Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium

Margherita Y. Turco1,2,13, Lucy Gardner1,2, Jasmine Hughes3, Tereza Cindrova-Davies2,4, Maria J. Gomez1, Lydia Farrell1,2, Michael Hollinshead1, Steven G. E. Marsh5, Jan J. Brosens6, Hilary O. Critchley7, Benjamin D. Simons8,9, Myriam Hemberger1,4,10, Bon-Kyoung Koo9,11, Ashley Moffett1,2,12 and Graham J. Burton2,4,12,13

In humans, the endometrium, the uterine mucosal lining, undergoes dynamic changes throughout the menstrual cycle and pregnancy. Despite the importance of the endometrium as the site of implantation and nutritional support for the conceptus, there are no long-term culture systems that recapitulate endometrial function in vitro. We adapted conditions used to establish human adult stem-cell-derived organoid cultures to generate three-dimensional cultures of normal and decidualized human endometrium. These organoids expand long-term, are genetically stable and differentiate following treatment with reproductive hormones. Single cells from both endometrium and decidua can generate a fully functional organoid. Transcript analysis confirmed great similarity between organoids and the primary tissue of origin. On exposure to pregnancy signals, endometrial organoids develop characteristics of early pregnancy. We also derived organoids from malignant endometrium, and so provide a foundation to study common diseases, such as endometriosis and endometrial cancer, as well as the physiology of early gestation.

Throughout adult reproductive life, the functional layer of the human endometrium undergoes a monthly cycle of regeneration, differentiation and shedding under the control of the hypothalamic–pituitary–ovarian axis. The mucosa contains simple glands lined by secretory columnar epithelium, separated by intervening stroma. During the oestrogen-dominated proliferative phase that follows menstruation, the mucosa regrows and then differentiates during the progesterone-dominated secretory phase. Implantation occurs ∼7 days post-ovulation onto the ciliated luminal epithelium and stimulates transformation into the gestational endometrium, the true decidua of pregnancy, that provides a microenvironment essential for placentation. Up to ∼10 weeks of gestation, uterine glands provide histotrophic nutrition for the conceptus before the definitive haemochorial placenta is established1,2. Animal models in mice and ruminants where glandular function is suppressed are unable to support implantation and pregnancy3,4. Such models have revealed the molecular interactions involved between the trophectoderm and the uterine surface, and the key cytokines secreted by the glands, such as leukaemia inhibitory factor5. However, the composition of the secretions, and the gland/conceptus signalling dialogue during human placentation are unknown due to their inaccessibility in vivo and the absence of in vitro models. Suboptimal glandular development and/or functions may result in human pregnancy failure or predispose to complications of later pregnancy, such as growth restriction6. Thus, model systems to study these essential processes of human early pregnancy would have many biological and clinical applications.

Although stem/progenitor cells within the stromal compartment of the endometrium have been identified, suitable markers for glandular progenitors are unknown7. In mice, stem cells are probably present at the base of the glands8; similarly in primates, cells in the basal layer that is not shed during menstruation can generate both glandular and luminal epithelia9,10. In humans, putative endometrial stem cells are the SSEA-1+, SOX9+ population with clonogenic ability11,12 but these are not fully characterized and it is unknown how they maintain uterine glands. Previous culture systems of human endometrial glandular cells, including three-dimensional (3D) cultures, do not fully recapitulate glandular features in vivo, and are not long-term

1Department of Pathology, University of Cambridge, Cambridge CB2 1QP, UK. 2Centre for Trophoblast Research, University of Cambridge, Cambridge CB2 3EG, UK. 3Department of Clinical Medicine, Addenbrooke’s Hospital, University of Cambridge, Cambridge CB2 2SP, UK. 4Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge CB2 3EG, UK. 5Anthony Nolan Research Institute, Royal Free Hospital, London, NW3 2OU, UK. 6Division of Reproductive Health, Clinical Sciences Research Laboratories, Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK. 7MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh EH16 4TJ, UK. 8Gordon Institute and Department of Physics, University of Cambridge, Cambridge CB2 1QG, UK. 9Wellcome Trust—Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge CB2 1QW, UK. 10Epigenetics Programme, The Babraham Institute, Babraham Research Campus, Cambridge CB22 3AT, UK. 11Department of Genetics, University of Cambridge, Cambridge CB2 3EH, UK. 12These authors jointly supervised this work.

Received 17 June 2016; accepted 16 March 2017; published online 10 April 2017; DOI: 10.1038/ncb3516
Organoids were self-organizing, genetically stable, 3D culture systems containing both progenitor/stem and differentiated cells that resemble the tissue of origin. Human organoids have been derived from tissue-resident adult epithelial stem cells from gut, liver, pancreas, prostate and fallopian tube. We have now generated long-term, chemically defined 3D glandular organoid cultures from non-pregnant endometrium and decidua. The organoids recapitulate features of uterine glands in vivo; the ability to respond to hormonal signals, secrete components of ‘uterine milk’ and differentiate into ciliated luminal epithelial cells. Human endometrial organoids can be used to answer questions about uterine/placental cross-talk during placentation, and will provide a system for studying the pathogenesis and treatment of common conditions affecting women, such as endometriosis and endometrial cancer.

RESULTS

Long-term genetically stable 3D organoid cultures can be established from human non-pregnant endometrium and decidua

To generate endometrial organoids, we used tissue isolates enriched for epithelial cells, and allowed these to self-organize within Matrigel droplets with the basal medium that supports development of other human tissue organoids, containing EGF, Noggin and R-spondin-1 (ENR) (Fig. 1a). Because the signalling pathways maintaining endometrial gland stem/progenitor cells are unknown, we tested factors secreted by surrounding stromal cells, FGF10 and HGF. Nicotinamide and the Alk3/4/5 inhibitor, A83-01, that blocks the TGFβ pathway were added as they are crucial in the establishment and/or long-term culture of other human organoid systems. Nicotinamide and the Alk3/4/5 inhibitor, A83-01, that blocks the TGFβ pathway were added as they are crucial in the establishment and/or long-term culture of other human organoid systems. Decidual samples were initially used to optimize the culture conditions as they yield high cell numbers. Glandular cells were cultured for 7 days and passaged at 1:3. Organoid numbers were counted after 7 days and passaged at 1:3 every 7–10 days for >6 months (reaching more than a 10^6-fold increase in the number of organoids). Markers of glandular epithelium (MUC1, E-cadherin, CK7 and EPCAM) are strongly expressed by the organoids (Fig. 1g,h,i). EPCAM and laminin are present at the baso-lateral membrane, showing that epithelial polarity is intact (Fig. 1i). EdU pulse-labelling shows that ~30% of cells are actively replicating (Fig. 1i). The organoids form cystic structures lined by columnar epithelium with secretions visible in the lumen. Electron microscopy reveals a microvillus, pseudostratified columnar epithelium supported by amorphous basement membrane material with basally located nuclei (Fig. 1j). The cytoplasm contains plentiful rough endoplasmic reticulum and Golgi bodies, numerous secretory vesicles, with evidence of secretory activity from the apical surface (Fig. 1k, arrowheads). A major component of endometrial glandular secretions, glycogen, was visualized by vivid periodic acid Schiff (PAS) staining (Fig. 1l). Thus, the appearances are highly similar to endometrial glands in vivo.

