lines have significant limitations. Recently, the emergence and advancement of organoids have aroused attention. Endometrial organoids and trophoblast organoids which recapitulate human decidua and trophoblasts in three-dimensional cultures provide new opportunities to study the physiological or pathological processes of decidualization and implantation. Furthermore, to recapitulate precise cell-cell communication in the decidual microenvironment *in vivo*, the co-culture of multicellular organoids will be a potential optimal model for future investigations of crosstalk at the maternal-fetal interface. Here, we focus on the latest development and advancement of endometrial organoids and trophoblast organoids.

**Key words:** organoids, endometrial, trophoblast, maternal-fetal interactions

## INTRODUCTION

In humans, the incidence of pregnancy loss is much higher than in other mammal species, with only about 30% of the natural pregnancies ending up with live birth.[1] Even with the wide application of assisted reproductive technology, the live birth rate of *in vitro* fertilization is still not optimistic.[2] A large portion of such fetal losses occurs in the initial period of pregnancy before being recognized clinically.[1] To solve this tough question, investigating the mysteries of maternal-fetal interaction in early pregnancy is one of the key points.

The establishment of a coordinated dialogue between the embryo and the maternal endometrium is indispensable for a successful conception. Decidualization, a critical step of pregnancy, refers to the process in which endometrial stromal cells proliferate and differentiate into secreting decidual cells. In humans, the transformation into the decidua completes after the blastocyst is embedded in the endometrium, because the success of embryo implantation guarantees the further decidual reaction.[3] In return, decidual cells affect the growth and development of the placenta and embryo by releasing some signals, like glandular secretions, which contribute to nutritional support until maternal blood penetrates into the intervillous space.[4,5] The decidua also regulates the maternal immune system which helps accept the semi-allogeneic fetus.[4] Besides, embryo implantation is another essential procedure, which is divided into three phases: apposition, adhesion and invasion. This complex

**Corresponding Author:**
Prof. Meirong Du
E-mail: mrdu@fudan.edu.cn
Peer review under responsibility of Scholar Media Publishing.

**Citation:**
Peng L, Yin T, Zhao W, Xu C, Du M. Endometrial organoids and trophoblast organoids: Novel models for investigation of maternal-fetal interactions. Placenta Reprod Med 2022;1:10.

**DOI:** 10.54844/prm.2022.0141

**Received:** 06 August 2022
**Accepted:** 23 November 2022
**Published:** 30 December 2022

physiological process requires receptive endometrium, decidualization and communication between the uterus and a healthy blastocyst.[6] Accordingly, maternal-fetal interaction is pivotal at the early stage of pregnancy, so that any aberrant factors during the process can lead to the termination of pregnancy or health risks to both maternal body and fetus.

Despite the importance of maternal-embryo crosstalk during decidualization, implantation and placentation, reliable models to study the potential cellular and molecular mechanisms have not been established. The widely used research models involve animal models and cell lines, both of which have significant limitations. Rodents, popular animal models used in endometrium and implantation research,[7–9] cannot recapitulate human endometrium with accurate features and functions. For instance, decidualization of endometrium in mice is only triggered by implanting blastocyst or mechanical stimulation, while a similar reaction is spontaneous and cyclic in human.[10,11] In addition, the primary syncytium originated from trophectoderm begins to form after complete embryo invasion, but this differentiation is not observed in mice, making the murine embryo a less suitable material for implantation study.[12–14] The deep invasion of the trophoblast cannot be realized in murine placentation, which may cause the discrepancy in experimental results.[15] Non-human primates with menstrual cycles seem to be ideal for endometrial and placental studies, however, the high cost, large labor, specific experimental infrastructure, and ethics become the main obstacles.[10,16] As for *in vitro* models, primary cells are inferior due to their disability of long-term expansion. Immortalized cell lines derived from endometrial cancer including ECC1 and Ishikawa cells are eligible for embryo implantation investigation, but they cannot recapitulate the physiological state of endometrial epithelium with transformed phenotypes.[17,18] Recently, an immortalized endometrial stromal cell line that helped identify many genes and chemical modulators in human decidualization has been depicted.[19] There are also many types of trophoblast cell lines that have been generated, such as BeWo, HPT-8, JEG-3 and others. However, a common shortcoming of these cell line systems is that they fail to mimic the cell-cell interactions in three-dimensional (3D) structures and microenvironments *in vivo*.

