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Mini-Review

Developments in reproductive biology and medicine

Endometrial organoids to model benign disorders affecting the endometrium

Marie-Madeleine Dolmans [1,2,*], Chloé Beaussart [1]

[1]Pôle de Recherche en Gynécologie, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium
[2]Gynecology Department, Cliniques Universitaires St-Luc, Brussels, Belgium

*Correspondence address. Gynecology Department, Cliniques Universitaires St-Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium.
E-mail: marie-madeleine.dolmans@uclouvain.be https://orcid.org/0000-0002-6331-3026

ABSTRACT

The endometrium is a highly dynamic and complex tissue lining the uterus, playing a central role in reproductive health. Despite its importance, the pathogenesis of many benign endometrial disorders remains poorly understood, largely due to limitations in current experimental models. Traditional *in vivo* models like murine and primate models fail to replicate key human-specific features like menstruation or spontaneous disease development. Similarly, conventional 2-dimensional *in vitro* cultures using cell lines or primary cells lack the structural and functional complexity of native endometrium, often losing physiological relevance over time. To address these challenges, endometrial epithelial organoids (EEOs), 3-dimensional self-organizing epithelial structures derived from endometrial biopsies, have emerged as a promising *in vitro* model. EEOs mimic many aspects of *in vivo* endometrial glands, including apical-basal polarity, hormone responsiveness, long-term preservation of epithelial identity, and retention of patient-specific genetic and molecular signatures. Their ability to reproduce cellular interactions and tissue architecture makes them an invaluable tool for studying endometrial physiology and disease. This review explores the application of EEOs in modeling various benign conditions affecting the endometrium, including endometriosis, adenomyosis, uterine fibroids, implantation failure, endometrial aging, endometritis, and endometrial hypoplasia, as well as systemic diseases and exposure to environmental or pharmacological agents. While EEOs do not yet fully replicate functional human endometrium, they represent a significant step forward in bridging the gap between basic research and clinical understanding of endometrial disorders.

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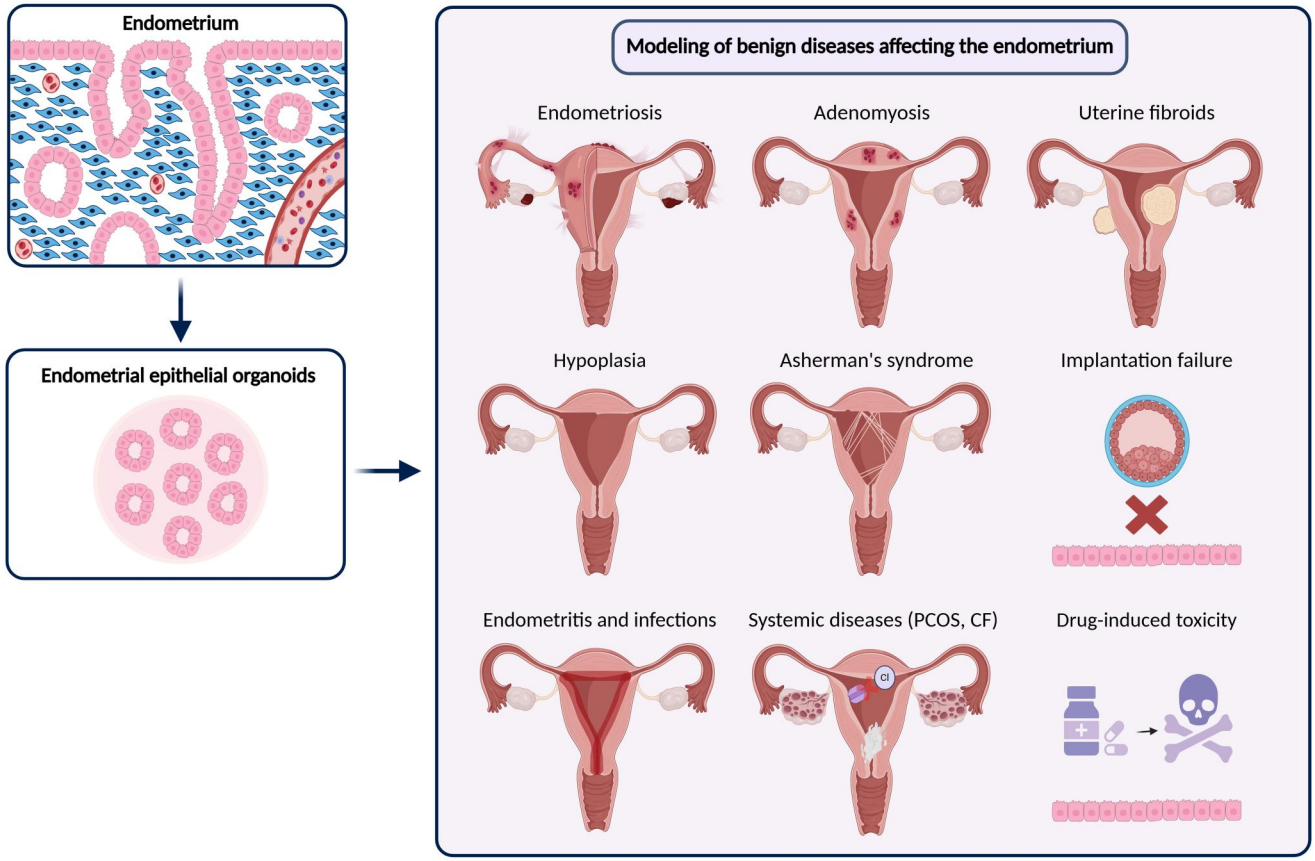
GRAPHICAL ABSTRACT



Modeling of benign diseases affecting the endometrium. Primary endometrial tissue can be used to generate endometrial epithelial organoids that replicate key structural and functional features of native endometrium. They provide a versatile *in vitro* platform to mimic a wide range of conditions affecting the endometrium, including endometriosis, adenomyosis, uterine fibroids, endometrial hypoplasia, Asherman's syndrome, implantation failure, endometritis and infections, systemic diseases, and drug-induced toxicity (CF, cystic fibrosis; PCOS, polycystic ovary syndrome). Created in BioRender. Dolmans, M. (2026) https://BioRender.com/eo50jgk.

Keywords: endometrium / endometrial cells / organoids / assembloids / endometriosis / adenomyosis / endometrial pathology / disease modeling

Introduction

The endometrium is a complex multicellular tissue lining the inner wall of the uterus, constituting one of the most dynamic organs in the human body. Disorders affecting its structure and function, like endometriosis, adenomyosis, and uterine fibroids, represent a major gynecological concern, yet their pathogenesis remains poorly understood. Thus far, use of cell lines has helped to further our grasp of endometrial biology and associated diseases, but is unable to replicate key physiological features of the endometrium in terms of its complex architecture and functions (Song and Fazleabas, 2021). One of the main factors hampering our understanding of the pathogenesis of endometrial pathologies has been the lack of reliable and reproducible models. A number of *in vivo* and *in vitro* models have been proposed over the years, but all of them have had drawbacks.

In vivo models: Various murine and even baboon models have been developed and, despite understandable ethical and biological shortcomings, have provided significant insights into endometrial physiology and pathology (Grümmer, 2006; Donnez *et al.*, 2015; Simitsidellis *et al.*, 2018; Marquardt *et al.*, 2020). However, these models remain limited. Indeed, mice do not menstruate, and neither mice nor baboons spontaneously reproduce the full spectrum of human endometrial disorders (Dehoux *et al.*, 2011).

In vitro models: Simple 2-dimensional (2D) cultures, like immortalized cell lines often derived from cancer cells, are straightforward to obtain and handle, but their inability to mimic individual patient characteristics seriously hinders their potential. Conversely, primary endometrial cells are able to maintain patient–patient heterogeneity and disease-specific characteristics. Unfortunately, primary endometrial epithelial cells only survive and propagate in 2D conditions for a given duration, as well as showing substantial differences from the normal 3-dimensional (3D) appearance of glands *in vivo*. Traditional 2D *in vitro* cultures trigger phenotypical changes in cells, failing to replicate physiological cell–cell and cell–microenvironment interactions.

To overcome these issues, 3D organoids were developed, quickly attracting the attention of the scientific community and generating more than 4,000 publications on PubMed during 2024

alone in various fields of translational research, 71 of which were on endometrial organoids. Organoids are small 3D structures, usually derived from stem or progenitor cells, designed to mimic the structure and function of human organs. They exhibit strong expandability thanks to their intrinsic stem/progenitor cell populations and optimized 3D culture conditions, allowing them to self-renew and be passaged multiple times while maintaining their epithelial identity. This overcomes the hurdle of insufficient quantities of primary biopsies.

When investigating human endometrium, primary tissue-derived organoids boast a range of advantages over 2D cultures, namely (i) formation of apical-basal polarity like glands *in vivo*, (ii) maintenance of the epithelial phenotype and molecular signature of glands of origin even after long-term culture, (iii) replication of cell–cell interactions and, above all, (iv) responsiveness to sex steroids and modification of their morphology and capacities accordingly (Boretto *et al.*, 2017; Turco *et al.*, 2017). Most importantly, they are able to capture disease heterogeneity and maintain key characteristics of the primary tissue, including its genetic background (Turco *et al.*, 2017; Boretto *et al.*, 2019; Ciprietti *et al.*, 2025).

Hence, endometrial epithelial organoid (EEO) cultures have emerged as a novel model to accurately mimic endometrial physiology, even if it is not yet possible to develop and examine fully functional human endometrium *ex vivo*. Nevertheless, these human EEOs can help us gain a deeper understanding of the complexity of the endometrium (Dias da Silva *et al.*, 2024).

In this review, we will discuss the use of EEOs as models for various benign endometrial pathologies, including endometriosis, adenomyosis, uterine fibroids, implantation failure, endometrial aging, endometritis, endometrial hypoplasia, systemic diseases, and environmental and drug-induced toxicity (Table 1).

Getting there: technical foundations of EEO culture

Organoid nomenclature

Organoids are defined as self-assembling 3D tissue constructs derived from adult stem or progenitor cells that mimic the architecture and function of the tissue of origin (Boretto *et al.*, 2017; Turco *et al.*, 2017). Figure 1A shows a picture of an endometrial organoid in culture. In the literature, the term 'organoid' is often used more broadly, which can lead to confusion when comparing studies. Indeed, models derived from pluripotent stem cells or cellular aggregates that form 3D structures without generating hollow epithelial glands can be more accurately described as 'spheroids' (Białkowska *et al.*, 2020; Wiwatpanit *et al.*, 2020; Stejskalová *et al.*, 2021). Acronyms for endometrial organoids also vary across studies. In this review, we apply the term EEOs to models derived from epithelial glands, in line with nomenclature recommendations from the World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonisation Project (EPHect) for experimental models in endometriosis research (Marr *et al.*, 2025).

