Stem Cell Research & Therapy 2013, 4(Suppl 1):S13 doi:10.1186/scrt374
http://www.woodrufflab.org

Recreating the female reproductive tract in vitro using iPSC technology in a linked microfluidics environment.

Monica M. Laronda[1], Joanna E. Burdette[2], J. Julie Kim[1], Teresa K. Woodruff[1]

[1] Division of Reproductive Biology, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois.
[2] Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois.

Abstract
The female reproductive tract produces hormones for reproductive function, cardiovascular, bone, and sexual health; it supplies a finite number of gametes, and supports fetal development. Diseases that affect each of the female reproductive tract organs, along with treatments that have direct, deleterious effects on the reproductive tract (e.g. chemotherapeutics), are understudied due to the lack of model systems that phenocopy in vivo function. This review describes a path toward developing female reproductive tract mimics. The models use isolated primary support cells cultured onto a biological scaffold and within a microfluidic system to create a niche and support the desired differentiation of epithelia, germ and somatic cells from patient-derived induced pluripotent stem cells (iPSCs). Improving our fund of knowledge about reproductive tract biology and creating reproductive organs for patients who have lost gonadal, uterine or vaginal/cervical function is a major step forward in women's health and an important advance in personalized medicine.

Abbreviations: DAZL, deleted in azoospermia-like protein; *Dppa3*, pluripotency associated 3; ECM, extracellular matrix; FOXJ1, forkhead box J1; iPSCs, induced pluripotent stem cells; PGCs, primordial germ cells; *Stra8*, stimulated by retinoic acid 8.

Introduction
The female reproductive tract produces hormones, supplies gametes and supports embryos through fetal development. Understudied and poorly understood diseases, including those contracted through sexual transmission, benign tumors and cancers, develop in or affect each of the female reproductive tract organs [1-4]. Additionally, treatments that are aimed to rid patients of their diseases, including radiation and chemical therapies, could result in premature loss of fertility and the side effects that accompany it [5, 6]. This is especially detrimental to women, who have a finite number of gametes and whose cardiovascular, bone and sexual health rely on the hormonal milieu supported by their reproductive tract [6, 7]. Advances in bioengineered tissue mimetics, including ovarian follicles, represent an important new avenue of investigation in the study of normal reproductive function and the regeneration of diseased tissues [8]. The mouse, while the standard of biological research models, does not adequately represent the human female. Mice have short four-day cycles with no endometrial shedding, are often resistant to pathogens of the reproductive tract, rarely contract reproductive tract tumors or cancers, and are polyovulatory. Reproductive tracts from young, healthy women are almost never removed, nor should they be. This results in analysis of human samples that are removed from diseased and/or aged patients that have undergone various types of hormonal therapy. Moreover, ethical concerns exist that prevent researchers from enrolling pregnant women in clinical trials, and even women of reproductive age are excluded from many studies resulting in little new knowledge on drug efficacy or interactions in young, reproductive age women. Not only are reproductive tract diseases inadequately studied, researchers lack the models to analyze potential therapies for these diseases or how existing treatments may affect normal

reproductive tissues. In vitro model systems that mimic disease processes associated with reproductive organs could meet this need.

Research into a personalized approach to understanding reproductive tract disease and restoring fertility or endocrine function would not only help the specific needs of patients in the future, but would allow for a model that could integrate a wider range of genetically and phenotypically diverse mimics into basic research. Great headway has been made in induced pluripotent stem cell (iPSC) derivation from human somatic cells for many organs, and new methods have been employed to derive these cells without integration of viral vector or transgene sequences [9-11]. Specifically, human iPSCs have been differentiated into cardiomyocytes, neurons and primordial germ cells [12-14]. Utilizing iPSCs to create the reproductive tract organ mimics would allow for new drug testing, and could provide personalized regenerative treatment options that restore fertility and/or endocrine function.

One specific example and urgent need for this technology is in the area of oncofertility – fertility restoration for young female cancer patients. Current technology includes autotransplantation of ovarian cortical strips laproscopically inserted into the ovary, once cancer treatments have been completed, to provide temporary fertility and hormonal support with some promising success [15-20]. However, a study in mice transplanted with ovarian tissue biopsied from patients with acute lymphoblastic leukemia (ALL) resulted in tumor formation creating the concern that tissue transplant may reintroduce cancer into the patient who recently survived the original disease [21]. To avoid this risk, iPSCs could be used as a personalized and purified source of somatic or germ cells for transplant. While biological offspring is a distant option, the more tangible restoration may be of endocrine function for children transitioning through puberty or for premenopausal women who lose gonadal function due to cancer treatment. Indeed, a piece of ovarian tissue was autotransplanted for the sole purpose of bringing a child with cancer through puberty, which carries the same intrinsic risk of reintroducing cancer cells as a transplant for the purpose of fertility [22]. Finally, transplants of uteri in non-human primates have been performed, with more success achieved in autotransplants where immunosuppression therapy is not required [23-28]. Uterine transplants in cancer patients are another utility for patient-specific, iPSC-derived reproductive tissue.

Somatic cells from a number of tissues have been used to induce embryonic stem cells (ESCs) into organ-specific cell types. For example, bladder mesenchyme can induce mouse ESCs into uteroepithelium, rat seminal vesicle mesenchyme can induce human ESCs into prostate epithelium and neonatal mouse uterine mesenchyme can induce human ESCs into female reproductive tract epithelium [29-31]. However, it is more desirable to utilize iPSCs than ESCs for reasons including, fewer ethical concerns, higher availability, and ability to utilize a patient source. As proof of principle, mouse iPSCs were differentiated into primordial germ cells (PGCs) and male or female germ cells through co-culture with testis or ovarian somatic cells, respectively [32, 33]. Human iPSC neural progenitors also differentiated once injected into a rat spinal cord [14].