Therefore, the emergence and advancement of organoid have aroused attention. Endometrial organoids and trophoblast organoids in 3D cultures offer new opportunities to study maternal-fetal interactions during crucial steps in pregnancy.

## EMERGENCE OF ORGANOIDS

The term "organoid" originally referred to a cellular cluster cultured from stem/progenitor cells, induced pluripotent stem cells or embryonic stem cells, which could organize into a 3D model with abilities of self-renewing and genetical stability.[20] Generally, the broader definition involves organoids derived from differentiated cells, such as epithelial cells. Until now, the three main approaches to establish organoid models include Matrigel medium, gas-liquid interface culture and rotating bioreactor.[21] Due to the rapidly improving culture technique since the 2010s, organoids from various types of tissues, like colon, pancreas, liver, fallopian tube, and prostate, have been developed.[22–26]

In two-dimensional (2D) cultures, complicated microenvironments with cell-cell interactions cannot be reconstructed, which results in a decrease in accuracy in experimental results. Compared with 2D models, 3D organoids closely recapitulate the anatomic structure and physiological function of original tissues. Thus, organoids can be widely applied in the investigation of organ development, disease and treatments.

### *Endometrial organoids*

The endometrium is the mucous membrane that covers the inner wall of the uterus and plays a vital role in reproduction. It consists of functional layer shedding monthly with the fluctuation of ovarian hormones and basal layer without periodic change. At present, organoids from human and murine endometrium have been established, presenting long-term expandability and epithelium characteristics.[27–29] Many kinds of endometrial epithelia can be the primary source of organoids, including one in the secretory, proliferative, and postmenopausal phase as well as decidua. The most widely used culture system of endometrial epithelial organoids (EEO) is the Matrigel. The endometrial specimens were minced and digested by enzyme and thus generated glandular pellets, which were subsequently embedded into different Matrigel as extracellular matrix. The Matrigel medium containing growth and signal factors, such as WNT activators (WNT3A, R-spondin 1), epithelial-cell mitogens (epidermal growth factor, fibroblast growth factor and hepatocyte growth factor), Noggin, Nicotinamide and so on, enabled endometrial tissue fragments to proliferate and form organoid structures.[27,28,30] After dissociated and re-seeded, the organoids could reconstitute and propagate for more than 14 consecutive passages, achieving long-term culture.[27] EEO organized a glandular-like structure, a spheroid with a central lumen and surrounding polarized columnar epithelium. Since the function of endometrium usually depends on the interaction between epithelial and stromal cells which is hard to model in human,[31,32] the porous collagen scaffolds and micro-molded agarose gel have been used to co-culture endometrial stromal and epithelial cells.[33,34] In the former culture system with the porous collagen scaffolds created using controlled lyophilization, stromal cells and EEO were seeded into the scaffold successively to form the multicellular organoid that exhibited apicobasal polarized

epithelial layer on the surface and adjacent stromal cells.[33] The micro-molded agarose gel is another system that can establish such organoids. Two types of endometrial cells were cultured on the base of a micro-molded agarose gel plate instead of an exogenous scaffold and developed into organoids showing the spherical surface composed of polarized epithelial cells and the inner side full of stromal cells.[34] Therefore, 3D endometrial models recapitulating epithelial-stromal microenvironment *in vivo* have been established by combining collagen scaffolds or agarose gel with EEO. One of the greatest characteristics of endometrial organoids is that they closely reflect the original source, with the specific markers and gene profiles of original cells.[20,27,28,34,35] Besides, endometrial organoids are sensitive to different hormonal treatments and thus show variant physiological responses.[27,28,33,34]