Cell origin

EEOs are derived from primary endometrial cells, which can be isolated from both animal and human tissues depending on the research context. They can be generated from biopsies from different phases of the menstrual cycle, atrophic endometrium including samples from postmenopausal women (Turco *et al.*, 2017), hormone-treated endometrium (Filby *et al.*, 2021), as well as decidual tissue acquired after early pregnancy termination (Turco *et al.*, 2017). These specimens are typically used fresh, but can also be cryopreserved, while retaining their organoid-forming capacity (Fitzgerald *et al.*, 2019; Heidari-Khoei *et al.*, 2022). Traditionally, biopsy remains the most commonly applied method to obtain tissue, but it is invasive and requires clinical expertise. More recently, endometrial cells recovered from menstrual effluent have emerged as a promising noninvasive and reliable source, maintaining similar hormone responsiveness to biopsy-derived organoids (Cindrova-Davies *et al.*, 2021; Filby *et al.*, 2021) (Fig. 2).

Extracellular matrix

A key component of EEO culture is the matrix that provides the scaffolding to enable 3D growth. The gold standard matrix for almost all organoid models is a basement membrane extract hydrogel, namely Matrigel, derived from Engelbreth–Holm–Swarm mouse sarcoma cells. Thanks to its complex composition and gelation properties at 37°C, it effectively supports organoid formation, growth, and differentiation, but has limitations because of its xenogeneic origin, undefined constituents, and lot-to-lot variability (Marr *et al.*, 2025). Matrigel also typically restricts culture duration to 7–14 days, shorter than the 28-day menstrual cycle *in vivo*. However, Jiang *et al.* (2024) successfully maintained EEOs for 28 days in a spinning bioreactor using Geltrex, a similar basement membrane extract.

Alternatives have been developed to address these drawbacks, giving rise to two main strategies for hydrogel matrices in EEO culture: (i) use of native endometrial extracellular matrix (ECM) proteins as a hydrogel biomaterial; and (ii) use of synthetic or defined ECM proteins to provide the minimal parameters needed for organoid culture. Among the former, decellularized human and animal ECMs have been exploited to promote proliferation of endometrial stem cells and support co-culture of epithelial and stromal cells (Francés-Herrero *et al.*, 2021; López-Martínez *et al.*, 2021; Jamaluddin *et al.*, 2022). Alternative approaches include collagen-based scaffolds from natural sources, such as lyophilized type I bovine collagen (Abbas *et al.*, 2020) or collagen I/III composites (Rawlings *et al.*, 2021). Fully synthetic hydrogels, like polyethylene glycol (PEG)-based matrices or fibrillar collagens and fibronectin, support organoid and stromal co-culture via cell adhesion and matrix remodeling, allowing organoids to interact with and deposit their own ECM. These hydrogels are highly tunable and animal-free, showing potential for clinical translation (Hernandez-Gordillo *et al.*, 2020; Gnecco *et al.*, 2023; Marr *et al.*, 2025). At the interface of these two strategies, semi-synthetic gelatin methacryloyl (GelMA) hydrogels offer a middle ground, combining intrinsic bioactivity with adjustable mechanical properties to support growth of both stromal cells and EEOs (Salisbury *et al.*, 2024).

Expansion medium

The most recent recommendations, extensively described by WERF, advise use of a phenol-free medium with adequate supplements. Indeed, phenol is known to show estrogenic activity. EEOs are cultured in a specialized serum-free medium based on DMEM/F12, supplemented with a combination of nutrients, growth factors, signaling modulators, and antioxidants to sustain progenitor-driven expansion and hormone responsiveness (Marr *et al.*, 2025). Core components include serum substitutes (N2, B27), epidermal growth factor, antibiotics/antimycotics, and antioxidants like N-acetyl-L-cysteine and nicotinamide. Organoid maintenance relies on Wnt/β-catenin stimulation (R-spondin-1) and inhibition of transforming growth factor beta (TGF-β)/bone morphogenetic protein (BMP) pathways (A83-01, Noggin). Inclusion of stromal cell-derived factors, such as fibroblast

Table 1. List of publications looking at endometrial organoids to model benign endometrial diseases.

First author, journal, year	Species	N (donors) and tissue type	Cell type	Disease model findings
		Endometriosis		
Boretto, *Nat Cell Biol*, 2019	Human	n = 15 healthy; n = 15 endometriosis, eutopic n = 21 ectopic; n = 11 hyperplastic	Epithelial	Multiple organoid models capture disease diversity to help advance drug screening and development
Luddi, *Cells*, 2020	Human	n = 5 healthy; n = 5 endometriosis, eutopic	Epithelial	EEOs reproduce key phase-dependent morphological, ultrastructural, and comprehensive molecular features, including specific glycodelin glycosylation patterns
Esfandiari, *Fertil Steril*, 2021	Human	n = 9 healthy; n = 16 endometriosis, matched eutopic and ectopic (endometriomas)	Epithelial	EEOs retain genomic and epigenetic signatures of the tissue of origin, including HOX gene clusters
Esfandiari, *RBMO*, 2021	Human	n = 3 healthy; n = 3 endometriosis, matched eutopic and ectopic (endometriomas)	Epithelial	Disrupted progesterone signaling in EEOs exhibits PRB downregulation linked to promoter hypermethylation in eutopic EEOs
Xu L, *J Reprod Immunol*, 2025	Human	n = 20 healthy; n = 20 endometriosis	Epithelial	EEOs treated with ACSL4 inhibitor PRGL493 have their endometrial receptivity and ultrastructure restored
Madasu, *PNAS*, 2024	Human	Endometriosis, eutopic	Epithelial	Potent selective EPHA2/4 inhibitors (CDD-2693, CDD-3167) curtail expansion of EEOs
Zhang R, *Sci Rep*, 2025	Human	n = 24 endometriosis, eutopic, 18 of which matched ectopic (endometriomas)	Epithelial	EEOs can be generated from endometriomas using varying estrogen and progesterone concentrations
Liao, *Commun Biol*, 2024	Human	n = 7 healthy; n = 7 endometriosis, eutopic	Epithelial + stromal	Assembloids treated with BMP-2 partially restore impaired decidualization
Ochoa, *Adv Sci*, 2025	Human	n = 5 confirmed endometriosis; n = 1 suspected endometriosis	Epithelial + (allogeneic) stromal or mesenchymal stem cells	Assembloids exposed to IL-1β mimicking the inflammatory 'ectopic' milieu show structural disruption and epithelial invasion of surrounding stroma
Haney, *BioRxiv*, 2025	Human	Eutopic and ectopic (endometriomas and peritoneal)	Epithelial + stromal + peripheral sensory brain organoids	Co-culture of EEOs with neuronal organoids sheds light on epithelial–fibroblast–neuronal interactions driving pain and inflammation
Gunther, *Hum Reprod*, 2026	Human	n = 23 ectopic (endometrioma, peritoneal, and deep-infiltrating)	Epithelial	Endometriotic EEOs can be established across all major surgical phenotypes with variable efficiency, and hormonal treatment at the time of biopsy significantly reduces organoid establishment success
		Adenomyosis		
Juárez-Barber, *J Pers Med*, 2022	Human	n = 6 healthy; n = 6 adenomyosis, eutopic	Epithelial	EEOs can mimic tissue- and disease-specific traits
Juárez-Barber, *RBMO*, 2023	Human	n = 4 adenomyosis, eutopic	Epithelial	EEO extravesicle cargo contains microRNAs involved in disease progression and impaired embryo implantation
Juárez-Barber, *Reprod Biol Endocrinol*, 2024	Human	n = 15 healthy; n = 15 adenomyosis, eutopic	Epithelial	Transcriptomic analysis of EEOs points to disease-specific pathways linked to implantation failure and pregnancy disorders
Stratopoulou, *Fertil Steril*, 2025	Human	n = 6 healthy; n = 6 adenomyosis, eutopic	Epithelial + stromal	Endometrial assembloids can model impaired endometrial receptivity
Fan, *Reprod Sci*, 2025	Human	n = 3 healthy; n = 3 adenomyosis	Epithelial	EEOs from adenomyosis patients exhibit increased DNMT1-mediated hypermethylation of Klotho and PPARγ,

(continued)

Table 1. Continued

First author, journal, year	Species	N (donors) and tissue type	Cell type	Disease model findings
				weakening their expression, but DNMT and HDAC can reactivate them
Xu QX, *FASEB Journal*, 2023	Mouse	Luminar and glandular epithelium from transgenic mice overexpressing Notch1	Epithelial	Notch1 overactivation increases proliferation of both LE and GE cells and reduces their transcriptional differences, indicating disrupted epithelial differentiation
Xu Y, *Sci China Life Sci*, 2025	Human	healthy and adenomyosis, eutopic and ectopic	Epithelial + stromal + smooth muscle cells	A three-layered assembloid model accurately mimics menstrual cycle dynamics and adenomyosis-specific epithelial and stromal abnormalities
		Uterine fibroids		
Zhang Y, *RBMO*, 2024	Human	n = 13 healthy; n = 13 endometrium adjacent to intramural myomas	Epithelial + stromal	Even small fibroids can alter hormonal signaling and ECM mechanotransduction, leading to dysregulation of genes involved in gland development, angiogenesis, and ciliary activity
		Implantation failure		
Turco, Nat Cell Biol, 2017	Human	n = 31 healthy; n = 3 endometrial carcinoma	Epithelial	Development of expandable long-term EEOs that stay are genetically stable and capable of differentiation after treatment with reproductive hormones
Zhou, Front Endocrinol, 2022	Human	Minimum n = 3 for each experiment, fertile and infertile women	Epithelial	Infertile EEOs respond aberrantly to estrogen and progesterone, showing dysregulated apical proteins, while trophoblast progenitor spheroids treated with apical secretions from infertile EEOs exhibit significantly less adhesion to epithelial monolayers
Luddi, Fertil Steril, 2021	Human	n = 5 women with proven fertility	Epithelial	Exposure to embryo-conditioned medium induces specific glycodelin A glycoforms, including a unique acidic glycoform in EEOs exposed to spent media from embryos that subsequently implant
Ahmad, *Reproduction*, 2024	Human	n = 2–4 healthy	Epithelial + *E. coli* or blastocyst	Apical-out EEOs expose the luminal epithelium to murine blastocysts and *E. coli*
Kagawa, *Nature*, 2022	Human	n = 3 healthy	Epithelial	Open-faced endometrial layers expose the luminal surface and allow direct depositing of human blastoids thereupon
Tian, *Adv Sci*, 2023	Human	n = 3 healthy	Epithelial + stromal	Air–liquid interface assembloids allow integrated analysis of epithelial–stromal interactions in tissue-mimetic settings
Shibata, *Sci Adv*, 2024	Human	Endometrium and decidua	Epithelial + stromal + HUVECs + blastoids or blastocysts	Apical-out assembloids enriched with an endothelial network derived from HUVECs can model the embryo–endometrial interface
Zhang H, *Adv Biol*, 2024	Human	n = 3 healthy; n = 3 women with RIF	Epithelial	EEOs derived from RIF patients preserve the distinct cellular and molecular signature of mid-secretory endometrium and show diminished responsiveness to sex steroids
Yang H, *Cell Prolif*, 2025	Human	n = 3 healthy; n = 3 women with RIF	Epithelial	Cilia-dependent processes, powered by mitochondrial activity and intraflagellar transport, are compromised in RIF EEOs
		Endometrial aging		
Lu, *Cell Prolif*, 2025	Human	Women below and above 35 years of age	Epithelial	The PI3K–AKT–FOXO1 signaling pathway is overactivated in aging endometrium and closely correlates with fibrosis and impaired receptivity characteristics. Blocking or