Next, the chromosomal stability of our endometrial organoids was checked by the Comparative Genomic Hybridization (CGH) array. Genomic DNAs were compared between the patient and established organoid cultures at early passage (p) (2–4p) and between early and late cultures (8–15p) (Supplementary Fig. 1d–f). No significant DNA copy number abnormalities were identified during derivation or after continuous passaging for up to 5 months. These organoids can be frozen, thawed and regrown, allowing bio-banking of human endometrial cultures.

Established human endometrial gland organoids recapitulate molecular signature of glands in vivo

To assess the similarity between organoids and the tissue of origin, we analysed the global gene expression profiles from established organoid lines (n = 7), initial glandular digests, and cultured stromal cells from the same biopsy. Staining for MUC1 (glands) and vimentin (stroma) confirmed enrichment of glands in our isolates and the purity of stromal cultures (Supplementary Fig. 2a–d). Hierarchical clustering analysis based on 15,475 probes (s.d./mean >0.1) shows that the organoid cultures cluster more closely to glands than to stroma, confirming their glandular epithelial nature (Fig. 2a).

To define an endometrial glandular genetic signature, we compared glands and organoids to stroma. Two hundred and eighty-seven genes were commonly upregulated in organoids and glands compared with stroma with a fold change of ≥1.5 (P ≤0.01) (Fig. 2b). Gene ontology (GO) analysis shows enrichment for ‘epithelial identity’ and ‘glandular function’ (Fig. 2c,d). Markers of epithelial cells (CDH1, CLDN10 and EPCAM), mucosal secretory cells (PAX8 and MUC1) and of uterine glandular products were all present (PAEP, KLK11 and MUC20) (Fig. 2e). Murine genes involved in endometrial glandular development and function (FoxA2, Sox17 and
Figure 1 Long-term 3D organoid cultures can be established from human non-pregnant endometrium and decidua. (a) Scheme for deriving organoids. (b) Screening conditions for generating organoids. FGF10, A83-01, HGF and nicotinamide added in combinations to generic organoid medium (ENR). Number of organoids derived under each condition (C2 to C9) shown relative to basal conditions (C1). Decidual digest from three different patients. Source data in Supplementary Table 5. (c) Representative images for conditions C1–C9 in b. Scale bars, 500 μm. (d) Images of decidual gland isolates (passage 0) and organoids after one passage in expansion medium (ExM) (passage 1). Scale bars, 200 μm. Representative of all samples, summarized in Supplementary Table 1. (e) Effect of withdrawal of growth factors from ExM. Organoids grown in ExM and each factor withdrawn: EGF, Noggin (NG), R-spondin-1 (RSPO1), FGF10, A83-01, HGF and nicotinamide (NIC). Organoids formed shown relative to ExM(%). Shown are decidual cultures derived from three different patients. Source data in Supplementary Table 5. (f) Images of organoids established in ExM from proliferative (Prol.) endometrium (n=3), secretory (Sec.) endometrium (n=11), decidua (n=25) and post-menopausal (atrophic) endometrium (n=1). Scale bars, 100 μm. (g) Immunohistochemistry of decidua (in vivo) and organoids for mucin 1 (MUC1). Scale bars, 50 μm. Representative of six decidual and endometrial samples, and organoids derived from two endometrial and two decidual samples from different patients. (h) Immunofluorescence staining of organoid for E-cadherin (E-cad) and cytokeratin-7 (CK7). Phase, phase-contrast image. Scale bar, 50 μm. Experiment repeated twice (one endometrial-derived and one decidua-derived organoid). (i) Immunohistochemistry of organoid for cell proliferation (uptake of EdU), epithelial marker EPCAM and basement membrane marker laminin (LAM). Scale bar, 50 μm. Experiment repeated twice with different donors. (j) Electron micrograph of organoid showing columnar epithelial cells with basally located nuclei. Scale bar, 5 μm. Experiment repeated twice with different donors. (k) Electron micrograph showing secretory activity (black arrowheads). Scale bar, 1 μm. Experiment repeated twice with different donors. (l) PAS staining for glycogen in endometrium and organoids. Scale bars, 50 μm (main image) and 10 μm (inset). Representative of three endometrial samples and three endometrial organoids.

Klf5 also emerged. Using immunohistochemistry, we verified nuclear presence of FOXA2, SOX17 and PAX8 in all organoids and endometrial glandular cells throughout the cycle (Fig. 2f). Markers (PROM1, AXIN2 and LRIG1) common to other epithelial progenitor cells were found (Fig. 2e), but in endometrium LRIG1 transcripts are present in glands and luminal epithelium throughout the cycle and so their significance is uncertain (Fig. 2g and Supplementary Fig. 3a). Analysis of expression of other putative endometrial stem cell markers, AXIN2 and SSEA-1 was inconclusive. Although AXIN2 transcripts were found in glands in vivo, lack of a reliable antibody prevented further analysis (Supplementary Fig. 3b). Only a few cells were SSEA-1+ in organoids, analysed by immunohistochemistry and flow cytometry (2–3%) and, after sorting SSEA-1+ cells, organoids emerged from the SSEA-1- fraction (Supplementary Fig. 3c,d). Overall the gene signature of decidual organoids (n=6) is also very similar to non-pregnant endometrium (Supplementary...
Figure 2. Established human endometrial organoids recapitulate the molecular signature of glands in vivo. (a) Unsupervised hierarchical clustering analysis of global gene expression profiles by microarray of gland digests, stromal cells and corresponding established organoids from endometrium (n=7 independent donors). Analysis based on 15,475 probes with s.d./mean >0.1. Expression profiles of organoids cluster with glands while those of the stroma cluster in a separate tree. (b) Venn diagram showing overlap of 287 genes significantly upregulated in glands and organoids with a fold change ≥1.5 (P≤0.01) relative to stroma. (c) Gene ontology (GO) analysis of the 287 genes from b using HumanMine v2.2 database for the GO terms associated with ‘biological processes’ and Benjamini Hochberg test correction with maximum P value of 0.05. The top ten significantly enriched GO terms for each category are shown with the −log of their P values and are enriched for terms describing epithelial tissue. (d) GO analysis of the 287 genes from b using the same method as in c. The top ten significantly enriched GO terms describe epithelial cells with secretory function. (e) Clustered heatmap of 287 genes commonly upregulated between organoids and glands compared with stroma from b. Genes of interest are listed on the right. Epithelial markers (blue) (EPCAM, CLDN10, CDH1), glandular products and markers of secretory cells (purple) (MUC20, PAX8, PAEP, MUC1), progenitor cell markers (cyan) (LRIG1, PROM1, AXIN2) and murine genes important for endometrial function (pink) (SOX17, KLF5, FOXA2). (f) Immunohistochemistry for genes selected from microarray, FOXA2, SOX17 and PAX8, in proliferative and secretory endometrium and organoids. Scale bars, 50 µm (main image) and 10 µm (insets). Representative of three proliferative and seven secretory endometrial samples and endometrial organoids derived from eight different patients. (g) In situ hybridization for LRIG1 on proliferative and secretory endometrium and organoids. Negative control probe is for the bacterial gene dapB. Scale bars, 50 µm (main image) and 10 µm (insets). Representative of three proliferative and three secretory endometrial samples and endometrial organoids derived from four different patients.
stomal interactions (integrin binding and extracellular matrix structural constituents), all absent in vitro. For organoids, in vitro proliferation (cell division and mitotic nuclear division) dominated. Thus, differential gene expression between gland samples and organoids reflects their contrasting microenvironments.