With the developed culture systems, it has been proved that endometrial organoids exert a huge impact on understanding the underlying mechanism of maternal-embryo interaction. Pregnant endometrium, also known as decidua, has been isolated and successfully generated organoids, which may contribute to the study on decidualization. In the same research, scientists found that human endometrial organoids cultured with additional hormones from decidualized stroma and placenta (prolactin, human chorionic gonadotropin, and human placental lactogen) as well as signal molecule (cAMP) have undergone further differentiation and acquired a decidual phenotype.[28] Similar findings were seen in a co-culture system of porous collagen scaffolds, in which hormones induced stromal cells to decidualize and transform into the secretory state.[33] Besides, Virginia *et al.* cocultured EEOs from decidual glands and endometrial stromal fibroblasts differentiated from human pluripotent stem cells (PSC-ESFs) in Matrigel.[36] Then, they observed that the cocultures of PSC-ESFs and EEOs could exhibit decidual characteristics in both gene expression and cell morphology in response to hormonal signals, self-organizing a 3D model of the human decidua. Collectively, appropriate treatment of hormones can stimulate the endometrial organoids to differentiate towards decidua, mimicking the biological process of human decidualization *in vivo* to some extent. Therefore, these endometrial organoids become potential *in vitro* models to study how decidual cells regulate the development of the placenta and how they interact with blastocyst.

In addition, endometrial organoids can be used to research on embryo implantation and the endometrium-embryo crosstalk at the implantation site. With the change in hormonal treatment, EEOs established by Luddi *et al.* demonstrated the distinct features of corresponding phases of the menstrual cycle in terms of both morphology and function.[37] Significantly, EEOs in the mid-secretory phase displayed the luminal cell surface with large protrusions named pinopodes, which is one of the critical markers of the implantation window. The expression of genes related to endometrial receptivity including progestagen associated endometrial protein (PAEP), vascular endothelial growth factor, insulin like growth factor 1 and matrix metallopeptidase 26 increased in EEOs of the mid-secretory phase compared to the proliferative phase, making them suitable indicators of the implantation window. Meanwhile, EEOs expressed a larger quantity of glycodelin A encoded by PAEP in the secretory phase, reflecting the physiological changes in the window of uterine receptivity *in vivo*. Furthermore, based on the prior knowledge that mechanosensitive ion channels participate in interactions between the endometrium and the blastocyst, expression of the PIEZO1 channel was described in endometrial organoids and primary endometrial epithelial cells.[37] Strong calcium responses were observed in EEOs under chemical and mechanical stimulation, which provided further evidence for the functional expression of mechanosensitive PIEZO1 channels in endometrial epithelial cells. Consequently, the role of the PIEZO1 channel in maternal-fetal crosstalk at the implantation interface was validated. All these factors mentioned above are important in the dialogue between the maternal uterus and blastocyst.

In general, the results of these studies confirm that endometrial organoids could closely recapitulate the characteristics of decidua and a receptive endometrium. Therefore, this novel kind of 3D model becomes an effective tool to investigate the physiological or pathological processes of decidualization and implantation, which can help understand maternal-embryo interactions.

## TROPHOBLAST ORGANOIDS

In human, the placenta is closely associated with decidua and connects the mother and fetus. It is a temporary extraembryonic organ that provides nutrients, secretes hormones and protects the fetus to support gestation. Trophoblasts, derived from the trophectoderm of the blastocyst, constitute a large part of the placenta and become one of the unique components. The trophoblast lineages of the placenta include three subtypes: villous cytotrophoblast (VCT) which can differentiate into the other two subtypes of trophoblasts, syncytiotrophoblast (SCT) with the responsibility for exchanging nutrients and producing pregnancy hormones and extravillous trophoblast (EVT) which makes the placenta attached by invading into the decidua and helps the transformation of spiral arteries.[5,38,39] To make up for the deficiencies of animal models and cell lines, trophoblast organoids have been developed and become a promising model of placental villi.[39–41] The original tissue of trophoblast organoids is the placenta in the first few weeks of pregnancy. Similar to the medium used in human trophoblast stem cells,[42] Matrigel medium containing growth factors and signaling inhibitors is used in the culture of trophoblast organoids.

