Biology of Reproduction, 2017, 97(6), 781–783
doi:10.1093/biolre/iox139
Research Highlight
Advance Access Publication Date: 2 November 2017

Research Highlight

Endometrial organoids: in vitro models for endometrial research and personalized medicine†

James A. Deane[1,2], Fiona L. Cousins[1,2] and Caroline E. Gargett[1,2,*]

[1]The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Victoria, Australia and [2]Department of Obstetrics and Gynaecology, Monash University, Monash Medical Centre, Melbourne, Victoria, Australia

*__Correspondence:__ The Ritchie Centre, Hudson Institute of Medical Research, 27–31 Wright Street, Melbourne, VIC 3168, Australia. E-mail: Caroline.Gargett@hudson.org.au

†__Grant Support:__ This work was supported by National Health and Medical Research Council of Australia Grants (ID 1085435) (CEG, JAD) and Senior Research Fellowship (ID 1042298) (CEG) and the Victorian Government's Operational Infrastructure Support Program.

Received 28 September 2017; Revised 21 October 2017; Accepted 1 November 2017

The endometrium is one of the most dynamic tissues in the human body, undergoing hormonally regulated cycles of growth and shedding each month. There is evidence that endometrial stem/progenitor cells are responsible for the regenerative properties of the endometrium, and there has been a great deal of interest in characterizing these cells so that they can be harnessed to treat infertility, disease, and injury, or modulated to treat proliferative disorders of the endometrium such as cancer and endometriosis [1].

Many of the tools and techniques used to study stem/progenitor cells in other tissues have proved invaluable in endometrial research. Colony-forming assays have been used to detect epithelial and stromal cells with the stem cell-related ability to expand clonally in vitro [2]. In vivo transplantation assays have demonstrated that certain endometrial populations are capable of reconstituting endometrial epithelium and stroma in vivo [3]. Label retention studies in the mouse have identified quiescent populations that are activated during regeneration [4]. Stem/progenitor markers have been used to identify cell types that may be involved in endometrial regeneration [5]. Several studies have also touched on the utility of three-dimensional culture systems in endometrial research [6]. However, recent studies by Turco et al [7] and Boretto et al [8] represent the first systematic examination of the conditions and protocols required to initiate, maintain, and cryopreserve endometrial epithelial organoids.

Organoids are defined as three-dimensional structures generated in vitro from stem cells that self-organize through cell sorting into multicellular structures that have functionality representative of the organ or tissue from which they were derived [9]. They are a miniature and simplified version of their parent organ/tissue and may contain multiple differentiated cells types. Organoids are often derived from tissue fragments containing tissue specific progenitor cells and can be clonally propagated. Organoids represent a powerful test of the regenerative and differentiation potential of the initiating cell type and the developmental processes they recapitulate, cell arrangement in the niche and lineage commitment, as the various cell types self-organize into mini-organs or tissues [9]. Organoid systems have proved to be an invaluable tool for investigating stem/progenitor cells in organs including intestine, liver, pancreas, prostate, fallopian tube, and kidney [7, 10].

Studies by Turco et al [7] and Boretto et al [8] are detailed examinations of methods for generating epithelial organoids from endometrium. Turco et al generated organoids from normal and malignant human endometrium, and Boretto et al from normal human and mouse endometrium. Both studies used matrigel and a defined culture medium supplemented with factors that promote organoid formation and maintenance by cells from other tissues (Figure 1A). Key factors promoting organoid formation and maintenance were shown to include activators/amplifiers of WNT signaling (WNT ligands and/or RSPO (R-spondin-1)), growth factors (EGF, FGF10) acting as epithelial mitogens, inhibitors of TGFβ (A83–01) and BMP (Noggin) signaling, and nicotinamide (commonly used in iPS and embryonic stem cell differentiation). Organoids generated could be maintained for many passages and several months (>6 months), and remained viable after cryopreservation. Both studies provide evidence that organoids are of clonal origin, and Turco et al also show that human endometrial organoids are genetically stable after passaging for more than 5 months. Endometrial organoids show physiologically relevant responses to ovarian hormones, with estrogen increasing proliferation and estrogen-regulated gene expression, and progesterone slowing proliferation, increasing progesterone-regulated gene expression and promoting differentiation of secretory and ciliated cells (Figure 1B). Mouse endometrial organoids survive when transplanted under the kidney capsule and expand in response to ovarian hormones [8].