A converse analysis to define a stromal cell signature (Supplementary Fig. 2e) revealed minimal contamination from endothelial cells (CD31 or CD34) or leukocytes (CD45). GO analysis showed ‘biological processes’ typical of fibroblasts and ‘molecular functions’ (Supplementary Fig. 2f). Gene sets were enriched for stromal cell markers (THY1, NT5E and IFITM1)34,35, extracellular matrix proteins (COL8A1, COL12A1, COL13A1 and LAMA1), and metalloproteinas (MMP11, MMP2, MMP12, MMP27, MMP3, TIMP2 and CTGF) (Supplementary Fig. 2e). Genes encoding for components of WNT (WNT2, WNT5A, RSPO3), BMP (BMP2, GREM1) and MAPK (FGF2) signalling pathways also emerged, pathways identified from our culture conditions.

**Human endometrial gland organoids respond to sex hormones**

Unlike other mucosal epithelia, the endometrium responds dramatically to ovarian hormones, oestrogen (E2) and progesterone (P4), which regulate cyclical proliferation and differentiation of endometrial glands with concomitant dynamic temporal and spatial expression of their receptors, ERα and PR (Fig. 3a)36–38. Following menstruation, glands increase expression of ERα in response to rising E2 levels (proliferative phase). After ovulation, ERα expression declines in the early secretory phase whereas PR is maintained until the mid-secretory phase (LH+7), after which both ERα and PR expression disappears37.

To mimic the response of the organoid cultures to hormones, we exposed organoids to E2 followed by P4 (Fig. 3b). Under ExM conditions most cells show weak expression of ERα (ERαlow) with some ERαhigh (Fig. 3c, arrowheads) and ERα cells (Fig. 3c, arrows) present. Although most organoids are PR−, a few cells are PRhigh; on serial sections these are also ERαhigh. After exposure to E2 and P4, high expression of both ERα and PR is seen in most organoids similar to the situation in vivo (Fig. 3c). Organoid cultures derived from decidua showed similar responses (Supplementary Fig. 6a).

We performed a microarray analysis of organoids in ExM, E2 alone or E2 and P4. Known genes upregulated by E2 and P4 in the mid-secretory phase 17βHSD2, PAEP, SPP1, LIF, IGFBP4, IGFBP5 and CYCLIN A1 were all upregulated in hormonally treated organoids (Fig. 3d)39–42. This was confirmed for several genes using quantitative real-time PCR (qRT-PCR) (Fig. 3e) and at the protein level for PAEP and SPP1 (Fig. 3f). We also confirmed that the addition of cyclic adenosine monophosphate (cAMP) to the differentiation medium, a component used typically in decidualization protocols, enhances the expression of differentiation markers shown by increased expression of PAEP and SPP1 (Supplementary Fig. 6b)43.

Other hormonally regulated endometrial genes emerged, including OLFM4, an intestinal stem cell marker. In ExM, organoid cells were OLFM4-negative but a subset became OLFM4+ after E2 treatment, similar to the proliferative phase in vivo (Fig. 3h, arrows). Collagen 1A2 (COL1A2), chromogranin A (CHGA) and OVOL2 were also upregulated, whilst HES1 and SOX9 were downregulated. In summary, the phenotypic response of glandular endometrial organoids to ovarian sex hormones is characteristic of the early–mid-secretory phase.

**Signals from decidualized stroma and the placenta can further stimulate differentiation of human endometrial gland organoids**

If implantation occurs, the endometrium forms the true decidua of pregnancy in response to P4; decidualized stromal cells characteristically secrete prolactin (PRL)45. Both PRL and signals from the conceptus are likely to stimulate uterine gland activity in early pregnancy (Fig. 4a)46,47. To mimic pregnancy, we added placental hormones (chorionic gonadotrophin, hCG and human placental lactogen, hPL) in combinations with PRL to ExM containing E2+E4+cAMP, referred to as differentiation medium (Fig. 4b).

The three hormones together stimulate maximal production of PAEP and a hypersecretory morphology characteristic of decidual glands in vivo (Fig. 4c). PRL has an additional effect by stimulating the formation of ciliated cells (identified by acetylated α-tubulin) (Fig. 4d). Similar findings were obtained using conditioned media from stromal cells decidualized in vitro for 10 days (Supplementary Fig. 6c). As ciliated cells are present in vivo only in the uterine luminal epithelium and in superficial glands, the organoids are undergoing both glandular and luminal differentiation.

SOX9, a marker of progenitor cells, is expressed in the base of endometrial glands in vivo and at high levels in the organoids but is absent from decidual glands in vivo. Organoids cultured with both ovarian and pregnancy hormones underwent differentiation as SOX9 was downregulated (Fig. 4e). Thus, appropriate hormonal stimulation induces organoids to acquire a decidua-like phenotype characteristic of early pregnancy.

**Human endometrial organoids have clonogenic ability and are bipotent**

To assess for stem cell activity, we measured clonogenic ability by plating single cells from established organoid cultures by limiting dilution; drops containing single cells were marked and followed by time-lapse photography. Some cells formed an entire organoid over 7–14 days; the rest either did not divide or formed small dying spheroids (Fig. 5a). The organoid-forming efficiency of these cells was 2–4% with 100 cells per drop and ~10-fold lower with 10 cells per drop (Supplementary Table 2). Single organoids can be expanded into clonal cultures and we now have grown 12 clonal lines from 5 independently derived organoids (Fig. 5b). A single cell has bipotent ability as it could generate the two main endometrial cell types: secretory (PAEP+) and ciliated (acetylated-α-tubulin+) cells (Fig. 5c). Formation of cilia was confirmed by electron microscopy (Fig. 5d).