Different conditions of WNT signaling determine the lineage specification and differentiation of these organoids.[40,43] Treatment with exogenous WNT activators maintains the growth of cytotrophoblast organoids, while the inhibition of the WNT signaling stimulates the organoids to develop into HLA-G$^+$ EVT. Moreover, the long-term expandability, the common nature of organoids, is also guaranteed, with sustainable passaging for more than a year.[41] Hence, the study on discrete procedures of EVT formation in sequence will be conducted with this model. Since both the maternal and fetal tissue existed in the placental material, it is necessary to ensure the fetal origin of trophoblast organoids. Researchers verified the derivation of organoids through Human leukocyte antigen (HLA) typing and confirmed their identities according to previous criteria of trophoblast.[39,44]

Literature indicates that trophoblast organoids display structural and functional characteristics of the human first-trimester placenta and showed genetic stability.[39–41] Pregnancy hormones and peptides, such as human chorionic gonadotropin, growth differentiation factor, pregnancy-specific glycoprotein, aldose reductase, and so on, were detected in the cultures, demonstrating the metabolic and endocrine function of trophoblast organoids. The HLA-G$^+$ EVT differentiated from the organoids vigorously migrated and invaded into the 3D cultures, resembling the process of trophoblast invasion. Besides, the similarity between the trophoblast organoids and villous placenta was also proved through analysis of the methylome.[39] As a novel 3D model recapitulating the human first-trimester placenta, it provides an alternative approach allowing researchers to study the trophoblast differentiation and placentation. Because this *in vitro* model closely mimicked the formation of cytotrophoblast cells and the differentiation of EVT, it will promote the investigation of the mutual effects of trophoblast and decidual microenvironment.[39] In summary, these studies indicate the potential applications of trophoblast organoids. The interaction between the decidua and placenta after implantation will be explored in depth with this powerful tool.

## FUTURE PERSPECTIVES

### *Co-culture models*

To reproduce precise cell-cell communications of native organs and tissues *in vivo*, co-culture of multicellular organoids is required. Reports show that leukocytes are highly represented in decidual cells, with a percentage of 30%–40%, at the early stage of pregnancy.[45,46] The decidual immune microenvironment consists of multiple kinds of immune cells, like lymphocytes (B cells and T cells), natural killer (NK) cells, dendritic cells and macrophages.[45] Decidual stromal cells, trophoblasts and immune cells contact with each other, developing a vast cellular network to maintain the conception.[47] However, there are still many unsolved questions about how maternal immune system coordinates with conceptus. Combining organoids with immune cells will be an effective method to illuminate the underlying mechanism. Research on intestinal homeostasis, digestive diseases, pulmonary disease and breast neoplasms has been conducted by coculturing the corresponding epithelial organoids and immune cells.[48] Recently, Dolat *et al.* cocultured infected endometrial organoids with neutrophils to build a primary immune cell response in a study of Chlamydia infections, showing evidence for the generation of the coculture system.[49] Furthermore, to understand the exact function of uterine natural killer (uNK) cells which are the major subsets of leukocytes within the decidua, it is possible to coculture the trophoblast organoids with uNK cells to identify related factors.[50] Therefore, endometrial organoids or trophoblast organoids cocultured with immune cells, such as NK cells, macrophages and so on, can potentially be used to explore how immune cells mediate the maternal-fetus tolerance in early pregnancy. Establishment of the multicellular organoids which better recapitulate the microenvironment at the maternal-fetal interface will be a tremendous advance.