(continued)

Table 1. Continued

First author, journal, year	Species	N (donors) and tissue type	Cell type	Disease model findings
				activating PI3K could attenuate the effect of aging or accelerate dysfunction of EEOs
		Endometritis and endometrial infections		
Bishop, *Front Cell Infect Microbiol*, 2020	Mouse	NA	Epithelial + *C. trachomatis*	Fragmentation-based infection shows that *C. trachomatis* completes its full developmental cycle within infected EEOs
Dolat and Valdivia, *J Cell Sci*, 2021	Mouse	NA	Epithelial + *C. trachomatis* + neutrophils	Microinjection enables luminal infection of EEOs with cytoskeletal and Golgi reorganization, and co-culture with neutrophils allows visualization of immune cell recruitment
Dolat, *Cell Host Microbe*, 2022	Mouse	NA	Epithelial + *C. trachomatis* or blastocyst	Chlamydial effector TepP manipulates the host actin regulator EPS8 to disrupt tight junctions, so EEOs from mice lacking EPS8 are resistant to this effect
Zhang X, *Front Rep Health*, 2024	Human	n = 3 healthy	Epithelial + *E. coli*	Air–liquid interface system allows infection of EEOs, while inflammatory responses are attenuated by E2
Zhang X, *Sci Rep*, 2025	Human	n = 3 healthy	Epithelial + *E. coli*	Comparative analyses of microinjection, air–liquid interface, and apical-out protocols for *E. coli* conclude that apical-out EEOs best replicate natural infection dynamics
		Endometrial hypoplasia		
Peng, *Hum Reprod*, 2024	Human	Healthy and refractory thin	Epithelial	Oxygen-glucose-deprived EEOs mimic endometrial hypoplasia, revealing ischemia-driven cytoskeletal and junctional defects
		Asherman's syndrome (AS)		
Santamaria, *Nat Commun*, 2023	Human	n = 3 healthy; n = 3 women with AS	Epithelial	EEOs from AS endometrium show epithelial loss, altered Wnt/Notch signaling, and disrupted epithelial communication, fostering a profibrotic, proinflammatory, and antiangiogenic environment
		Systemic diseases		
Ye, *Redox Biol*, 2025	Human	Endometrium from PCOS patients	Epithelial	EEOs modeling PCOS endometrial dysfunction show that GPX4 deficiency induces ferroptosis, fibrosis, and ECM remodeling via TGF-β1/Smad2/3 signaling, with GSH supplementation alleviating fibrotic phenotypes
Luyckx, *Hum Reprod*, 2025	Human	n = 3 lean healthy; n = 3 overweight healthy; n = 3 lean PCOS; n = 3 overweight PCOS	Epithelial	PCOS EEOs capture endometrial abnormalities, including increased inflammation and decreased receptivity-related gene expression
De Pauw, *Cell Mol Life Sci*, 2025	Human and mouse	Endometrium from cystic fibrosis patients	Epithelial	EEOs replicating CF characteristics show molecular and pathway differences in cycle-mimicking hormone responses
		Environmental and drug-induced toxicity		
Abady, *Reprod Toxicol*, 2024	Pig	n = 5	Epithelial	BPA destabilizes cytoskeletal organization and metabolic homeostasis in EEOs, but treatment with melatonin or resveratrol can mitigate these alterations
Abady, *Reprod Toxicol*, 2025	Pig	n = 5	Epithelial	Endometrial lamotrigine exposure leads to reduced EEO growth, oxidative stress, apoptosis, and changes to implantation-related microRNAs

BPA, bisphenol A; CF, cystic fibrosis; ECM, extracellular matrix; EEOs, endometrial epithelial organoids; EnSCs, endometrial stromal cells; HUVEC, human umbilical vein endothelial cells; NA, not applicable or not found; PCOS, polycystic ovary syndrome; PRB, progesterone receptor B; RIF, recurrent implantation failure.

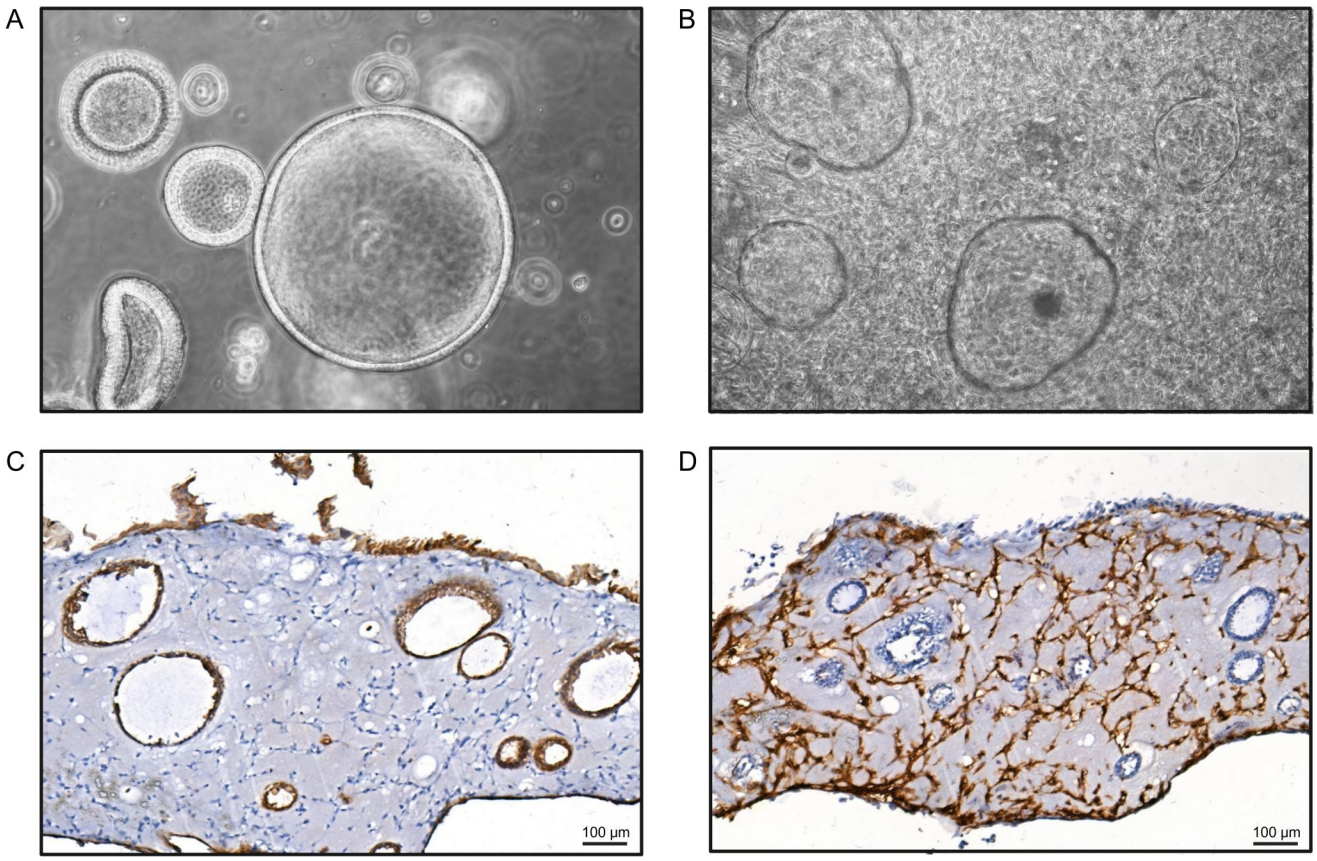


Figure 1. Endometrial epithelial organoids (EEOs) and assembloids. (**A**) EEOs in culture (light microscope, magnification 20×). (**B**) Assembloids in culture (light microscope, magnification 20×). (**C**) Histological appearance of endometrial assembloids using CK22 immunohistochemistry (magnification 10×), a marker of epithelial cells. (**D**) Histological appearance of endometrial assembloids using CD10 immunohistochemistry (magnification 10×), a marker of stromal cells. Cultures performed and captured by Beaussart, C. Image created in BioRender. Dolmans, M. (2026) https://BioRender.com/qzzae89.

growth factor 10 and hepatocyte growth factor, can further improve organoid growth and physiological relevance. Media compositions vary across studies depending on experimental goals, reflecting the flexibility of EEO culture for different experimental objectives.

Hormonal stimulation

EEOs enable *in vitro* modeling of the menstrual cycle. Stimulation with estrogen promotes proliferation and primes organoids for progesterone-mediated responses, while combined treatment with estrogen, progesterone, and cAMP enhances secretory phenotypes within shorter culture timeframes. Additional pregnancy-related hormones like human chorionic gonadotropin, prolactin, and human placental lactogen further drive early pregnancy-like differentiation, as evidenced by progesterone-associated endometrial protein (PAEP) secretion and loss of progenitor markers (Turco *et al.*, 2017). Very recently, some teams have also been able to mimic the menstrual phase by means of hormone withdrawal, associated with either mechanical disruption (Nikolakopoulou *et al.*, 2025, preprint, not yet peer-reviewed) or RU-486 (Ren *et al.*, 2025, preprint, not yet peer-reviewed). However, these models still lack a functional vascular compartment, which is essential to fully replicate endometrial breakdown and repair dynamics as they occur *in vivo*.

Endometrial assembloids

While epithelial organoids do provide valuable information, epithelial-stromal crosstalk is particularly crucial *in vivo* (Hantak *et al.*, 2014; Fitzgerald *et al.*, 2023). By adding stromal cells to epithelial organoid cultures, researchers can generate so-called endometrial assembloids, enabling functional study of key interactions (Rawlings *et al.*, 2021; Stratopoulou *et al.*, 2025). Figure 1B–D shows assembloids in culture. While 'assembloids' is a broader term referring to any self-organizing system combining organoids or specialized cell types (Pașca *et al.*, 2022; Marr *et al.*, 2025), in the context of this review, the term endometrial assembloids is used specifically for epithelial organoids combined with stromal cells.

Generating assembloids remains challenging due to the inherent properties of cells and the demands of their culture environment. To address this, assembloids have been created using fully synthetic PEG-based matrices (Gnecco *et al.*, 2023) or air–liquid interface culture (Tian *et al.*, 2023), while approaches like hanging- or floating-drop cultures within Matrigel offer rapid and accessible alternatives (Beaussart *et al.*, 2024; Bajetto *et al.*, 2025). More recently, a multi-compartment technique was introduced, in which organoids are embedded in Matrigel and surrounded by a dense, collagen-rich layer supporting stromal cells (Ren *et al.*, 2025, preprint, not yet peer-reviewed). Despite these advances, additional cell types and microenvironmental cues are still needed to fully replicate the complexity of endometrium *in vivo*.