**Organoid cultures can be derived from endometrial cancer**

Endometrial cancer is the commonest gynaecological tumour. Organoids were derived from samples of tumours and the normal adjacent endometrium from post-menopausal women. The morphology of the organoids shown in Fig. 6 resembles the primary tumour (FIGO grade I endometrioid carcinoma) showing pleomorphic cells with hyperchromic nuclei and disorganized epithelium. In places, breaching of the basement membrane is obvious, and isolated cells are seen in the surrounding Matrigel. The organoids are positive for glandular markers such as MUC1 and SOX17, confirming their glandular origin.
Figure 3 Human endometrial organoids respond to sex hormones. (a) Ovarian hormones, oestrogen (E2) (solid red), and progesterone (P4) (solid blue), and the cycling endometrium. Expression of oestrogen receptor (ERα) (dashed red) and progesterone receptor (PR) (dashed blue) is specific for glands of the functional layer. Data taken from refs 36–38. (b) Hormonal stimulation. Organoids grown in ExM, day 0, are primed with E2 for 48 h on day 4 followed by stimulation with P4 and cyclic AMP (cAMP) for 48 h. (c) Immunohistochemistry for ERα and PR on organoids after hormonal stimulation. In ExM expression of ERα is weak, but some cells are either ERα+ (arrowheads) or ERα− (arrows). Few cells are positive for PR (arrowheads). After E2 and P4 treatment, levels of ERα and PR are higher. Scale bars, 50 µm (main image), 10 µm (insets). Representative of endometrial organoids from six patients and decidual organoids from nine patients. (d) Clustered heatmap of selected genes from organoids grown in ExM, ExM+E2 or ExM+E2+P4+cAMP. Shown are the mean ± s.e.m. levels of expression relative to housekeeping genes and ExM conditions (kM). Data from endometrial organoids from n=6 different patients. Source data in Supplementary Table 5. (f) Western blot for PAEP in organoids after hormonal stimulation. Levels of glycosylated and non-glycosylated PAEP increase following exposure to E2 and E2+P4+cAMP. Ponceau S staining (Ponc S) for loading control. Experiment repeated twice using endometrial organoids from two patients. Unprocessed original scans of blots are shown in Supplementary Fig. 7. (g) Enzyme-linked immunosorbent assay for SPP1 production by endometrial organoids following exposure to hormones. Three independent experiments (donors 1–3). SPP1 secretion increases following exposure to E2 and further after E2+P4+cAMP. Source data in Supplementary Table 5. (h) Immunohistochemistry for OLFM4 on organoids under ExM, ExM+E2 and ExM+E2+P4+cAMP, and proliferative and secretory endometrium. Scale bars, 50 µm (main image) and 10 µm (insets). Representative of two proliferative and two secretory endometrial tissues and organoids derived from three different patients.
DISCUSSION

Here, we describe a robust chemically defined method for establishing genetically stable endometrial organoids from human non-pregnant endometrium and decidua that can be cultured long-term and recapitulate the molecular signature of endometrial glands in vivo. Several murine genes important for glandular development and function (Foxa2, Klf5 and Sox17) are also expressed. The organoids functionally respond to sex hormones, E2 and P4, and when further stimulated with pregnancy (hCG, hPL) and stromal cell (PRL) signals, acquire characteristics of gestational endometrium, synthesizing abundant PAEP (glycodelin) and SPP1 (osteopontin). PAEP and SPP1, components of glandular secretions, ‘uterine milk’, provide histotrophic support to the trophoblast before the haemochorial placenta is established.

Clonal organoid cultures generated from a single cell contain cells with extensive proliferative capacity, and both ciliated and secretory cells. Their gene signature includes markers of epithelial stem cells, LRIG1, PROM1, AXIN2 and SOX9. Because we could generate SOX9- expressing organoids from non-proliferative, SOX9- differentiated secretory phase endometrium and decidua, the few SOX9+ cells present mainly in the basal layer might expand11. Alternatively, plasticity of endometrial cells allows SOX9- differentiated cells to self-renew and reacquire SOX9 expression in our cultures. A similar reversion occurs in the liver, where non-Lgr5+ cells reacquire Lgr5 stem cell marker expression following tissue injury50.

Although organoids have been established from human fallopian tube with differentiation into both ciliated and secretory cells, neither the dramatic cyclical changes in response to E2 and P4, nor the process
of decidualization induced by pregnancy occurs in the fallopian tube, a mucosal surface contiguous to endometrium. Furthermore, the crucial site of embryo attachment is the luminal surface of the endometrium.

Endometrial organoids can be maintained and expanded in ExM, recapitulating pathways essential for culturing organoids from other organs—the FGF-MAPK, WNT–R-spondin, BMP–Noggin and TGFβ signalling pathways. The contribution of endometrial stromal cells to these signalling pathways is revealed from our microarray analysis showing stromal transcripts encoding R-spondin-3 and FGF2. Further refinement of the method to replace Matrigel with a chemically defined extracellular matrix would enhance the model in future studies.

The glands of gestational endometrium continue to differentiate and display a hypersecretory appearance with abundant PAEP secretion. In our organoid system, addition of trophoblast hormones (hCG and hPL) resulted in a similar appearance. This culture system will therefore allow further investigation of the essential (but understudied) period of histotrophic nutrition in the first trimester of pregnancy before the haemochorial placenta is established. Additionally, we were able to derive organoids from endometrial adenocarcinomas. These common tumours in post-menopausal women are associated with increased exposure to oestrogen that is a feature of obesity, nulliparity, treatment with tamoxifen and late menopause. These can be used in the future to build a biobank to screen drugs and investigate the mutational changes, as has been done for colon cancers.

In summary, we describe a method for reliable chemically defined, long-term culture of endometrial glands from non-pregnant endometrium and decidua that closely recapitulates the molecular and functional characteristics of their cells of origin. The organoid cultures can be frozen down without loss of their proliferative ability after thawing, allowing the possibility to build up patient-specific...
bio-banks. This method will be an invaluable research tool to study new therapies for common pathologies of the endometrium, such as endometriosis and endometrial cancer, as well as investigating problems of implantation and the secretion of uterine histotroph during early pregnancy.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

**ACKNOWLEDGEMENTS**

The authors are grateful to patients for donating tissue for research. We thank D. Moore, R. Remadevi, M. Baumgarten, M. Jimenez-Linan, D. S. Charnock-Jones, Department of Obstetrics and Gynaecology and NHS Tissue Bank staff at Addenbrooke’s Hospital, Cambridge; H. Skelton for her invaluable histological services and technical advice; I. Pshenichnaya, K. Bird and A. Starling at the Stem Cell Institute for their histological services; J. Bauer and Cambridge Genomic Services for microarray analysis; I. Simonic at Medical Genetics Laboratory, Cambridge University Hospital for CGH analysis; J. N. Skepper for electron microscopic analysis; N. Miller for flow cytometry sorting; H. W. Yung and A. Sharkey for technical help and advice; J. Cross and Y. W. Loke provided much helpful discussion and all members of the Moffett laboratory were supportive throughout. This work was supported by the Medical Research Council (MR/L020041/1), the Centre for Trophoblast Research, University of Cambridge and the Wellcome Trust (RG60992). M.Y.T. has received funding from the E.U. 7th Framework Programme for research, technological development and demonstration under grant agreement no PIEF-GA-2013-629785. J.H. was supported by a Wellcome Trust vacation scholarship. B.-K.K. is supported by a Sir Henry Dale Fellowship under grant agreement no PIEF-GA-2013-629785. M.Y.T., A.M. and G.J.B. wrote the manuscript.