Endometrial epithelium has historically been difficult to maintain in vitro, and the ability to reliably produce, maintain, and cryopreserve physiologically relevant epithelial organoids from

© The Authors 2017. Published by Oxford University Press on behalf of Society for the Study of Reproduction. All rights reserved.
For permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/biolreprod/article-abstract/97/6/781/4587925
by Biomedical Library user
on 18 June 2018

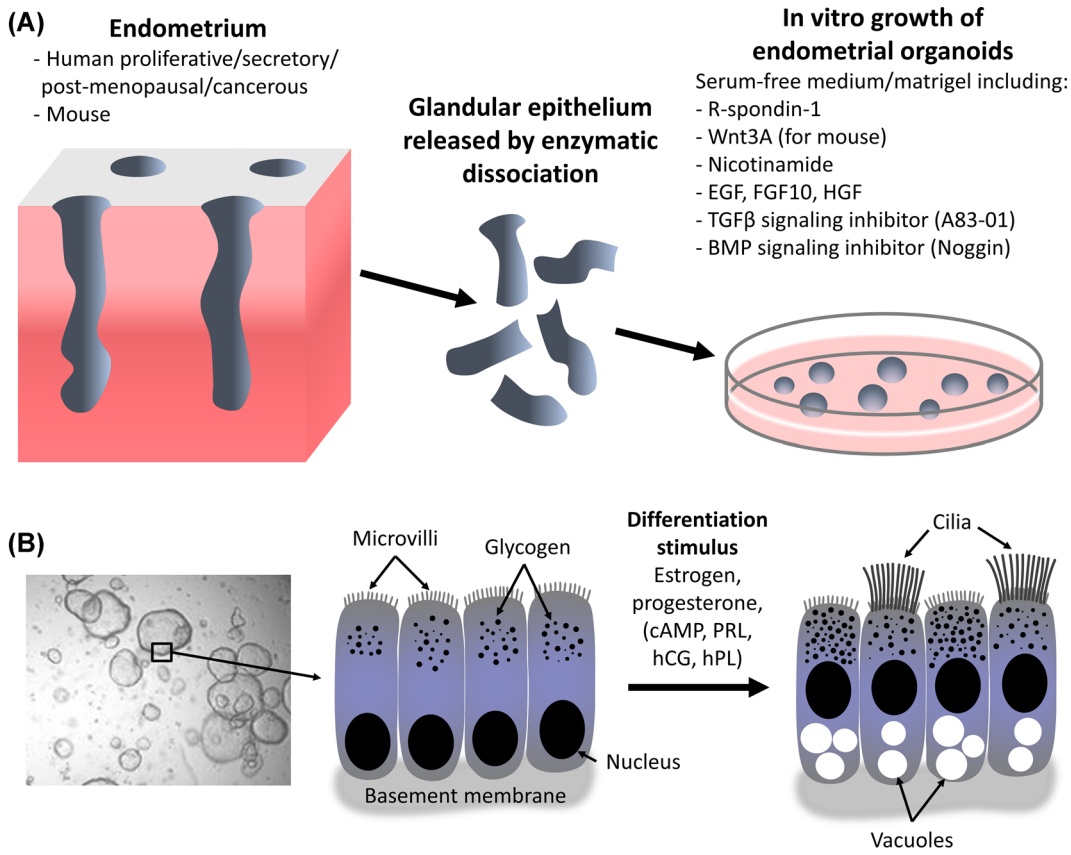

Figure 1. The derivation and properties of endometrial organoids reported in [7, 8]. (A) Endometrial organoids are produced by enzymatic dissociation of endometrium to obtain glandular epithelial fragments that are grown in serum-free medium and matrigel suplemented with key factors allowing the initiation and growth of organoids. (B) Endometrial organoids replicate pseudostratified endometrial epithelium and contain both secretory and ciliated epithelial cells when subjected to differentiation stimuli. Abbreviations: BMP, Bone morphogenetic protein; EGF, epidermal growth factor; FGF10, Fibroblast growth factor 10; hCG, human chorionic gonadotropin; hPL human placental lactogen; HGF, hepatocyte growth factor; PRL, prolactin; TGFβ, transforming growth factor β. Micrograph in panel B is from Boretto et al [8] and used with permission.