Endometriosis

Endometriosis is defined as the 'presence of endometrial epithelial and stromal cells in ectopic sites', but this definition was

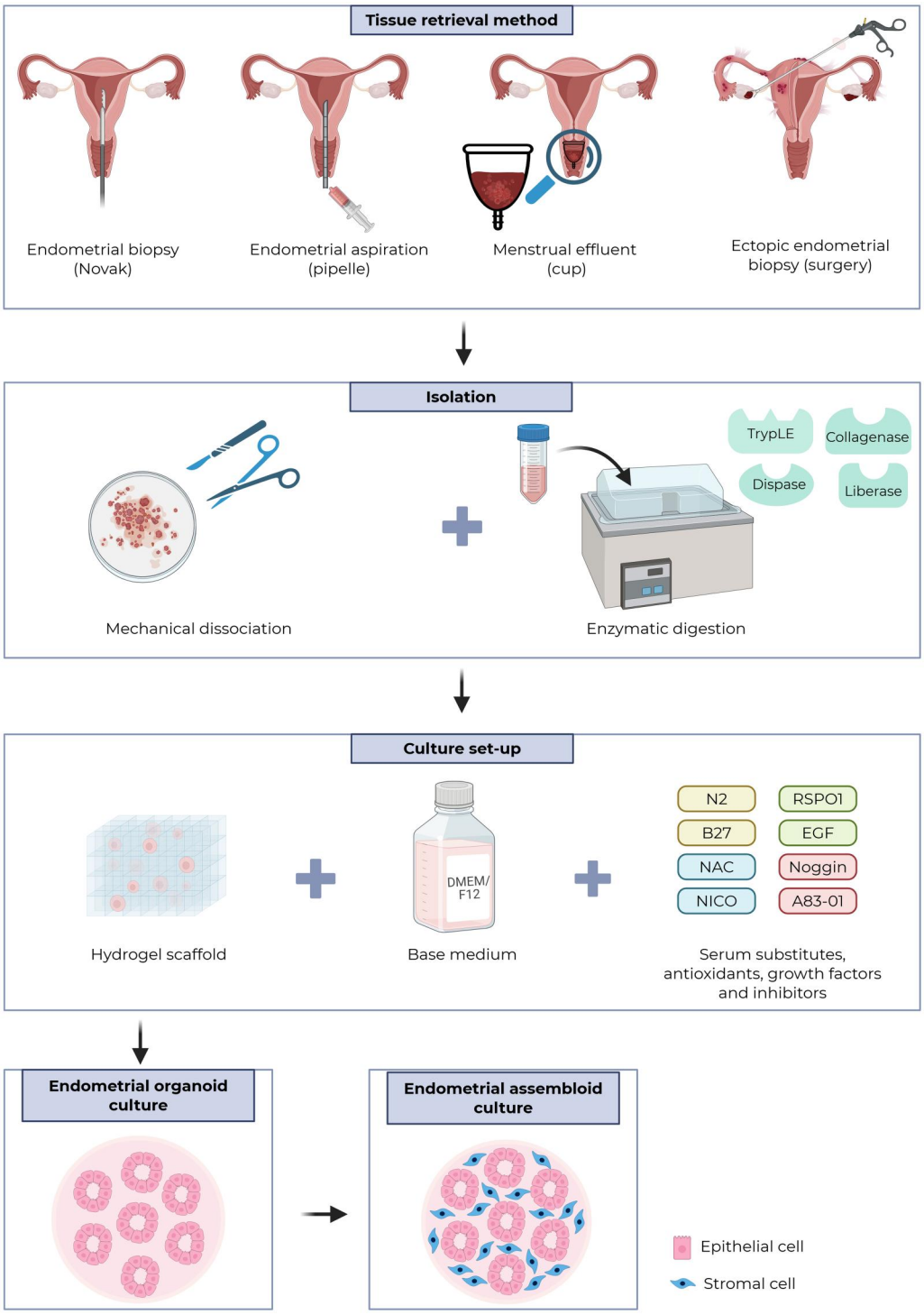


Figure 2. Schematic overview of the main steps involved in generation of human endometrial epithelial organoids and assembloids. Endometrial tissue can be acquired through various clinical procedures, including Novak biopsy, pipelle aspiration, hysteroscopic sampling, or surgical resection. Retrieved tissue is mechanically minced and enzymatically dissociated to isolate epithelial glands by commonly applied digestion strategies, such as collagenase, dispase, TrypLE, or liberase-based protocols (Boretto *et al.*, 2017; Turco *et al.*, 2017; Hwang *et al.*, 2024). Following isolation, epithelial fragments are embedded inside a 3D extracellular matrix and cultured in a specific medium typically based on DMEM/F12 and supplemented with niche factors supporting epithelial stem cell maintenance and proliferation. These supplements often include N2, B27, nicotinamide (NICO), N-acetylcysteine (NAC), R-spondin 1 (RSPO1), epidermal growth factor (EGF), noggin, and TGF-β pathway inhibitor A83-01, although their exact composition and concentrations may vary between protocols. Such conditions allow for long-term expansion of endometrial epithelial organoids. Incorporation of additional stromal components enables generation of endometrial assembloids, yielding more complex modeling of epithelial–stromal interactions. The schematic structure is adapted from Jabri *et al.* (2025). Created in BioRender. Dolmans, M. (2026) https://BioRender.com/adrg9mu.

reworded by Vigano *et al.* (2018) to 'a fibrotic condition in which endometrial stroma and epithelium can be identified'. Even after decades of research, the pathogenesis of endometriosis continues to elude us and the search for effective 3D models has now led us to organoid systems to investigate the mechanistic aspects of the disease (Koninckx *et al.*, 2021). Boretto *et al.* (2019)

were the first to develop EEOs using eutopic and ectopic tissue exhibiting the typical features of endometriosis, including altered *HOX* gene expression and dysregulation of pathways involved in tissue adhesion/invasion (like *MMP2, SNAI2*, and *TIMP4*) and hormonal responses (like *PGR, LIFR*, and *PRLR*). More recently, Esfandiari *et al.* investigated the epigenetic landscape of endometriosis using organoids derived from both eutopic and ectopic tissue, alongside normal endometrium. They found that these organoids retained the genomic and methylation profiles of their original tissues, including *HOX* gene clusters, providing a platform to unravel the mechanistic and epigenetic drivers of endometriosis (Esfandiari *et al.*, 2021a). In follow-up work, the same team demonstrated disrupted progesterone signaling in EEOs derived from endometriosis patients. Indeed, these EEOs failed to upregulate progesterone-regulated genes (like *17HSDβ2, SPP1, LIF*, and *PAEP*) to the extent observed in control endometrium organoids. Moreover, progesterone receptor B (PRB) was significantly downregulated in EEOs from endometriosis patients compared to control endometrium organoids. DNA hypermethylation, operating as an epigenetic mechanism for suppression of transcription, was detected in the PRB promoter in these organoids from endometriosis patients (Esfandiari *et al.*, 2021b).

Xu *et al.* further used EEOs from endometriosis patients to investigate the involvement of ferroptosis in endometrial receptivity defects, identifying long-chain acyl-CoA synthetase 4 (ACSL4) as one of its key regulators via transcriptomic analysis. These EEOs exhibited ACSL4 overexpression, as well as mitochondrial abnormalities, fewer secretory vesicles, and decreased receptivity markers. Treatment with the ACSL4 inhibitor PRGL493 restored mitochondrial and secretory ultrastructure and normalized receptivity marker expression, providing functional evidence that ACSL4-mediated ferroptosis contributes directly to impaired endometrial receptivity in endometriosis (Xu *et al.*, 2025). Another team showed that, much like in certain cancers where ephrin system signaling plays a role in cell migration and invasion (Pasquale, 2024), ephrin receptors are also overexpressed in endometriotic lesions compared to normal eutopic endometrium. Targeting these receptors in EEOs from endometriosis patients was found to be sufficient to limit their growth (Madasu *et al.*, 2024).

Endometrial assembloids generated from tissue derived from women with endometriosis have also displayed impaired decidualization. Addition of BMP-2 was shown to potentially correct this defect by boosting decidual gene expression in cultures from these individuals (Liao *et al.*, 2024). Another team modeled the ectopic inflammatory milieu by treating decidualized assembloids with interleukin 1B, which disrupted the glandular architecture and induced epithelial infiltration of the surrounding stroma (Ochoa *et al.*, 2025). Taking these models one step further, co-culture with neuronal organoids was subsequently developed to incorporate the neural component of the tissue microenvironment. This advanced system allows functional investigation of epithelial–fibroblast–neuronal interactions, yielding deeper insights into cellular networks driving pain and inflammation in endometriosis (Haney *et al.*, 2025, preprint, not yet peer-reviewed).

In endometriosis research, both eutopic endometrium and lesions themselves require scrutiny (Gunther *et al.*, 2025). Most organoid studies focus on eutopic tissue, while organoids from endometriotic lesions have been generated mainly from ovarian endometriomas (Esfandiari *et al.*, 2021a; Zhang *et al.*, 2025b) and, more recently, peritoneal implants (Haney *et al.*, 2025 preprint, not yet peer-reviewed). Importantly, a new study has now shown that organoids can in fact be established from all major surgical phenotypes, including deep-infiltrating lesions, albeit with variable efficiency (Gunther *et al.*, 2026).

As organoid models become increasingly complex and widely used, standardization of methods is essential to ensure reproducibility, comparability, and meaningful interpretation of findings. Recognizing this, WERF convened a dedicated working group (EPHect) to harmonize documentation and protocols for EEO research in endometriosis (Marr *et al.*, 2025), an initiative that was both timely and critically needed.

Adenomyosis

Like its sister disease endometriosis, adenomyosis is also somewhat of an enigma to clinical and research communities all over the world. Both conditions involve the uterine endometrium and contain similar lesion cell types, but in different locations, namely outside and inside the uterus respectively (Donnez *et al.*, 2024; Giudice *et al.*, 2025). The high prevalence of adenomyosis, diversity of morphological and symptomatic presentations, range of potential pathogenic mechanisms, and variable response to existing therapies make it particularly challenging to replicate the human disorder in animals. However, as with endometriosis, adenomyosis can be modeled using EEOs as a means of conceptualizing physiological processes, an approach known as physiomimetics (Gnecco *et al.*, 2020).

Primary assembloids can be generated from endometrial biopsies derived from women affected by adenomyosis from different phases of the menstrual cycle. One of the latest studies (Stratopoulou *et al.*, 2025) reports that assembloids mimicking adenomyosis appear to be less sensitive to hormonal treatment, showing reduced expression of LIF and HOXA10 in the stromal compartment and failing to form pinopodes. In fact, endometrial assembloids are a legitimate advanced preclinical model of adenomyosis-related impaired endometrial receptivity.

Juárez-Barber *et al.* (2022, 2023) previously developed EEOs from eutopic endometrium of adenomyosis patients, and found that the extracellular vesicle cargo of these organoids contained microRNAs involved in disease progression and defective embryo implantation. In a later study, the same team explored dysregulated genes participating in impaired endometrial receptivity and implantation failure, demonstrating that women with adenomyosis show altered secretory- and gestational-phase endometrium (Juárez-Barber *et al.*, 2024).

EEOs can also be developed from mouse tissue and were used to study endometrial epithelial proliferation, disrupted luminal-glandular differentiation, and impaired receptivity (Xu *et al.*, 2025). These processes were shown to be pathological and involved in endometriosis and probably adenomyosis pathogenesis (Zipponi *et al.*, 2024). Xu *et al.* (2023b) evaluated Notch1 overactivation in a transgenic mouse model. Since expression of Notch1 and its intracellular domain N1ICD were found to be stronger in eutopic endometrium from women with adenomyosis (Qi *et al.*, 2015), EEOs from the reported transgenic mouse model could be used to investigate this phenomenon.