**AUTHOR CONTRIBUTIONS**

M.Y.T. and L.G. designed and carried out all experiments and data analyses; J.H. and T.C.-D. assisted with experiments and data analyses; M.J.G. performed microarray analysis; M. Hollinshead performed EM analysis and assisted with confocal analysis; J.J.B. and H.O.C. provided endometrial specimens and input for the manuscript; L.F. and S.G.E.M. assisted with experiments; A.M. and B.-K.K. assisted with experimental design, analyses of results and preparation of manuscript; B.D.S. and M. Hemberger assisted with analyses of results and preparation of manuscript; M.Y.T., A.M. and G.J.B. wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

Published online at http://dx.doi.org/10.1038/ncb3516

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**METHODS**

**Patient samples.** All tissue samples used for this study were obtained with written informed consent from all participants in accordance with the guidelines in The Declaration of Helsinki 2000 from multiple centres. Endometrial and cervicovaginal samples were obtained from elective terminations of normal pregnancy at Addenbrooke's Hospital between 8 and 12 weeks gestation under ethical approval from the Cambridge Local Research Ethics Committee (04/Q0108/23). Secretory phase (6 and 10 d after pre-ovulatory luteinizing hormone surge) endometrial samples were obtained from subjects recruited from the Implantation Clinic at University Hospitals Coventry and Warwickshire National Health Service Trust with ethical approval from NHS National Research Ethics—Hammersmith and Queen Charlotte's & Chelsea Research Ethics Committee (1997/5065). Endometrial biopsies were obtained using a Wallach Endocell sampler, starting from the uterine fundus and moving downward to the internal cervical ostium. None of the subjects were on hormonal treatments for at least 3 months prior to the procedure.

**Proliferative and secretory endometrial samples were obtained from Addenbrooke's Hospital under ethical approval from the East of England—Cambridge South Research Ethics Committee (08/H0305/40).** Endometrial carcinoma samples were obtained from Addenbrooke's Hospital Tissue Bank.

Proliferative and secretory human endometrial tissue sections for immunohistochemistry and in situ hybridization studies were available with research ethical committee approval (Lothian Research Ethics Committee: 10/S1402/59; 16/ES0007). Endometrial tissues were staged on the basis of standard histological criteria and circulating oestradiol and progesterone levels at the time of collection and no exogenous hormone exposure.

**Isolation of glands, derivation and culture of organoids from human uterine tissue samples.** Endometrial/decidual/carcinoma tissues were chopped using scalpels into approximately 0.5 mm² cubes and enzymatically digested in 20–30 ml 1.25 U/ml Dispase II (Sigma, D4693)/0.4 mg/ml 1- collagenase V (Sigma, C-9263) solution in RPMI 1640 medium (Thermo Fisher Scientific, 21875-034)/10% FCS (Biosera, FB-1001) with gentle shaking at 37 °C for 30–60 min. The supernatant was passed through one or more 100 μm cell sieves (Corning, 431752) and the sieve washed several times with medium. The flow-through was collected for stromal cell culture in Advanced DMEM/F12 (Thermo Fisher Scientific, 12634010) +10% FBS+ pen/strep (Sigma, P0781) +1-glutamine (Sigma, 25030-024) for several days and subsequent analysis. The sieves were inverted over a Petri dish and retained glandular elements were backwashed from the sieve membranes, pelleted by centrifugation and resuspended in ice-cold Matrigel (Corning, 356231) at a ratio of 1:20 (vol/vol). Twenty-microlitre drops of Matrigel-cell suspension were plated into 48-well plates (Costar, 3548), allowed to set at 37 °C and overlaid with 250 μl organoid Expansion Medium (ExM). See Supplementary Table 3 for ExM composition. The medium was changed every 2–3 d. Cultures were passaged by manual picking using 7–10 d for freezing organoids, Matrigel was removed using Cell Recovery Solution (Corning, 354253) and organoids were resuspended in Recovery cell culture freezing medium (Thermo Fisher Scientific, 12648-010). A step-by-step protocol of the derivation and maintenance of human endometrial organoid cultures can be found at Nature Protocol Exchange.

**Organoid formation efficiency assays.** Organoids were removed of Matrigel using Cell Recovery Solution and pipetted several hundred times before trypsinizing with TrypLE Express (Invitrogen, 1247701). Cells were washed in medium and passed through a 40 μm cell strainer (Corning, 352340) to ensure single-cell suspension. Cells were diluted in trypsin blue to exclude dead cells and counted using a haemocytometer. For the growth factor requirement experiment (Fig. 1e), 5,000 cells were plated per 20 μl Matrigel drop into 48-well plate, per culture condition and the relative positions of the drops containing one cell were stored using the ZEISS 780 Confocal microscope and ZEN Observer software.

**Differentiation of endometrial organoids.** For hormonal stimulation of organoids with β-oestradiol (E2, Sigma E4389), progesterone (P4, Sigma P7556) and 8-bromoadenosine 3′, 5′-cyclic monophosphate (cAMP, Sigma B7880), organoids were passaged routinely and after 4 d of growth in ExM, they were primed with 10 nM E2. After 48 h, medium was replaced with the following conditions: untreated (ExM); 10 nM E2; or 10 nM E2 + 1 μM P4 + 1 μM cAMP. After 96 h, the organoids were collected for downstream experiments.

**For differentiation of organoids using human pregnancy hormones, 20 ng ml⁻¹ prolactin (PRL, Peprotech 100-07), 1 μg ml⁻¹ human chorionic gonadotropin (hCG, Source Bioscience ABC403) and 20 ng ml⁻¹ human placental lactogen (hPL, R&D 5757-PL) were used.** Organoids were passaged routinely and after 4 d in ExM, the medium was switched to differentiation medium (DM), which is ExM containing 10 nM E2 + 1 μM P4 + 1 μM cAMP. DM was added with a combination of HCG, hPL and/or PRL for another 8 d. See Supplementary Table 3 for further information.

**Enzyme-linked immunosorbent assay.** Organoids from nine wells of a 48-well plate were pooled and transferred onto three 35 mm ibidi μ-dishes (Thermo Scientific, 81156) thinly coated with Matrigel diluted 1:2 in DMEM/F12. Matrigel was removed with Matrigel cell recovery solution on ice for 1 h. Organoids were washed in DMEM/F12 and resuspended in 1.2 ml ExM. Organoid suspension (400 μl) was plated into each dish and incubated at 37 °C for 2 h to allow organoids to attach. Dishes were flooded with ExM and cultured for 2–4 d until complete monolayers of cells had grown out. Cells were then treated as described above for E2 and P4 stimulation. Supernatants were harvested after a further 96 h and centrifuged to remove any cellular material prior to concentrating to 250 μl with Vivaspin 2 concentrators (Generon, V50291). Concentrated supernatants were stored at –80 °C until use. Human Osteopontin (SPP1) Platinum ELISA (eBioscience, BMS2066) was performed using 50 μl concentrated supernatant with 50 μl sample buffer in duplicate and using the standard plots using Microsoft Excel.