In addition, the combination of different types of organoids might provide a novel model for some research. For example, organoids of the trophoblast and endometrium could be cultured together to study interactions of trophoblast cells with the maternal environment during the trophoblast differentiation and invasion.[28] Another literature pointed out that the coculture of endometrial models with trophoblast organoids and blastoids (blastocyst organoids) could develop into an *in vitro* model specific for implantation study.[51] However, ethical implications must be considered before the creation of these coculture systems, and strict scrutiny is required.[52,53] Although there are many challenges in the coculture of multiple organoids, this kind of multicellular model will provide incredible opportunities to investigate the early events of pregnancy in human.

### *Microfluidics*

Recently, a new technology named microfluidics has been developed to improve the co-culture of organoids and other cell types. Microfluidics is a tool to operate fluids through submillimeter channels to further manipulate the location of cells and tissues, fluid flow parameters, gradient parameters and mechanical cues.[54] Microfluidic organ-on-a-chip models which can co-culture living cells ideally mimic organs in physiological or pathological state at micrometer diameters.[55] Furthermore, it has been proved that microfluidics can be applied in cancer studies by recapitulating interactions between immune cells and cancer cells and metastatic microenvironment.[56–58] Similarly, with the support of a microfluidics tool, co-culturing endometrial or trophoblast organoids with decidual immune cells will become a potential approach to investigating the maternal-

fetal microenvironment. Therefore, the combination of microfluidics technology and organoids provides a novel model for future study in many fields.

### *Genome engineering*

With the rapid development of gene technology, the application of genetic engineering in organoid models will enable scientists to study physiological and pathological phenomena with higher accuracy. CRISPR/Cas9 gene editing system can be used to disrupt or modify the targeted genetic locus. With such a technique, the chloride channel function of intestinal stem cell organoids from patients with cystic fibrosis has been repaired, retaining the normal function of organoids.[59] Besides, Kopper *et al.* introduced the fallopian tube organoids genetically modified for the investigation of ovarian cancer.[60] In the future, as an efficient bioengineering tool, genome engineering might be combined with organoids of endometrium or trophoblast to further analyze the outstanding questions of pregnancy.

### *Tissue engineering*

Although organoids recapitulate human tissue *ex vivo*, the morphogenesis process which exists in internal developing tissues is not reproduced in organoids.[61,62] Morphogenesis is regulated by biophysical and biochemical factors which coordinate the cellular composition and structure of *in vivo* tissues spatiotemporally.[63] Therefore, tissue engineering methodologies have been developed to regulate these factors and instruct morphogenesis in organoids. The novel methodologies include engineered hydrogel scaffolds, controlling organoid assembly, spatiotemporal regulating of morphogen gradients as well as artificial gene circuits through genetic engineering.[64] To precisely establish models recapitulating the microenvironment of embryo development and maternal-fetal crosstalk, integrating tissue engineering methods and endometrial and trophoblast organoids is necessary to control morphogenesis *in vitro*. Such integrated models with organoids and tissue engineering will become optimal approaches in wild fields.

## CONCLUSION

Maternal-fetal crosstalk affects the entire process of early pregnancy, including decidualization, embryo implantation, placental development and so on. It is very challenging to study pregnancy physiology in human, considering specimen accessibility and ethical factors. Although much research has been done, much is unknown about the underlying cellular and signaling mechanism of these events. Recently, endometrial organoids and trophoblast organoids that both closely recapitulate the microenvironment at the maternal-fetal interface have been reported as novel 3D models to investigate the dialogue between the decidua and blastocyst or embryo. However, there are also some common questions about organoid models to be solved. Thus, it is necessary to take into account the reproducibility, standardization, and validation of organoids to further improve this culture system.[65] Moreover, when establishing the implantation model, ethical issues should not be ignored.[52] In conclusion, organoids of endometrium and trophoblast are opening new avenues for the investigation of maternal-fetal interactions, which will make huge contributions to female reproductive health.