One hypothesis on the pathogenesis of adenomyosis involves tissue injury and repair mechanisms (García-Solares *et al.*, 2018). Cellular senescence is known to be implicated in tissue repair, but its role in adenomyosis remains unclear. Indeed, EEOs derived from adenomyosis patients exhibit decreased expression of Klotho and PPARγ, both involved in fibrosis and tissue aging (Fan *et al.*, 2025). Since adenomyosis is defined by invasion of underlying myometrium by endometrial tissue, modeling this invasive process is essential. Major methodological progress has been

made with development of three-layered assembloids that incorporate a myometrial compartment (Xu *et al.*, 2026). This approach allows direct visualization of tissue invasion and provides a powerful platform to investigate the mechanisms driving lesion formation and progression.

Although there are very few articles on the topic so far in the literature, recent publications and growing interest in the field firmly indicate that the EEO model will prove an asset in our continued research into adenomyosis.

Uterine fibroids

Uterine fibroids, also known as (leio)myomas, are benign smooth muscle tumors of the uterus that can alter uterine structure and function (Dolmans *et al.*, 2021). While larger fibroids that distort the uterine cavity are clearly associated with reduced fertility, the effects of smaller intramural fibroids on the surrounding endometrium remain less well understood. To investigate this, Zhang *et al.* (2024b) generated patient-derived EEOs from endometrium adjacent to small intramural fibroids. Their work demonstrated that even these small fibroids can alter hormonal signaling and ECM mechanotransduction, leading to dysregulation of genes involved in gland development, angiogenesis, and ciliary activity (Zhang *et al.*, 2024b). It is noteworthy that organoids have also been generated from fibroids themselves using myometrial stem cells, mimicking key features of fibroid pathophysiology and responding to reproductive hormones (Banerjee *et al.*, 2022; Omran *et al.*, 2025).

Implantation failure

Early reproductive failure is the most common complication of pregnancy, with only about 30% of conceptions resulting in live births (Dimova *et al.*, 2025). A major hurdle lies in the implantation process, a complex and finely tuned dialogue between the blastocyst and endometrium. Because it cannot be directly observed *in vivo*, implantation has often been referred to as the 'black box' of human reproduction. EEOs and assembloids now offer a way to access this box, providing a controlled model to explore the cellular and molecular events that govern endometrial receptivity and embryo–maternal communication (Nikolakopoulou and Turco, 2021; Guo *et al.*, 2023; Jabri *et al.*, 2025).

Endometrial receptivity

EEOs retain the key hallmarks of endometrial receptivity when stimulated to mimic the mid-secretory phase. They respond to hormonal cues by upregulating classic receptivity markers like PAEP, 17βHSD2, and LIF, thereby replicating the transcriptional program of the implantation window (Turco *et al.*, 2017; Luddi *et al.*, 2020). EEOs also enable investigations into mechanosensitivity, reproducing ion channel activity and responsiveness to physical cues (Hennes *et al.*, 2019). Ultrastructurally, EEOs exhibit the apical features of receptivity, including pinopodes and motile cilia, thought to facilitate embryo adhesion (Luddi *et al.*, 2020; Stratopoulou *et al.*, 2024). This is why organoid models have been used to explore endometrial receptivity alterations in conditions like endometriosis (Xu *et al.*, 2025), adenomyosis (Stratopoulou *et al.*, 2025), and fibroids (Zhang *et al.*, 2024b). Endometrial aging also compromises receptivity, with patient-derived EEOs revealing fibrosis, inflammatory imbalance, and impaired gene expression linked to PI3K–AKT–FOXO1 signaling. Interestingly, pharmacological inhibition of this pathway was found to rescue receptive gene expression and reduce fibrosis, whereas its activation aggravated senescence and receptivity loss, underscoring the potential for targeted interventions to restore endometrial receptivity in aging women (Lu *et al.*, 2025).

Embryo–endometrium interactions

EEOs derived from infertile women show aberrant hormonal responses and altered secretomes, which reduce trophoblast adhesion (Zhou *et al.*, 2022). This interaction is bidirectional, as embryos also release factors that modulate epithelial behavior. Indeed, using their EEO model, Luddi *et al.* (2021) demonstrated that exposure to embryo-conditioned medium induces specific glycodelin A glycoforms, including a unique acidic variant found only in organoids treated with spent media from embryos that subsequently implanted, illustrating dynamic embryo–epithelium crosstalk. In this context, development of trophoblast organoids provides a complementary embryonic platform that, combined with endometrial organoids, enables more integrated modeling of the bidirectional dialogue underlying human implantation (Beristain and McNeill, 2025).

Luminal epithelium models

Modeling the luminal epithelium has further boosted the utility of these systems. A number of strategies have been developed to recreate the luminal epithelium and, in so doing, epithelium–embryo interactions. One approach involved generation of open-faced endometrial layers (OFELs) from organoids, exposing the luminal surface and enabling direct depositing of human blastoids thereupon (Kagawa *et al.*, 2022). Another team established a method to create polarity-reversed, apical-out organoids using ultra-low attachment plates, maintaining their apicobasal orientation and hormonal responsiveness, while facilitating co-culture with embryonic entities (Ahmad *et al.*, 2024). By exposing the luminal surface, apical-out structures encourage embryo attachment and facilitate investigation of multiple sequential stages of implantation. Moving beyond epithelium-only models, Tian *et al.* (2023) developed air–liquid interface assembloids allowing integrated analysis of epithelial–stromal interactions in tissue-mimetic settings. Shortly after, Shibata *et al.* (2024) generated apical-out assembloids enriched with an endothelial network derived from human umbilical vein endothelial cells (HUVECs), introducing a vascular dimension.

Recurrent implantation failure

EEOs derived from patients with recurrent implantation failure preserve the distinct cellular and molecular signature of mid-secretory endometrium typically observed in these individuals, while also showing diminished responsiveness to sex steroids (Zhang *et al.*, 2024d). Further studies have demonstrated that cilia-dependent processes, powered by mitochondrial activity and intraflagellar transport, are compromised in these organoids, resulting in defective vesicle transport and disrupted epithelial–stromal communication (Yang *et al.*, 2025).

Endometritis and endometrial infections

Endometritis, an inflammatory disorder of the endometrium often caused by *Chlamydia (C.) trachomatis* or pathogenic *Escherichia* (E.) *coli* infection, is a major contributor to both infertility and poor reproductive outcomes. Understanding how these pathogens traverse the epithelial barrier and disrupt immune functions is essential, and EEOs provide a physiologically relevant system to examine such host–pathogen interactions (Kaya *et al.*, 2023; Jabri *et al.*, 2025).

Before the advent of organoid technologies, Łaniewski *et al.* developed 3D endometrial epithelial spheroids in a bioreactor system to model ascending infections. They showed that while commensal bacteria colonized without major impact, *Neisseria gonorrhoeae* triggered strong proinflammatory responses and profound epithelial alterations, underscoring the added value of 3D systems over traditional 2D cultures (Łaniewski *et al.*, 2017).

Building upon these advances, organoid-based models have since enabled more physiologically complex infection studies. Chlamydia, a leading cause of pelvic inflammatory disease and infertility, has been modeled in murine EEOs using different infection strategies. Bishop *et al.* (2020) applied a fragmentation-based infection approach, showing that *C. trachomatis* completes its full developmental cycle within the generated EEOs. Dolat and Valvidia later introduced a microinjection model, in which luminal infection of EEOs revealed cytoskeletal and Golgi apparatus reorganization. It also allowed visualization of immune cell recruitment when organoids were co-cultured with bone marrow-derived neutrophils (Dolat and Valdivia, 2021). Based on these findings, the same team focused on how the chlamydial effector TepP participates in epithelial barrier breakdown. Using their EEOs, they demonstrated that TepP manipulates the host actin regulator EPS8 to disrupt tight junctions, so EEOs derived from mice lacking EPS8 were resistant to this effect (Dolat *et al.*, 2022).

Pathogenic *E. coli* infections have also been modeled in EEOs. Ahmad *et al.* (2024) used human-derived apical-out EEOs not only to expose the luminal epithelium to murine blastocysts, as mentioned earlier, but also directly infect them with *E. coli*, evidencing efficient apical adhesion and invasion. In a complementary approach using an air–liquid interface system, Zhang *et al.* (2024c) achieved robust inflammatory responses that were attenuated by estradiol. This effect was later confirmed in bovine tissue aggregates by another team and shown to be associated with Wnt/β-catenin pathway activation (Zhang *et al.*, 2024e). In a subsequent study, Zhang *et al.* (2025c) systematically compared microinjection, air–liquid interface, and their newly published apical-out protocol for *E. coli* infection, concluding that polarity-reversed apical-out EEOs best replicate natural infection dynamics (Zhang *et al.*, 2025a). Together, these studies show that EEOs effectively model pathogen–endometrium interactions, offering insights into how infections disrupt epithelial integrity and affect fertility.

Endometrial hypoplasia and adhesive syndromes

Endometrial hypoplasia and intrauterine adhesions, as witnessed in Asherman's syndrome (AS), represent major clinical challenges, often resulting in infertility, menstrual abnormalities, and recurrent pregnancy loss. Indeed, the pathophysiology of these conditions is not fully understood and current therapies (primarily surgical removal of adhesions) show limited long-term success. Recent organoid-based studies have provided new information on the mechanisms behind underdeveloped endometrium and AS. Santamaria *et al.* (2023) generated EEOs from AS endometrium, documenting epithelial cell loss, altered Wnt/Notch signaling, and disrupted epithelial cell communication, which fostered a profibrotic, proinflammatory, and antiangiogenic environment. Peng *et al.* (2024) modeled endometrial hypoplasia by exposing EEOs to oxygen-glucose deprivation, revealing ischemia-driven cytoskeletal and junctional defects that compromise epithelial growth.

Beyond modeling, organoids are also being explored as therapeutic tools. In parallel with progenitor/stem cell therapies (Cousins *et al.*, 2022), EEO-based approaches are being investigated because they can overcome some of the key limitations of stem cell transplantation, including low engraftment rates, rapid senescence, and the risk of tumorigenesis. These impediments have long hindered efficient and effective application of stem cell-based technologies in endometrial repair. With this in mind, Xu *et al.* (2023a) developed multi-lineage endometrial organoids combining epithelial and mesenchymal components. When seeded onto a human acellular amniotic membrane scaffold, these components promoted endometrial regeneration and improved fertility *in vivo* (Xu *et al.*, 2023a). Hwang *et al.* (2024) then transplanted human and mouse EEOs into murine AS models, where they markedly reduced fibrosis, restored metabolic balance, and enhanced proliferation, angiogenesis, and embryo implantation. More recently, Qin *et al.* (2025) engineered a sprayable hydrogel loaded with extracellular vesicles derived from EEOs, achieving enhanced tissue repair, prevention of fibrosis, and restoration of fertility in preclinical models.

Endometrial dysfunction in systemic and environmental contexts

Endometrial structure and function can be profoundly affected not only by local uterine conditions, but also systemic disorders and environmental or pharmacological exposures. Such disruptors can contribute to infertility, implantation failure, and abnormal menstrual patterns. EEOs provide a physiologically relevant 3D platform to model these effects.