**Immunohistochemistry.** Tissue sections of 4 μm were cut from formalin-fixed paraffin wax-embedded human endometrial and decidual tissues and organoids. Prior to paraffin embedding, organoids were removed from Matrigel using Cell Recovery Solution, fixed in formalin (Sigma, F5554) and embedded into 1% agarose (Melford, MB1200). Sections were dewaxed with Histoclear (National Diagnostics, HS-200), cleared in 100% ethanol and rehydrated through gradients of ethanol to PBS. Heat-induced epitope retrieval (HIER) was performed in Access Revelation (AR) pH 6.4 buffer (A.Menarini, MP-607-PG1) or Access Super (A). (Menarini, MP-606-PG1), at 125 °C in an antigen Access pressure cooker unit (A.Menarini, MP-2008-CE). Sections were blocked with 2% serum (of species in which the secondary antibody was made) in PBS, primary antibody incubation was 30 min at room temperature or overnight at 4 °C and slides were washed in PBS. Biotinylated horse anti-mouse or goat anti-rabbit secondary antibody was used, followed by Vectastain ABC-HRP reagent (Vector, PK-6100) and developed with 3,3'-diaminobenzidine (DAB) substrate (Sigma, DAB94). Sections were counterstained with Carazzi's haematoxylin and mounted in glycerol/gelatin mounting medium (Sigma, GG1-10). Primary antibody was replaced with an equivalent concentration of mouse or rabbit IgG for negative controls. See Supplementary Table 4 for antibody information. Periodic acid Schiff (PAS) staining was performed on paraffin sections following standard protocols provided by Surgipath. Tissue sections were imaged using a Zeiss Axiosvert Z1 microscope and Axiosview imaging software SE64 V4.8.

**Immunofluorescence (IF) and confocal microscopy.** Endometrial organoids were grown in 35 mm ibidi μ-dishes (Thermo Scientific, 81156). Organoids were incubated for 2 h at 37 °C in 10 μM EdU in ExM. Organoids were fixed for 30 min in 0.5% Triton/PBS. EdU staining was done using Click-iT EdU Alexa Fluor 594 Imaging Kit (Thermo Scientific, C10339) following the manufacturer’s instructions. Organoids were washed in PBS and blocked in 5% GS/1%BSA in PBS for 40 min at room temperature. Primary antibodies were incubated in blocking buffer with 0.05% Triton at 4 °C overnight. For antibodies used, see Supplementary Table 4. Negative controls were prepared by omitting primary antibody and omitting EdU incubation. Organoids were washed 3 times for 15 min in PBS. Organoids were incubated for 3 h at room temperature in PBS with secondary antibodies (all from Thermo Fisher Scientific): Alexa Fluor 488 goat anti-mouse IgG1 (A21121), Alexa Fluor goat anti-rabbit 568 (A11011) or Alexa Fluor 647 (A21244) at 1:400 and DAPI (Sigma, D9542). Organoids were washed in PBS for 30 min 3 times, mounted in ibidi mounting medium (Thermo Fisher Scientific, 400241) and imaged using the ZEISS 780 Confocal microscope and ZEN Microscope Software.

**Flow cytometry.** Organoids were processed as described above in the ‘Organoid formation efficiency assays’ section to obtain single-cell suspensions. Cells were blocked in 1% FBS in DPBS without calcium and magnesium (Thermo Fisher Scientific, 10270022)
14190136) with human IgG (Sigma, 14506) and then incubated at 4 °C with SSEA-1 PE at 1:10 (Miltenyi, 130-104-936). 7-AAD was used for live/dead discrimination. Cells were sorted using a DakoCytomation MoFlo cytometer and Summit software.

In situ hybridization assays. In situ hybridization assays for LRIG1 and AXIN2 were performed on 4-μm-thick paraffin sections using the RNAscope 2.0 High definition assay (Advanced Cell Diagnostics) following the manufacturer’s instructions. Briefly, the tissue sections were baked at 60 °C for 1 h and dewaxed with xylene, cleared in 100% ethanol and air-dried. For tissue sections, the slides were treated according to the standard protocol: 10 min in Pretreat buffer 1, 15 min in Pretreat buffer 2 and 30 min at 37 °C in Pretreat buffer 3. For organoid sections, milder treatments were necessary to avoid non-specific signal: Pretreat 2 for 5 min and Pretreat 3 for 15 min. Sections were then incubated with LRIG1 probe (Cat. no. 407241) or AXIN2 (Cat. no. 400241), positive control probe PPIB (Cat. no. 313901), negative control probe dapB (Cat. no. 310043) for 2 h at 40 °C. Positive and negative controls were performed for each sample. For the visualization of signal, the samples were incubated using the amplification kit and then treated with DAB for 10 min. Sections were then dehydrated, mounted in DPX (Sigma, 45681) and imaging using a Zeiss Axiosvert Z1 microscope and Axiosvision imaging software SE64 V4.8.

Electrolysis reaction (EM). For Fig. 1k, organoids were fixed in 4% glutaraldehyde in 0.1 M Hepes buffer (pH 7.4) for 12 h at 4 °C, rinsed in 0.1 M Hepes buffer ×3, treated with 1% osmium tetroxide at room temperature for 12 h, followed by 5 washes in deionized water. They were then treated with 2% uranyl acetate in 0.05 M maleate buffer (pH 5.5) for 12 h at room temperature, rinsed in deionized water and dehydrated in an ascending series of ethanol solutions from 70% to 100% treated twice with dry acetone and infiltrated with Quetol epoxy resin. Images were taken in an FEI Tecnai G2 operated at 120 kV using an AMT XR60B digital camera running Deben software. For Fig. 5d, organoids were fixed in 0.5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) for 30 min, washed in sodium cacodylate buffer, treated with reduced osmium tetroxide 1% OsO4, 1.5% potassium ferricyanide at room temperature for 60 min, washed in water, treated with 0.5% magnesium uranyl acetate at 4 °C for 16 h, dehydrated with ethanol rinsed in propylene oxide and embedded in Epon resin. Ultrathin sections were examined in an FEI Tecnai G2 TEM at 80 kV. Images were acquired with a MegaView III CCD and Soft Imaging Systems program.

Western blotting analysis. Organoids were incubated in Matrigel cell recovery solution on ice for 1 h to remove Matrigel, washed in ice-cold PBS and resuspended in ice-cold buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM EDTA, and complete mini proteases inhibitor cocktail (Roche, 0469359001). Western blots were performed as previously described6. Equivalent amounts of protein were resolved by SDS–PAGE, and transferred onto nitrocellulose membranes. Membranes were blocked in 5% milk in TBST for 1 h at room temperature, rinsed in deionized water and dehydrated in an ascending series of ethanol solutions from 70% to 100% ethanol and dehydrated in an ascending series of ethanol solutions from 70% to 100% ethanol and dehydrated in an ascending series of ethanol solutions from 70% to 100%..

DNA extraction and quantification. DNA was extracted from donor patients’ blood, endometrial biopsies, decidua tissue and organoids using QiAamp DNA Blood Mini kit (Qiagen) by digestion with ATL buffer (Qiagen, 19076) and Proteinase K (Sigma, P4850), followed by purification steps with RNeasy A (Sigma, R6513) and Protein Precipitation Solution (Qiagen, 158910), precipitation with isopropanol and washing with 70% ethanol. DNA quality and concentration were determined using the Nanodrop ND-1000 Spectrophotometer.