## Source of Funding

The work was supported by the National Key R&D Program of China (2017YFC0206500) and the National Nature Science Foundation of China (82271713, 31970859 and 81630036).

## Conflict of Interest

Meirong Du is an editorial board member of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of this editor and her research groups.

## REFERENCE

1. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. Hum Reprod Update 2002;8:333–343.
2. Gleicher N, Kushnir VA, Barad DH. Worldwide decline of IVF birth rates and its probable causes. Hum Reprod Open 2019;2019:hoz017.
3. Zhang S, Lin H, Kong S, Wang S, Wang H, Wang H, *et al.* Physiological and molecular determinants of embryo implantation. Mol Aspects Med 2013;34:939–980.
4. Chavan AR, Bhullar BA, Wagner GP. What was the ancestral function of decidual stromal cells? A model for the evolution of eutherian pregnancy. Placenta 2016;40:40–51.
5. Turco MY, Moffett A. Development of the human placenta. Development 2019;146:dev163428.
6. Fukui Y, Hirota Y, Matsuo M, Gebril M, Akaeda S, Hiraoka T, *et al.* Uterine receptivity, embryo attachment, and embryo invasion: Multistep processes in embryo implantation. Reprod Med Biol 2019;18:234–240.
7. Zuberi A, Lutz C. Mouse Models for Drug Discovery. Can New Tools and Technology Improve Translational Power? Ilar j 2016;57:178–185.
8. Matsumoto H. Molecular and cellular events during blastocyst implantation in the receptive uterus: clues from mouse models. J Reprod Dev 2017;63:445–454.
9. Wu SP, Emery OM, DeMayo FJ. Molecular Studies on Pregnancy with Mouse Models. Curr Opin Physiol 2020;13:123–127.
10. Ochoa-Bernal MA, Fazleabas AT. Physiologic Events of Embryo Implantation and Decidualization in Human and Non-Human Primates. Int J Mol Sci 2020;21:1973.
11. Finn CA, Martin L. Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. Biol Reprod 1972;7:82–86.
12. Aplin JD, Ruane PT. Embryo-epithelium interactions during implantation at a glance. J Cell Sci 2017;130:15–22.
13. James JL, Carter AM, Chamley LW. Human placentation from nidation to 5 weeks of gestation. Part II: Tools to model the crucial first days. Placenta 2012;33:335–342.
14. Pollard RM, Finn CA. Influence of the trophoblast upon differentiation of the uterine epithelium during implantation in the mouse. J Endocrinol 1974;62:669–674.
15. Carter AM. Animal models of human placentation--a review. Placenta. 2007;28 Suppl A:S41–S47.