Polycystic ovary syndrome

Wiwatpanit *et al.* developed stromal-epithelial cell aggregates in an exogenous matrix and cultured them for 14 days with physiological concentrations of estradiol and testosterone. They were looking to mimic either normal cycles or the hyperandrogenic profile characteristics of polycystic ovary syndrome (PCOS). Exposure to androgen increased epithelial proliferation and dysregulated gene expression, reproducing the proliferative and transcriptional alterations encountered in the endometrium of PCOS patients (Wiwatpanit *et al.*, 2020).

More recently, two separate research teams derived EEOs directly from endometrial biopsies of PCOS patients. First, Ye *et al.* (2025) used single-cell RNA sequencing, metabolomics, and phase-specific EEOs to show that glutathione peroxidase 4 deficiency in PCOS endometrium induces ferroptosis, leading to fibrosis through TGF-β1/Smad2/3-mediated ECM remodeling. This in turn exposes ferroptosis as a mechanistic contributor to impaired endometrial receptivity. The authors then supplemented EEOs with glutathione, alleviating this profibrotic phenotype, thereby aligning with previous evidence on the therapeutic relevance of antioxidant strategies in PCOS (Viña *et al.*, 2025). Not long after, another team generated EEOs from two disease subtypes (overweight/obese vs lean women) and reproduced the inflammatory gene expression profile of PCOS endometrium, while showing subtype-specific differential responses to ovarian sex hormones (Luyckx *et al.*, 2025).

Cystic fibrosis

The inherited disorder cystic fibrosis (CF), caused by CFTR dysfunction, is another systemic condition linked to female subfertility. While multifactorial, the role of the endometrium remains unclear in this context. De Pauw *et al.* recently derived EEOs from CF patients, which revealed aberrant hormone responses, ECM

remodeling, hypoxia signatures, and impaired ciliary pathways. Interestingly, these defects were partially rescued by CFTR modulators. These findings point to the endometrium as a contributor to CF-associated subfertility and position EEOs as a tractable model to explore therapeutic strategies (De Pauw *et al.*, 2025).

Environmental exposures

EEOs can also be used to study environmental toxicants. Bisphenol A (BPA), an endocrine-disrupting chemical, destabilizes cytoskeletal organization, Wnt/β-catenin signaling, epithelial–mesenchymal markers, and metabolic homeostasis in porcine EEOs. Treatment with melatonin or resveratrol was found to effectively mitigate these BPA-induced alterations (Abady *et al.*, 2024).

Pharmacological testing

EEOs yield insights into potential responses to pharmaceuticals. Boretto *et al.* (2019) demonstrated that EEOs exhibit patient-specific responses to different chemotherapeutic agents, highlighting their potential for personalized drug screening. On the other hand, EEOs can also be applied to study the reproductive toxicity of medicines. Lamotrigine, a neurotherapeutic drug, induced morphological changes, reduced organoid growth, oxidative stress and apoptosis, and altered implantation-related microRNAs in porcine EEOs (Abady *et al.*, 2025). Likewise, chemotherapeutic agents elicit patient-specific responses in human EEOs, supporting their use in personalized drug screening (Boretto *et al.*, 2019).

Limitations of current organoid and assembloid models

Endometrial organoid and assembloid research is still in its infancy and a number of technical and biological limitations should be acknowledged (Supplementary Table S1). Most models do not fully capture the cellular complexity of the endometrium, often lacking stromal, immune, and vascular compartments essential to *in vivo*-like physiology. Organoids are highly patient-dependent, yet many studies rely on a limited number of patient-derived biopsies, potentially reducing generalizability. Culture conditions vary widely, including differences in ECM composition and culture medium, both of which can hamper physiological fidelity. The majority of studies rely on Matrigel, which has known limitations, as listed above. Culture medium also influences organoid behavior. Phenol red may introduce estrogenic effects (Berthois *et al.*, 1986), while use of Wnt activators and TGF-β inhibitor, commonly contained in expansion medium, is in direct contrast with the *in vivo* mid-secretory phase, where Wnt signaling is downregulated and TGF-β is active (Guo *et al.*, 2023). The timing and concentration of hormonal stimulation are not standardized across studies either, further contributing to variability in organoid responses.

Although previous studies have shown that the cycle phase at the time of biopsy does not significantly affect organoid phenotype (Boretto *et al.*, 2017; Cindrova-Davies *et al.*, 2021), this is not consistently reported or uniform across studies. It is actually unclear whether the cycle phase influences organoid responses to hormonal stimulation at all. Similarly, the number of passages prior to experimentation is rarely standardized, even though it can alter the balance of stem and progenitor cell populations and thereby affect organoid properties (Zhang *et al.*, 2024a). The stability of organoids derived from the same patient across multiple menstrual cycles has not in fact been characterized. Moreover, most studies are descriptive, with limited functional validation or *in vivo* correlation, attenuating mechanistic insights and translational applicability.

Conclusion and perspectives

EEOs represent a significant step forward in bridging the gap between basic research and clinical understanding of endometrial disorders. While most organoids are currently generated using epithelial cells, these are not representative of the multicellular state of the endometrium *in vivo*. There is still much work to be done in terms of reproducing functional human endometrium to model the numerous disorders that affect so many women and girls around the world. By improving existing methodologies and unifying protocols, as proposed by the WERF EPHect guidelines, we can ensure that the translational potential is fulfilled. EEOs have enabled researchers to crack open the black box to many gynecological pathologies, and we now need to delve deeper into its manifold contents to advance our understanding of these complex and multifactorial diseases.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

Apart from newly generated figures available via links in the legends, no new data were acquired or analyzed for this review.

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Authors' roles

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Conflict of interest

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References

Abady MM, Saadeldin IM, Han A, Bang S, Kang H, Seok DW, Kwon HJ, Cho J, Jeong JS. Melatonin and resveratrol alleviate molecular and metabolic toxicity induced by bisphenol A in endometrial organoids. *Reprod Toxicol* 2024;**128**:108628.

Abady MM, Saadeldin IM, Han A, Bang S, Kang H, Seok DW, Kwon HJ, Cho J, Jeong JS. Modeling lamotrigine-induced reprotoxicity in

porcine endometrial organoids: integrated multi-platform profiling. *Reprod Toxicol* 2025;**135**:108926.

Abbas Y, Brunel LG, Hollinshead MS, Fernando RC, Gardner L, Duncan I, Moffett A, Best S, Turco MY, Burton GJ *et al.* Generation of a three-dimensional collagen scaffold-based model of the human endometrium. *Interface Focus* 2020;**10**:20190079.

Ahmad V, Yeddula SGR, Telugu B, Spencer TE, Kelleher AM. Development of polarity-reversed endometrial epithelial organoids. *Reproduction* 2024;**167**:e230478.

Bajetto A, Pattarozzi A, Corsaro A, Tremonti B, Gatti M, Pisaturo V, Campagnolo L, Colia D, Pastine E, Alteri A *et al.* A floating endometrial organoid model recapitulates epithelial-stromal cell interactions in vitro. *Exp Cell Res* 2025;**452**:114749.

Banerjee S, Xu W, Chowdhury I, Driss A, Ali M, Yang Q, Al-Hendy A, Thompson WE. Human myometrial and uterine fibroid stem cell-derived organoids for intervening the pathophysiology of uterine fibroid. *Reprod Sci* 2022;**29**:2607–2619.

Beaussart C, Rossi M, Stratopoulou CA, Zipponi M, Cacciottola L, Donnez J, Dolmans MM. Refining endometrial assembloids: a novel approach to 3-dimensional culture of the endometrium. *F S Sci* 2024;**5**:331–334.

Beristain AG, McNeill GL. Organoids in modeling human trophoblast development. *Fertil Steril* 2025;**124**:1155–1161.

Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS. Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc Natl Acad Sci USA* 1986;**83**:2496–2500.

Białkowska K, Komorowski P, Bryszewska M, Miłowska K. Spheroids as a type of three-dimensional cell cultures-examples of methods of preparation and the most important application. *Int J Mol Sci* 2020;**21**:6225.

Bishop RC, Boretto M, Rutkowski MR, Vankelecom H, Derré I. Murine endometrial organoids to model chlamydia infection. *Front Cell Infect Microbiol* 2020;**10**:416.

Boretto M, Cox B, Noben M, Hendriks N, Fassbender A, Roose H, Amant F, Timmerman D, Tomassetti C, Vanhie A *et al.* Development of organoids from mouse and human endometrium showing endometrial epithelium physiology and long-term expandability. *Development* 2017;**144**:1775–1786.

Boretto M, Maenhoudt N, Luo X, Hennes A, Boeckx B, Bui B, Heremans R, Perneel L, Kobayashi H, Van Zundert I *et al.* Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. *Nat Cell Biol* 2019;**21**:1041–1051.

Cindrova-Davies T, Zhao X, Elder K, Jones CJP, Moffett A, Burton GJ, Turco MY. Menstrual flow as a non-invasive source of endometrial organoids. *Commun Biol* 2021;**4**:651.

Ciprietti M, Bueds C, Vankelecom H, Vriens J. Organoids as powerful models of endometrium epithelium in transcriptomic, cellular and functional mimicry. *Cell Mol Life Sci* 2025;**82**:272.

Cousins FL, Filby CE, Gargett CE. Endometrial stem/progenitor cells-their role in endometrial repair and regeneration. *Front Reprod Health* 2022;**3**:811537.

Dehoux JP, Defrère S, Squifflet J, Donnez O, Polet R, Mestdagt M, Foidart JM, Van Langendonckt A, Donnez J. Is the baboon model appropriate for endometriosis studies? *Fertil Steril* 2011;**96**:728–733.e3.

De Pauw E, Gommers B, Ensinck MM, Timmerman S, De Vriendt S, Bueds C, Wei M, Hermans F, Arnauts K, Ramalho AS *et al.* Endometrium-derived organoids from cystic fibrosis patients and mice as new models to study disease-associated endometrial pathobiology. *Cell Mol Life Sci* 2025;**82**:109.

Dias Da Silva I, Wuidar V, Zielonka M, Pequeux C. Unraveling the dynamics of estrogen and progesterone signaling in the endometrium: an overview. *Cells* 2024;**13**:1236.

Dimova T, Alexandrova M, Vangelov I, You Y, Mor G. The modeling of human implantation and early placentation: achievements and perspectives. *Hum Reprod Update* 2025;**31**:133–163.

Dolat L, Carpenter VK, Chen YS, Suzuki M, Smith EP, Kuddar O, Valdivia RH. Chlamydia repurposes the actin-binding protein EPS8 to disassemble epithelial tight junctions and promote infection. *Cell Host Microbe* 2022;**30**:1685–1700.e10.

Dolat L, Valdivia RH. An endometrial organoid model of interactions between *Chlamydia* and epithelial and immune cells. *J Cell Sci* 2021;**134**:jcs252403.

Dolmans MM, Isaacson K, Zhang W, Gordts S, Munro MG, Stewart EA, Bourdon M, Santulli P, Donnez J. Intramural myomas more than 3-4 centimeters should be surgically removed before in vitro fertilization. *Fertil Steril* 2021;**116**:945–958.