human and mouse endometrium opens up exciting new avenues for research. Patient-derived endometrial organoids may offer insight into the epithelial biology underlying infertility and proliferative diseases of the endometrium such as endometriosis and endometrial cancer. Endometrial organoids offer a new platform for drug discovery and therapeutic development for endometrial disorders as well as enabling personalized medicine, as an individual's cryopreserved organoids can be tested against a range of drugs to identify the most efficacious treatment. The clonal nature of organoids will allow endometrial epithelial stem/progenitor populations in the endometrium to be investigated in greater detail. This is particularly pertinent to the recent identification of N-cadherin as a human endometrial epithelial progenitor cell marker [5]. The ability to produce organoids from genetically altered and mutant mice will allow the dissection of the pathways regulating endometrial stem/progenitor cells and their niche. Inclusion of stromal cells may enable the generation of more complex endometrial organoids, an important goal given the key roles of stromal–epithelial interactions in endometrium. These two recent studies [7, 8] describe methods that are a notable addition to the toolkit available to the endometrial research community.

References

1. Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 10 years. *Hum Reprod Update* 2016; **22**:137–163.
2. Chan RWS, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells1. *Biol Reprod* 2004; **70**: 1738–1750.
3. Masuda H, Matsuzaki Y, Hiratsu E, Ono M, Nagashima T, Kajitani T, Arase T, Oda H, Uchida H, Asada H, Ito M, Yoshimura Y et al. Stem cell-like properties of the endometrial side population: implication in endometrial regeneration. *PLoS One* 2010; **5**: e10387.
4. Chan RWS, Kaituúu-Lino T, Gargett CE. Role of label-retaining cells in estrogen-induced endometrial regeneration. *Reprod Sci* 2012; **19**:102–114.
5. Nguyen HPT, Xiao L, Deane JA, Tan K, Cousins FL, Masuda H, Sprung CN, Rosamilia A, Gargett CE. N-cadherin identifies human endometrial epithelial progenitor cells by in vitro stem cell assays. *Hum Reprod* 2017; **32**:2254–2268.
6. Rinehart CA, Lyn-Cook BD, Kaufman DG. Gland formation from human endometrial epithelial cells in vitro. *In Vitro Cell Dev Biol* 1988; **24**:1037–1041.
7. Turco MY, Gardner L, Hughes J, Cindrova-Davies T, Gomez MJ, Farrell L, Hollinshead M, Marsh SGE, Brosens JJ, Critchley HO, Simons BD, Hemberger M et al. Long-term, hormone-responsive organoid cultures of human endometrium in a chemically defined medium. *Nat Cell Biol* 2017; **19**:568–577.
8. Boretto M, Cox B, Noben M, Hendriks N, Fassbender A, Roose H, Amant F, Timmerman D, Tomassetti C, Vanhie A, Meuleman C, Ferrante M et al. Development of organoids from mouse and human endometrium

Downloaded from https://academic.oup.com/biolreprod/article-abstract/97/6/781/4587925
by Biomedical Library user
on 18 June 2018

showing endometrial epithelium physiology and long-term expandability. *Development* 2017; **144**:1775–1786.

9. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 2014; **345**:1247125.
10. Sato T, Stange DE, Ferrante M, Vries RGJ, Van Es JH, Van Den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and barrett's epithelium. *Gastroenterology* 2011; **141**:1762–1772.

Downloaded from https://academic.oup.com/biolreprod/article-abstract/97/6/781/4587925
by Biomedical Library user
on 18 June 2018