16. Song Y, Joshi NR, Vegter E, Hrbek S, Lessey BA, Fazleabas AT. Establishment of an Immortalized Endometriotic Stromal Cell Line from Human Ovarian Endometrioma. Reprod Sci 2020;27:2082–2091.
17. Mo B, Vendrov AE, Palomino WA, DuPont BR, Apparao KB, Lessey BA. ECC-1 cells: a well-differentiated steroid-responsive endometrial cell line with characteristics of luminal epithelium. Biol Reprod 2006;75:387–394.
18. Zhang D, Lv P, Zhang R, Luo Q, Ding G, Yin L, *et al*. A new model for embryo implantation: coculture of blastocysts and Ishikawa cells. Gynecol Endocrinol 2012;28:288–292.
19. Haller M, Yin Y, Ma L. Development and utilization of human decidualization reporter cell line uncovers new modulators of female fertility. Proc Natl Acad Sci U S A 2019;116:19541–19551.
20. Song Y, Fazleabas AT. Endometrial Organoids: A Rising Star for Research on Endometrial Development and Associated Diseases. Reprod Sci 2021;28:1626–1636.
21. Ashok A, Choudhury D, Fang Y, Hunziker W. Towards manufacturing of human organoids. Biotechnol Adv 2020;39:107460.
22. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, *et al*. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology 2011;141:1762–1772.
23. Huch M, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M, *et al*. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. Embo J 2013;32:2708–2721.
24. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, *et al*. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 2015;160:299–312.
25. Kessler M, Hoffmann K, Brinkmann V, Thieck O, Jackisch S, Toelle B, *et al*. The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. Nat Commun 2015;6:8989.
26. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J, *et al*. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. Cell 2014;159:163–175.
27. Boretto M, Cox B, Noben M, Hendriks N, Fassbender A, Roose H, *et al*. Development of organoids from mouse and human endometrium showing endometrial epithelium physiology and long-term expandability. Development 2017;144:1775–1786.
28. Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, *et al*. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. Nat Cell Biol 2017;19:568–577.
29. Marinić M, Rana S, Lynch VJ. Derivation of endometrial gland organoids from term placenta. Placenta 2020;101:75–79.
30. Haider S, Gamperl M, Burkard TR, Kunihs V, Kaindl U, Junttila S, *et al*. Estrogen Signaling Drives Ciliogenesis in Human Endometrial Organoids. Endocrinology 2019;160:2282–2297.
31. Kelleher AM, Milano-Foster J, Behura SK, Spencer TE. Uterine glands coordinate on-time embryo implantation and impact endometrial decidualization for pregnancy success. Nat Commun 2018;9:2435.
32. Hantak AM, Bagchi IC, Bagchi MK. Role of uterine stromal-epithelial crosstalk in embryo implantation. Int J Dev Biol 2014;58:139–146.
33. Abbas Y, Brunel LG, Hollinshead MS, Fernando RC, Gardner L, Duncan I, *et al*. Generation of a three-dimensional collagen scaffold-based model of the human endometrium. Interface Focus 2020;10:20190079.
34. Wiwatpanit T, Murphy AR, Lu Z, Urbanek M, Burdette JE, Woodruff TK, *et al*. Scaffold-Free Endometrial Organoids Respond to Excess Androgens Associated With Polycystic Ovarian Syndrome. J Clin Endocrinol Metab 2020;105:769–780.
35. Fitzgerald HC, Dhakal P, Behura SK, Schust DJ, Spencer TE. Self-renewing endometrial epithelial organoids of the human uterus. Proc Natl Acad Sci U S A 2019;116:23132–23142.
36. Cheung VC, Peng CY, Marinić M, Sakabe NJ, Aneas I, Lynch VJ, *et al*. Pluripotent stem cell-derived endometrial stromal fibroblasts in a cyclic, hormone-responsive, coculture model of human decidua. Cell Rep 2021;35:109138.
37. Hennes A, Held K, Boretto M, De Clercq K, Van den Eynde C, Vanhie A, *et al*. Functional expression of the mechanosensitive PIEZO1 channel in primary endometrial epithelial cells and endometrial organoids. Sci Rep 2019;9:1779.
38. Hamilton WJ, Boyd JD. Development of the human placenta in the first three months of gestation. J Anat 1960;94:297–328.
39. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, *et al*. Trophoblast organoids as a model for maternal-fetal interactions during human placentation. Nature 2018;564:263–267.
40. Haider S, Meinhardt G, Saleh L, Kunihs V, Gamperl M, Kaindl U, *et al*. Self-Renewing Trophoblast Organoids Recapitulate the Developmental Program of the Early Human Placenta. Stem Cell Reports 2018;11:537–551.
41. Sheridan MA, Fernando RC, Gardner L, Hollinshead MS, Burton GJ, Moffett A, *et al*. Establishment and differentiation of long-term trophoblast organoid cultures from the human placenta. Nat Protoc 2020;15:3441–3463.
42. Okae H, Toh H, Sato T, Hiura H, Takahashi S, Shirane K, *et al*. Derivation of Human Trophoblast Stem Cells. Cell Stem Cell 2018;22:50–63.e56.
43. Kretzschmar K, Clevers H. Organoids: Modeling Development and the Stem Cell Niche in a Dish. Dev Cell 2016;38:590–600.
44. Lee CQ, Gardner L, Turco M, Zhao N, Murray MJ, Coleman N, *et al*. What Is Trophoblast? A Combination of Criteria Define Human First-Trimester Trophoblast. Stem Cell Reports 2016;6:257–272.
45. Faas MM, de Vos P. Uterine NK cells and macrophages in pregnancy. Placenta 2017;56:44–52.
46. Bulmer JN, Williams PJ, Lash GE. Immune cells in the placental bed. Int J Dev Biol 2010;54:281–294.
47. Yang F, Zheng Q, Jin L. Dynamic Function and Composition Changes of Immune Cells During Normal and Pathological Pregnancy at the Maternal-Fetal Interface. Front Immunol 2019;10:2317.
48. Bar-Ephraim YE, Kretzschmar K, Clevers H. Organoids in immunological research. Nat Rev Immunol 2020;20:279–293.
49. Dolat L, Valdivia RH. An endometrial organoid model of interactions between Chlamydia and epithelial and immune cells. J Cell Sci 2021;134.
50. Nikolakopoulou K, Turco MY. Investigation of infertility using endometrial organoids. Reproduction 2021;161:R113–R127.
51. Ban Z, Knöspel F, Schneider MR. Shedding light into the black box: Advances in in vitro systems for studying implantation. Dev Biol 2020;463:1–10.
52. Bredenoord AL, Clevers H, Knoblich JA. Human tissues in a dish: The research and ethical implications of organoid technology. Science 2017;355:eaaf9414.
53. Boers SN, Bredenoord AL. Consent for governance in the ethical use of organoids. Nat Cell Biol 2018;20:642–645.
54. Yu F, Hunziker W, Choudhury D. Engineering Microfluidic Organoid-on-a-Chip Platforms. Micromachines (Basel) 2019;10:165.
55. Bhatia SN, Ingber DE. Microfluidic organs-on-chips. Nat Biotechnol 2014;32:760–772.
56. Sontheimer-Phelps A, Hassell BA, Ingber DE. Modelling cancer in microfluidic human organs-on-chips. Nat Rev Cancer 2019;19:65–81.
57. Puschhof J, Pleguezuelos-Manzano C, Clevers H. Organoids and organs-on-chips: Insights into human gut-microbe interactions. Cell Host Microbe 2021;29:867–878.
58. Aboulkheyr Es H, Montazeri L, Aref AR, Vosough M, Baharvand H. Personalized Cancer Medicine: An Organoid Approach. Trends Biotechnol 2018;36:358–371.
59. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, *et al*. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 2013;13:653–658.
60. Kopper O, de Witte CJ, Lõhmussaar K, Valle-Inclan JE, Hami N, Kester L, *et al*. An organoid platform for ovarian cancer captures intra- and interpatient heterogeneity. Nat Med 2019;25:838–849.
61. Yin X, Mead BE, Safaee H, Langer R, Karp JM, Levy O. Engineering Stem Cell Organoids. Cell Stem Cell 2016;18:25–38.
62. Brassard JA, Lutolf MP. Engineering Stem Cell Self-organization to

Build Better Organoids. Cell Stem Cell 2019;24:860–876.
63. Ashton RS, Keung AJ, Peltier J, Schaffer DV. Progress and prospects for stem cell engineering. Annu Rev Chem Biomol Eng 2011;2:479–502.
64. Marti-Figueroa CR, Ashton RS. The case for applying tissue engineering methodologies to instruct human organoid morphogenesis. Acta Biomater 2017;54:35–44.
65. Huch M, Knoblich JA, Lutolf MP, Martinez-Arias A. The hope and the hype of organoid research. Development 2017;144:938–941.