Donnez O, Orellana R, Van Kerk O, Dehoux JP, Donnez J, Dolmans MM. Invasion process of induced deep nodular endometriosis in an experimental baboon model: similarities with collective cell migration? *Fertil Steril* 2015;**104**:491–497.e2.

Donnez J, Stratopoulou CA, Dolmans MM. Endometriosis and adenomyosis: similarities and differences. *Best Pract Res Clin Obstet Gynaecol* 2024;**92**:102432.

Esfandiari F, Favaedi R, Heidari-Khoei H, Chitsazian F, Yari S, Piryaei A, Ghafari F, Baharvand H, Shahhoseini M. Insight into epigenetics of human endometriosis organoids: DNA methylation analysis of HOX genes and their cofactors. *Fertil Steril* 2021a;**115**:125–137.

Esfandiari F, Heidari Khoei H, Saber M, Favaedi R, Piryaei A, Moini A, Shahhoseini M, Ramezanali F, Ghaffari F, Baharvand H. Disturbed progesterone signalling in an advanced preclinical model of endometriosis. *Reprod Biomed Online* 2021b;**43**:139–147.

Fan J, Liu X, Guo SW. Hypermethylation of Klotho and peroxisome proliferator-activated receptor γ concomitant with overexpression of DNA methyltransferase 1 in adenomyosis. *Reprod Sci* 2025;**32**:668–683.

Filby CE, Wyatt KA, Mortlock S, Cousins FL, McKinnon B, Tyson KE, Montgomery GW, Gargett CE. Comparison of organoids from menstrual fluid and hormone-treated endometrium: novel tools for gynecological research. *J Pers Med* 2021;**11**:1314.

Fitzgerald HC, Dhakal P, Behura SK, Schust DJ, Spencer TE. Self-renewing endometrial epithelial organoids of the human uterus. *Proc Natl Acad Sci USA* 2019;**116**:23132–23142.

Fitzgerald HC, Kelleher AM, Ranjit C, Schust DJ, Spencer TE. Basolateral secretions of human endometrial epithelial organoids impact stromal cell decidualization. *Mol Hum Reprod* 2023;**29**:gaad007.

Francés-Herrero E, Juárez-Barber E, Campo H, López-Martínez S, de Miguel-Gómez L, Faus A, Pellicer A, Ferrero H, Cervelló I. Improved models of human endometrial organoids based on hydrogels from decellularized endometrium. *J Pers Med* 2021;**11**:504.

García-Solares J, Donnez J, Donnez O, Dolmans MM. Pathogenesis of uterine adenomyosis: invagination or metaplasia? *Fertil Steril* 2018;**109**:371–379.

Giudice LC, Liu B, Irwin JC. Endometriosis and adenomyosis unveiled through single-cell glasses. *Am J Obstet Gynecol* 2025;**232**:S105–S123.

Gnecco JS, Brown A, Buttrey K, Ives C, Goods BA, Baugh L, Hernandez-Gordillo V, Loring M, Isaacson KB, Griffith LG. Organoid co-culture model of the human endometrium in a fully synthetic extracellular matrix enables the study of epithelial-stromal crosstalk. *Med* 2023;**4**:554–579.e9.

Gnecco JS, Brown AT, Kan EL, Baugh L, Ives C, Loring M, Griffith LG. Physiomimetic models of adenomyosis. *Semin Reprod Med* 2020; **38**:179–196.

Grümmer R. Animal models in endometriosis research. *Hum Reprod Update* 2006;**12**:641–649.

Gunther K, Fisher T, Liu D, Abbott J, Ford CE. Endometriosis is not the endometrium: reviewing the over-representation of eutopic endometrium in endometriosis research. *Elife* 2025;**14**:e103825.

Gunther K, Liu D, Cortesi M, Powell E, Nesbitt-Hawes E, Abbott JA, Ford CE. Patient-derived epithelial cell organoids mimic the phenotypic complexity of endometriosis subtypes. *Hum Reprod* 2026; **41**:262–274.

Guo J, Zhou W, Sacco M, Downing P, Dimitriadis E, Zhao F. Using organoids to investigate human endometrial receptivity. *Front Endocrinol (Lausanne)* 2023;**14**:1158515.

Haney CM, Alizadeh E, Sullivan M, Lee J, Kuljancic J, Flynn WF, Robson P, White BS, Luciano DE, Courtois ET. High-resolution spatial transcriptomics reveals fibroblast and neuroimmune microenvironments in endometriosis lesions. 2025. bioRxiv. 2025:2025.08.20.671344, preprint: not peer reviewed.

Hantak AM, Bagchi IC, Bagchi MK. Role of uterine stromal-epithelial crosstalk in embryo implantation. *Int J Dev Biol* 2014;**58**:139–146.

Heidari-Khoei H, Esfandiari F, Moini A, Yari S, Saber M, Ghaffari Novin M, Piryaei A, Baharvand H. Derivation of hormone-responsive human endometrial organoids and stromal cells from cryopreserved biopsies. *Exp Cell Res* 2022;**417**:113205.

Hennes A, Held K, Boretto M, De Clercq K, Van den Eynde C, Vanhie A, Van Ranst N, Benoit M, Luyten C, Peeraer K *et al.* Functional expression of the mechanosensitive PIEZO1 channel in primary endometrial epithelial cells and endometrial organoids. *Sci Rep* 2019;**9**:1779.

Hernandez-Gordillo V, Kassis T, Lampejo A, Choi G, Gamboa ME, Gnecco JS, Brown A, Breault DT, Carrier R, Griffith LG. Fully synthetic matrices for in vitro culture of primary human intestinal enteroids and endometrial organoids. *Biomaterials* 2020; **254**:120125.

Hwang SY, Lee D, Lee G, Ahn J, Lee YG, Koo HS, Kang YJ. Endometrial organoids: a reservoir of functional mitochondria for uterine repair. *Theranostics* 2024;**14**:954–972.

Jabri A, Alsharif M, Abbad T, Taftafa B, Mhannayeh A, Elsalti A, Attia F, Mir TA, Saadeldin I, Yaqinuddin A. Endometrial organoids and their role in modeling human infertility. *Cells* 2025;**14**:829.

Jamaluddin MFB, Ghosh A, Ingle A, Mohammed R, Ali A, Bahrami M, Kaiko G, Gibb Z, Filipe EC, Cox TR, *et al.* Bovine and human endometrium-derived hydrogels support organoid culture from healthy and cancerous tissues. *Proc Natl Acad Sci USA* 2022;**119**: e2208040119.

Jiang Y, Palomares AR, Munoz P, Nalvarte I, Acharya G, Inzunza J, Varshney M, Rodriguez-Wallberg KA. Proof-of-concept for long-term human endometrial epithelial organoids in modeling menstrual cycle responses. *Cells* 2024;**13**:1811.

Juárez-Barber E, Corachán A, Carbajo-García MC, Faus A, Vidal C, Giles J, Pellicer A, Cervelló I, Ferrero H. Transcriptome analysis of adenomyosis eutopic endometrium reveals molecular mechanisms involved in adenomyosis-related implantation failure and pregnancy disorders. *Reprod Biol Endocrinol* 2024;**22**:10.

Juárez-Barber E, Francés-Herrero E, Corachán A, Vidal C, Giles J, Alamá P, Faus A, Pellicer A, Cervelló I, Ferrero H. Establishment of adenomyosis organoids as a preclinical model to study infertility. *J Pers Med* 2022;**12**:219.

Juárez-Barber E, Segura-Benítez M, Carbajo-García MC, Bas-Rivas A, Faus A, Vidal C, Giles J, Labarta E, Pellicer A, Cervelló I *et al.* Extracellular vesicles secreted by adenomyosis endometrial organoids contain miRNAs involved in embryo implantation and pregnancy. *Reprod Biomed Online* 2023;**46**:470–481.

Kagawa H, Javali A, Khoei HH, Sommer TM, Sestini G, Novatchkova M, Scholte Op Reimer Y, Castel G, Bruneau A, Maenhoudt N *et al.* Human blastoids model blastocyst development and implantation. *Nature* 2022;**601**:600–605.

Kaya YA, de Zoete MR, Steba GS. Advanced technologies for studying microbiome-female reproductive tract interactions: organoids, organoids-on-a-chip, and beyond. *Semin Reprod Med* 2023; **41**:160–171.

Koninckx PR, Ussia A, Martin DC. How organoids from endometrium and endometriosis could help to understand the pathogenesis of endometriosis. *Fertil Steril* 2021;**115**:78–79.

Łaniewski P, Gomez A, Hire G, So M, Herbst-Kralovetz MM. Human three-dimensional endometrial epithelial cell model to study host interactions with vaginal bacteria and Neisseria gonorrhoeae. *Infect Immun* 2017;**85**:e01049-16.

Liao Z, Tang S, Jiang P, Geng T, Cope DI, Dunn TN, Guner J, Radilla LA, Guan X, Monsivais D. Impaired bone morphogenetic protein (BMP) signaling pathways disrupt decidualization in endometriosis. *Commun Biol* 2024;**7**:227.

López-Martínez S, Campo H, de Miguel-Gómez L, Faus A, Navarro AT, Díaz A, Pellicer A, Ferrero H, Cervelló I. A natural xenogeneic endometrial extracellular matrix hydrogel toward improving current human *in vitro* models and future *in vivo* applications. *Front Bioeng Biotechnol* 2021;**9**:639688.

Lu M, Han Y, Zhang Y, Yu R, Su Y, Chen X, Liu B, Li T, Zhao R, Zhao H. Investigating aging-related endometrial dysfunction using endometrial organoids. *Cell Prolif* 2025;**58**:e13780.

Luddi A, Pavone V, Governini L, Capaldo A, Landi C, Ietta F, Paccagnini E, Morgante G, De Leo V, Piomboni P. Emerging role of embryo secretome in the paracrine communication at the implantation site: a proof of concept. *Fertil Steril* 2021; **115**:1054–1062.

Luddi A, Pavone V, Semplici B, Governini L, Criscuoli M, Paccagnini E, Gentile M, Morgante G, Leo V, Belmonte G *et al.* Organoids of human endometrium: a powerful in vitro model for the endometrium-embryo cross-talk at the implantation site. *Cells* 2020;**9**:1121.

Luyckx L, Wei M, Saarela U, Myllykangas M, Kinnunen J, Arffman R, Lie Fong S, Vriens J, Vankelecom H, Piltonen TT. PCOS endometrium-derived epithelial organoids as a novel model to study endometrial dysfunction. *Hum Reprod* 2025;**40**:1535–1549.

Madasu C, Liao Z, Parks SE, Sharma KL, Bohren KM, Ye Q, Li F, Palaniappan M, Tan Z, Yuan F, *et al.* Identification of potent pan-ephrin receptor kinase inhibitors using DNA-encoded chemistry technology. *Proc Natl Acad Sci USA* 2024;**121**:e2322934121.

Marquardt RM, Jeong JW, Fazleabas AT. Animal models of adenomyosis. *Semin Reprod Med* 2020;**38**:168–178.