Genetic analysis. The DNA was analysed using the Agilent SurePrint G3 unrestricted CGH ISCA 8 × 60K array (Agilent, G4500A) by the Medical Genetics Laboratory at Addenbrooke’s Hospital. Five independent organoid samples were analysed and DNA from patient’s blood, biopsied tissue or early passage organoids was used as hybridization controls. DNA was diluted to 50 ng μl-1 and labelled using the Agilent kit following the manufacturer’s instructions. Data analysis for segmentation and copy number calls was performed at a genome-wide resolution of 500 kb using the default analysis method—CGH v2 from the Agilent CytoGenomics software Edition 2.5.8.11 (Build 37).

RNA extraction, quantification and cDNA synthesis. Total RNA was extracted using the RNeasy Mini kit with on column DNAse treatment (Qiagen, 79254), following the manufacturer’s instructions. RNA quality and concentration were determined using the Nanodrop ND-1000 Spectrophotometer. Total RNA (500 ng–1 μg) was reverse transcribed using Superscript VILO Reverse Transcriptase (Thermo Fisher Scientific, 11754050) with random hexamers and Rnase inhibitor according to the manufacturer’s instructions. An RNA sample without reverse transcriptase was used as a control for genomic DNA contamination.

Real-time RT-PCR. Quantitative real-time RT-PCR (qRT-PCR) was performed on a 7900HT (Fast Real-Time PCR system, Applied Biosystems) with Taqman Mix and Taqman gene expression assays and following the manufacturer’s protocol. The cycling conditions were: 95 °C for 20 s and 40 cycles of 95 °C for 3 s followed by 60 °C for 30 s. TaqMan Gene expression assays (Applied Biosystems) used: LIF (Hs01055668_m1), PAEP (Hs01046125_m1), SP1 (Hs00959010_m1) and HSID17B2 (Hs01057993_m1). Expression levels were calculated applying the comparative Cycle threshold (Ct) method. Relative expression levels were normalized to the geometric mean of three housekeeping genes HPRT1 (Hs00980695_m1), TOP1 (Hs002432257_m1) and TBP (Hs00427620_m1) using Microsoft Office Excel. All qRT-PCR experiments were carried out with a non-template control.

Microarray expression profiling and data analysis. Microarray experiments were performed using the HumanHT-12 v4 expression BeadChip (Illumina, BD-103-0204) according to the manufacturer’s instructions to the Cambridge Genomic Services University of Cambridge. A total of 7 endometrial samples were analysed and for each, the starting glandular digest, stromal cells and established organoids were used for analysis. Six organoid cultures derived from decidua were also analysed. RNA samples for microarray analysis were assessed for concentration and quality using a SpectroStar and a Bioanalyzer. Briefly, 200 ng of Total RNA underwent linear amplification using the Illumina TotalPrep RNA Amplification Kit (Thermo Fisher Scientific, AM1719) following the manufacturer’s instructions. The concentration, purity and integrity of cRNA were measured by SpectroStar and Bioanalyzer. cRNA was hybridized to the HumanHT-12 v4 BeadChip overnight following by washing, staining and scanning using the Illumina Bead Array Reader. After scanning, the data were loaded into GenomeStudio software. No background correction or normalization is applied at this stage. The data are processed in R using the limma package and the limma package. Across all samples probes for which the intensity values were not significantly different (P > 0.01) from the negative controls were removed from the analysis. Following filtering the data were transformed using the Variance Stabilization Transformation (VST) from lumi and then normalized to remove technical variation between arrays using quantile normalization. Comparisons were performed using the limma package with results corrected for multiple testing using False Discovery Rate (FDR) testing. Finally, the quality of the data was assessed and the correlation of the samples in the groups compared. Heatmaps were generated using the heatmap.2 function of the R package ‘gplots’, which uses the Euclidean method to obtain the distance matrix and the complete agglomeration method for clustering. For the sample heatmaps, the input is a correlation matrix based on samples’ expression profile. For the gene heatmaps, the input is the vst transformed and normalized intensity matrix. The cluster analysis for the hormone stimulation microarray was done using the R sva package; expression values were trimmed by removing the baseline differences between samples due to patient origin. Then, cluster analysis was done on a selected group of genes (those differentially expressed between control groups (ExM) and stimulated with oestrogen (ExM+E2) with adjusted P values ≤0.05) using the R stats package. The distance matrix was computed using 1-correlation as the distance measure, and hierarchical clustering was performed using the complete linkage method.

Statistics and reproducibility. All experiments reported in this study have been reproduced with similar results using independent samples (tissues and organoids) from multiple patients. The origin of derivation of organoids (proliferative or secretory endometrium, decidua, post-menopausal or carcinoma) and the number of times the experiments were repeated are reported in the figure legends and summarized in Supplementary Table 1. Given the descriptive nature of the work and biological variation between human samples, the experimental data points for each patient sample are shown separately unless stated otherwise. Statistical analyses used to analyse microarray data are reported in the Methods above and in the figure legends.

Data availability. Microarray data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession codes GSE61303 (Fig. 2 and 4) and GSE94723 (Fig. 3). Source data for Figs 1b,c and 3e,g and Supplementary Fig. 6b have been provided as Supplementary Table 5. All other data supporting the findings of this study are available from the corresponding authors on reasonable request.