Marr EE, Gnecco JS, Missmer SA, Hawkins SM, Osteen KG, Hummelshoj L, Greaves E, Bruner-Tran KL; EPHect Experimental Models Working Group. WERF endometriosis phenome and biobanking harmonisation project for experimental models in endometriosis research (EPHect-EM-Organoids): endometrial organoids as an emerging technology for endometriosis research. *Mol Hum Reprod* 2025;**31**:gaaf024.

Nikolakopoulou K, Turco MY. Investigation of infertility using endometrial organoids. *Reproduction* 2021;**161**:R113–R127.

Nikolakopoulou K, Ybañez W, Klaeylé L, Frugoli L, Hotz HR, Soneson C, Turco MY. An organoid model of the menstrual cycle reveals the role of the luminal epithelium in regeneration of the human endometrium. 2025. bioRxiv. 2025.07.03.663000, preprint: not peer reviewed.

Ochoa S, Rasquel-Oliveira FS, McKinnon B, Haro M, Subramaniam S, Yu P, Coetzee S, Anglesio MS, Wright KN, Meyer R, Porter AE, Gargett CE, Mortlock S, Montgomery GW, Rogers MS, Lawrenson K. M2 macrophages are major mediators of germline risk of endometriosis and explain pleiotropy with comorbid traits. *Adv Sci (Weinh)* 2025;**12**:e15285.

Omran MM, Vafaei S, Alkhrait S, Ali FL, Bariani MV, Bai T, Thompson WE, Yang Q, Ali M, Al-Hendy A. Utilising human myometrial and uterine fibroid stem cell-derived three dimentional organoids as a robust model system for understanding the pathophysiology of uterine fibroids. *Cell Prolif* 2025;**58**:e70025.

Pașca SP, Arlotta P, Bateup HS, Camp JG, Cappello S, Gage FH, Knoblich JA, Kriegstein AR, Lancaster MA, Ming GL *et al.* A nomenclature consensus for nervous system organoids and assembloids. *Nature* 2022;**609**:907–910.

Pasquale EB. Eph receptors and ephrins in cancer progression. *Nat Rev Cancer* 2024;**24**:5–27.

Peng T, Yang S, Lian W, Liu X, Zheng P, Qin X, Liao B, Zhou P, Wang Y, Liu F *et al.* Cytoskeletal and inter-cellular junction remodelling in endometrial organoids under oxygen-glucose deprivation: a new potential pathological mechanism for thin endometria. *Hum Reprod* 2024;**39**:1778–1793.

Qi S, Zhao X, Li M, Zhang X, Lu Z, Yang C, Zhang C, Zhang H, Zhang N. Aberrant expression of Notch1/numb/snail signaling, an epithelial mesenchymal transition related pathway, in adenomyosis. *Reprod Biol Endocrinol* 2015;**13**:96.

Qin X, Hu KL, Li Q, Sun Y, Peng T, Liu X, Li J, Nan W, Yu Y, Qi X, *et al.* In situ sprayed hydrogel delivers extracellular vesicles derived from human endometrial organoids for uterine function preservation and fertility restoration. *Adv Healthc Mater* 2025;**14**:e2403604.

Rawlings TM, Makwana K, Taylor DM, Molè MA, Fishwick KJ, Tryfonos M, Odendaal J, Hawkes A, Zernicka-Goetz M, Hartshorne GM, *et al.* Modelling the impact of decidual senescence on embryo implantation in human endometrial assembloids. *Elife* 2021;**10**:e69603.

Ren KA, Duarte-Alvarado V, Zhou XD, Ho B, Forjaz A, Crawford AJ, Alicea-Rebecca GM, Joshi S, Nair P, Hanna EA *et al.* 3D map-guided modeling of functional endometrial tissue using multi-compartment assembloids. 2025. bioRxiv. 08.22.671545, preprint: not peer reviewed.

Salisbury E, Rawlings TM, Efstathiou S, Tryfonos M, Makwana K, Fitzgerald HC, Gargett CE, Cameron NR, Haddleton DM, Brosens JJ *et al.* Photo-cross-linked gelatin methacryloyl hydrogels enable the growth of primary human endometrial stromal cells and epithelial gland organoids. *ACS Appl Mater Interfaces* 2024;**16**:39140–39152.

Santamaria X, Roson B, Perez-Moraga R, Venkatesan N, Pardo-Figuerez M, Gonzalez-Fernandez J, Llera-Oyola J, Fernández E, Moreno I, Salumets A *et al.* Decoding the endometrial niche of Asherman's syndrome at single-cell resolution. *Nat Commun* 2023;**14**:5890.

Shibata S, Endo S, Nagai LAE, H Kobayashi E, Oike A, Kobayashi N, Kitamura A, Hori T, Nashimoto Y, Nakato R, *et al.* Modeling embryo-endometrial interface recapitulating human embryo implantation. *Sci Adv* 2024;**10**:eadi4819.

Simitsidellis I, Gibson DA, Saunders PTK. Animal models of endometriosis: replicating the aetiology and symptoms of the human disorder. *Best Pract Res Clin Endocrinol Metab* 2018;**32**:257–269.

Song Y, Fazleabas AT. Endometrial organoids: a rising star for research on endometrial development and associated diseases. *Reprod Sci* 2021;**28**:1626–1636.

Stejskalová A, Vankelecom H, Sourouni M, Ho MY, Götte M, Almquist BD. In vitro modelling of the physiological and diseased female reproductive system. *Acta Biomater* 2021;**132**:288–312.

Stratopoulou CA, El Grari I, Camboni A, Donnez J, Dolmans MM. Expression of endometrial receptivity markers throughout the menstrual cycle in women with and without uterine adenomyosis. *J Clin Med* 2024;**13**:5016.

Stratopoulou CA, Rossi M, Beaussart C, Zipponi M, Camboni A, Donnez J, Dolmans MM. Generation of epithelial-stromal assembloids as an advanced in vitro model of impaired adenomyosis-related endometrial receptivity. *Fertil Steril* 2025;**123**:350–360.

Tian J, Yang J, Chen T, Yin Y, Li N, Li Y, Luo X, Dong E, Tan H, Ma Y, *et al.* Generation of human endometrial assembloids with a luminal epithelium using air-liquid interface culture methods. *Adv Sci (Weinh)* 2023;**10**:e2301868.

Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, Hollinshead M, Marsh SGE, Brosens JJ, Critchley HO *et al.* Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol* 2017;**19**:568–577.

Vigano P, Candiani M, Monno A, Giacomini E, Vercellini P, Somigliana E. Time to redefine endometriosis including its pro-fibrotic nature. *Hum Reprod* 2018;**33**:347–352.

Viña I, Viña JR, Carranza M, Mariscal G. G. Efficacy of N-acetylcysteine in polycystic ovary syndrome: systematic review and meta-analysis. *Nutrients* 2025;**17**:284.

Wiwatpanit T, Murphy AR, Lu Z, Urbanek M, Burdette JE, Woodruff TK, Kim JJ. Scaffold-free endometrial organoids respond to excess androgens associated with polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2020;**105**:769–780.

Xu Y, Cai S, Wang Q, Cheng M, Hui X, Dzakah EE, Zhao B, Chen X. Multi-lineage human endometrial organoids on acellular amniotic membrane for endometrium regeneration. *Cell Transplant* 2023a;**32**:9636897231218408.

Xu Y, Cheng T, Wang J, Qiu Z, Guan X, Yu Y, Shen J, Xu F, Jiang X, Bai D *et al.* Decoding adenomyosis pathogenesis using an assembloid model. *Sci China Life Sci* 2026;**69**:136–150.

Xu Q-X, Madhavan M, Wei S-W, Zhang W-Q, Lu L, Wang K-Z, Genna M, Song Y, Zhao Y, Shao H-T, *et al.* Aberrant activation of Notch1 signaling in the mouse uterine epithelium promotes hyper-proliferation by increasing estrogen sensitivity. *FASEB J* 2023b;**37**:e22983.

Xu L, Wei H, Shang Y, Yang Q. Association of elevated ACSL4 expression with impaired endometrial receptivity in endometriosis and restoration by ACSL4 inhibitor PRGL493. *J Reprod Immunol* 2025;**171**:104630.

Yang H, Zhang J, Yan F, Chen Y, Wu Y, Luo J, Duan L, Zou J, Guo J, Pang J, *et al.* Ciliary IFT-B transportation plays an important role in human endometrial receptivity establishment and is disrupted in recurrent implantation failure patients. *Cell Prolif* 2025;**58**:e13819.

Ye Z, Cheng M, Lian W, Leng Y, Qin X, Wang Y, Zhou P, Liu X, Peng T, Wang R *et al.* GPX4 deficiency-induced ferroptosis drives endometrial epithelial fibrosis in polycystic ovary syndrome. *Redox Biol* 2025;**83**:103615.

Zhang Y, Chen W, Dong X, Shang W, Shao S, Zhang L. Long-term maintenance of human endometrial epithelial organoids and their stem cell properties. *Reprod Toxicol* 2024a;**123**:108522.

Zhang X, Fan L, Zhang L, Liu Z. Comparative analysis of organoid, air-liquid interface, and direct infection models for studying pathogen-host interactions in endometrial tissue. *Sci Rep* 2025a; **15**:8531.

Zhang Y, Lu M, Han Y, Liu B, Zhao R, Liu P, Zhao H. Deciphering endometrial dysfunction in patients with uterine myoma using endometrial organoids: a pilot study. *Reprod Biomed Online* 2024b; **49**:104355.

Zhang R, Yang Y, Li R, Ma Y, Ma S, Chen X, Li B, Li B, Qi X, Ha C. Construction organoid model of ovarian endometriosis and the function of estrogen and progesterone in the model. *Sci Rep* 2025b;**15**:6636.

Zhang X, Zhang L, Fan L, Liu Z. The establishment of 3D polarity-reversed organoids from human endometrial tissue as a model for infection-induced endometritis. *Bio Protoc* 2025c;**15**:e5349.

Zhang X, Zhang L, Li T, Zhang Z, Shang X, Bai H, Liu Y, Zong X, Shang C, Song D *et al*. Investigating bacteria-induced inflammatory responses using novel endometrial epithelial gland organoid models. *Front Reprod Health* 2024c; **6**:1490520.

Zhang H, Zhang C, Zhang S. Single-cell RNA transcriptome of the human endometrium reveals epithelial characterizations associated with recurrent implantation failure. *Adv Biol (Weinh)* 2024d; **8**:e2300110.

Zhang Y, Zhuang Y, Zhou J, Xie X, Sun M, Zheng M, Yuan K, Zhang Z, Zhang J. Effect of estradiol after bacterial infection on the Wnt/β-catenin pathway in bovine endometrium epithelial cells and organoids. *Theriogenology* 2024e;**219**:75–85.

Zhou W, Barton S, Cui J, Santos LL, Yang G, Stern C, Kieu V, Teh WT, Ang C, Lucky T *et al*. Infertile human endometrial organoid apical protein secretions are dysregulated and impair trophoblast progenitor cell adhesion. *Front Endocrinol (Lausanne)* 2022; **13**:1067648.

Zipponi M, Cacciottola L, Dolmans MM. Overview of crosstalk between stromal and epithelial cells in the pathogenesis of adenomyosis and shared features with deep endometriotic nodules. *Hum Reprod* 2024;**39**:1608–1617.

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