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Supplementary Figure 1  Growth factor requirements of established endometrial organoids (a) Bright field (BF) images of spheroid formation assay for endometrial organoids at d 7. Single factors were omitted from ExM (control) as indicated. Noggin (NG), Rspo1 (RSPO1) and Nicotinamide (NIC). Scale bar, 500 μm. Representative of experiments performed with 3 decidual organoids derived from different donors. (b) BF images of endometrial organoids after 4 passages in ENR, ENR+A83-01, ENR+Nicotinamide, ENR+A83-01+Nicotinamide and ExM. Scale bar, 500 μm. Representative of experiments performed with 3 endometrial organoids derived from different donors. (c) BF images of organoids derived from atrophic post-menopausal endometrium 10 d after plating and cultured under ENR, ENR+A83-01+Nic and ExM conditions. Scale bar, 500 μm. Only 1 tissue sample obtained for this experiment. (d) BF images of endometrial organoids at early passage 2, (p2) and late passage 8 (p8). Scale bar, 100 μm.  
(e) Analysis of genetic stability of cultures with CGH array. A representative whole-genome array CGH plot generated using Agilent Cytogenomics software. Genomic DNA from early passage (p2) endometrial organoids (red) is compared to genomic DNA from original sample (blue). Each spot is a single probe. Log ratios of the average signal intensity of each probe on the Y-axis along its position on the chromosomes (1-22, X and Y) on the x-axis. A log signal ratio of 0 represents equivalent copy number. High signal in the Y chromosome region is not significant, and is due to absence of the Y chromosome in the samples. Repeated with 4 different decidual organoids and 2 endometrial organoids. (f) Genomic DNA from late passage (p8) endometrial organoids (red) compared to genomic DNA from early passage (p2) organoids (blue). The plot represents data described in the same way as in (e). Repeated with 4 different decidual organoids and 2 endometrial organoids.
Supplementary Figure 2 Cell isolation procedure from endometrium enriches for glands and stromal cells. (a) BF image of gland fragments generated from endometrium. Scale bar, 500 μm. (b) IF staining on cytospin smears of gland isolates shows enrichment for MUC1-positive fragments. Scale bar, 100 μm. Experiment repeated twice with endometrial digests from different donors. (c) Phase-contrast image of endometrial stromal cultures isolated from endometrium showing typical fibroblast morphology (passage 2). Scale bar, 200 μm. (d) IF staining of endometrial stromal cultures shows cells are uniformly positive for mesenchymal marker VIMENTIN (far left, scale bar, 50 μm). Image at higher magnification (centre, scale bar 50 μm) and zoom-in image (far right) show filamentous staining pattern typical of VIMENTIN (white arrowheads). Experiment repeated twice with stromal cells isolated from endometrial samples from different patients. (e) Clustered heatmap of 376 genes upregulated in stromal cells compared to gland digests and organoids derived from endometrium (n=7 independent donors). Genes descriptive of fibroblast function (magenta), genes that encode for signalling pathways (blue) and genes that encode for fibroblast markers (cyan). (f) Gene ontology (GO) analysis of upregulated genes from (e) using HumanMine v2.2 database for GO Terms Molecular Function with Benjamini Hochberg test correction with maximum p-value of 0.05. The top eight significantly enriched GO terms for each category are shown with the –log of their p-values on the x-axis. (g) analysis of upregulated genes from (e) using HumanMine v2.2 database for GO Terms Cellular Components with Benjamini Hochberg test correction with maximum p-value of 0.05. The top eight significantly enriched GO terms are shown with the –log of their p-values on the x-axis.
Supplementary Figure 3  Expression of stem cell markers LRIG1, AXIN2 and SSEA-1 in human endometrium. (a) ISH for LRIG1 on functional and basal layers of proliferative endometrium. LRIG1 transcripts are detected in both luminal (LE) and glandular (GE) compartments (positive signal, in brown, DAB and counterstain in purple with Carazzi’s hematoxylin). Stroma (St) and myometrium (M) are negative. Positive control probe is for PPIB. Negative control probe is for the bacterial gene dapB. Scale bars, 50 μm. Representative of 3 different endometrial samples. (b) ISH to localize AXIN2 expression in human endometrium (positive signal, in brown, DAB). AXIN2 transcripts are found within GE and not in St. Scale bars, 50 μm (main image) and 10 μm (inset). Representative of 3 different endometrial samples. (c) IHC for SSEA-1 on endometrial organoids showing a small population of positive cells. Representative of 3 different endometrial samples. (d) FACS sorting gates for SSEA-1 on endometrial organoids. Organoids were processed to obtain single cells and stained for SSEA-1-PE and 7-AAD. Gating strategy (from left to right); debris was gated out on forward/side scatter (cells, R1), singlets were gated on (R2) and live cells based on negativity for 7-AAD (R3). To ensure sorted SSEA-1+/- cells are pure, gating between them was distanced by a log difference. Negative control using mouse IgG-PE was used for gating positive populations. Specificity of SSEA-1 staining was tested on peripheral blood where it is expressed on granulocytes and monocytes. Percentages of SSEA-1+ cells from organoid cultures derived from 4 different donors are shown in table below.
Supplementary Figure 4 Organoids derived from gestational endometrium (decidua) are similar to endometrial organoids. (a) Unsupervised hierarchical clustering analysis of global gene expression profiles by microarray of initial glandular digests, primary isolates of stromal cells and corresponding established organoids from endometrial biopsies. Highlighted in red, endometrial organoid_1 to organoid_7, highlighted in yellow: decidual organoid_8 to organoid_13. Analysis based on 20611 probes with sd/mean >0.1. Glands and organoids derived from endometrium and decidua cluster together whilst the stroma clustered separately. (b) IHC for FOXA2, SOX17 and PAX8 expression identified from the microarray as characteristic of endometrial glands. All gland cells in the decidua and organoids derived from decidua are positive for these markers. Scale bars, 50 μm (main image) and 10 μm (insets). Representative images of negative controls on decidua and organoids using mouse or rabbit IgGs are shown. Representative of 7 decidual samples and organoids derived from 5 different patients. (c) ISH for LRIG1 on decidua and organoids derived from decidua. Positive control probe is for PPIB. Negative control probe is for the bacterial gene dapB. Scale bars, 50 μm (main image) and 10 μm (insets). Representative of 3 decidual samples and organoids derived from 3 different patients.
Supplementary Figure 5 Analysis of genes that are differentially expressed between isolated glands and organoids in comparison to stromal cells. Genes that are only expressed in one or other group without restriction to a fold change of ≥1.5 were examined. For glands, these are 421/652, and for organoids 286/484. Gene ontology analysis using R package TopGO (stringent Weight method) of 421 genes expressed only in isolated glands show terms that describe interaction of cells with their microenvironment, such as integrins and extracellular matrices. The 268 genes expressed only in organoids contain GO terms describing proliferation and DNA replication, reflecting the expanding in vitro system.
Supplementary Figure 6 Further characterization of endometrial and deciduval organoid differentiation. (a) IHC for ERα and PR on decidual organoids cultured in ExM and after hormonal stimulation. Expression and upregulation of ERα and PR in ExM and after hormonal stimulation is similar to that of endometrial organoids (Figure 3c). Scale bars, 50 μm (main image) and 10 μm (insets). Representative of stimulation of decidual organoid cultures derived from 3 different patients. (b) QRT-PCR for differentiation markers PAEP and SPP1 in endometrial organoids stimulated with differentiation protocol described in Figure 3b, in the presence and absence of cAMP. Addition of cAMP to differentiation medium enhances upregulation of these markers. Results from endometrial organoids derived from 3 different donors are shown individually. (c) Effect of conditioned medium from endometrial stromal cells stimulated with hormones on cilia formation in endometrial organoids. Acetylated-α tubulin are shown in brown (DAB) and cells are counterstained with Carazzi’s Hematoxylin. Ciliated cells are present in both ExM+E2+P4+cAMP+PRL and d 10 stromal cell conditioned medium. Scale bars, 50 μm (main image) and 10 μm (insets). Representative of treatment of endometrial organoids derived from 3 different patients.
Supplementary Figure 7 Unprocessed scans of western blots for Figure 3f.
Supplementary Table Legends

Supplementary Table 1 Summary of established organoid cultures used for this study. Organoids were derived from 43 samples: proliferative phase endometrium (n=3), secretory endometrium (n=11), decidua (n=25), atrophic endometrium (n=1) and endometrial adenocarcinomas (n=3). Each organoid culture was derived from a different patient sample. Shown below are the samples used in this study (n=34). For each organoid culture, the type of endometrial tissue from which it was derived and the characterization performed are indicated (grey box).

Supplementary Table 2 Human endometrial organoids have clonogenic ability. Organoid formation efficiency is shown as percentage of plated cells from established organoid cultures ± SD (plated at 100 and 10 organoid cells/5 μL Matrigel drop into 96-well plates). From endometrial organoids derived from 3 different patients.

Supplementary Table 3 Expansion medium composition for the culture of organoids from human endometrium and hormones used for their differentiation.

Supplementary Table 4 List of antibodies used.

Supplementary Table 5 Source data for Figures 1b, 1e, 3e, 3g and Supplementary Figure 6b.