Based on the urgent need, both at the basic research level and for patient use, this review describes a path for developing female reproductive tract mimics using isolated primary support cells cultured onto a biological scaffold and within a microfluidic system to provide a niche and support the desired differentiation of epithelia and germ cells. A variety of steps are necessary before these tissues are ready for patients, but recent advances make the possibility of regenerative reproductive organs an important area of investigation.

Recreating the Female Reproductive Tract.

A three-dimensional (3D) culture system is essential for developing and maintaining the functionality, physiology and integrity of an organ culture mimic. The importance of the niche extends beyond nutrient and ligand support. Intracellular connections and signal transduction between cells cultured in a traditional versus a 3D culture system have shown significant

changes in morphology, differentiation capacity and gene expression profiles. For example, mammary epithelia cultured in a 3D system develop acinar structures and a basement membrane through integrin interactions with the extracellular matrix (ECM) [34]. Additionally, varying degrees of ECM density can be observed in an ovary, and each portion harbors follicles at different stages of activation or differentiation [8]. Likewise, primordial and secondary follicles require different degrees of rigidity within a 3D culture in order to maintain their integrity [35, 36]. Each organ within a reproductive tract mimic must be maintained in a suitable 3D culture system.

The female reproductive tract organs are dynamic and require synchronization of movement and differentiation to guide ovulated oocytes, prepare for implantation and nurture a fetus to develop as an independent organism. It is necessary to not only see these organs as unique entities, but as one cohesive system that rely on biochemical interactions with each other for proper reproductive function. A schematic of how each reproductive tract tissue is affected in response to changing hormone levels during the menstrual cycle is presented in Figure 1. Recently developed high-throughput drug screens utilize 3D systems-level models that incorporate microfluidics to create a microenvironment that chemically and physically imitates the desired system [reviewed in 9]. Likewise, it is important to develop these organs in a connected microfluidic system in order to provide the sequence of hormones that control biological function in a dynamic manner. Additionally, studies in postmenopausal women and women who have undergone premature ovarian failure demonstrate that hormone replacement regimens could improve the patients overall health, quality of life and is recommended by the American Association of Clinical Endocrinologists [37-40]. These non-reproductive tract effects of the endocrine hormones produced by the ovaries are important to program into other organ systems in order to ensure normal function. Thus, while we have focused on the role of female sex hormones on the adjacent reproductive tissues, it is important to keep the impact of the overall influence of estrogens and progesterones on all tissues of the body [6].

The following sections focus on the individual organs of the female reproductive tract and describe how one may mimic these organs by utilizing patient-derived iPSCs differentiated in a tissue-specific niche. A representative schematic is diagramed in Figure 2. In general, iPSCs derived from a diverse patient population could be initially induced *in vitro* with defined factors. One way to perform this induction down a differentiation path of choice is to initially induce the iPSCs into the primary embryonic lineage in which the desired cell type could arise [41, 42]. For example, the human fibroblast-derived iPSCs would be induced toward the mesodermal lineage for uterine epithelium differentiation with factors such as bone morphogenetic protein 4 (BMP4) and retinoic acid (RA) [43]. The desired cells would be identified and combined with uterine mesenchyme niche to induce further differentiation. The success of this differentiation would be analyzed by the presence of specific uterine epithelium markers and, most importantly, response to hormonal cues. In the future, this uterine mimic could be used to restore uterine function by replacing damaged or diseased sections of the uterus (Figure 2A). Additionally, this uterine mimic could be used within a microfluidic system, downstream of the ovarian source of hormones, to screen potential drugs that may prevent miscarriages (Figure 2B).

Each section below describes how the dynamic cell type within each female reproductive tract tissue could be replaced by patient-derived iPSCs that have been differentiated by the paracrine factors and cytokines of the supportive cell type or niche.

Female Reproductive Tract Organ Mimics

The Ovary: Germ Cells and Somatic Endocrine Cells

The ovary is the central organ of the female reproductive tract as it produces a haploid gamete that can be fertilized to develop into a viable embryo. Oocytes do not develop in

isolation but require close interactions with granulosa and theca cells to activate and mature. This somatic cell with germ cell unit is called the follicle. Follicles are maintained in a hierarchy of developmental stages that regulate a woman's fertility during her reproductive life. The meiotically arrested primordial follicles delineate the fertility reserve of a female and rely on intraovarian factors for survival and eventual activation. Select follicles are recruited during each cycle and a dominant follicle is chosen in response to follicle stimulating hormone (FSH). This gonadotropin is important for development and prepares the mature oocyte to be terminally matured and released in response to luteinizing hormone (LH). The dominant follicle secretes estradiol and androstenedione in changing levels throughout the cycle [44]. After ovulation, the remaining somatic cells (granulosa and theca cells) form the corpus luteum (CL) and produce high levels of progesterone and estrogens to support endometrial function and implantation. If fertilization does not occur, the CL regresses, steroid production ceases and the endometrium is shed.

The ability to recreate the germ cell and somatic cells of the follicle has progressed rapidly in recent years. Human iPSCs cultured with BMP-4, -7 and -8b for 1 to 2 weeks differentiated down the PGC lineage as measured by VASA and deleted in azoospermia-like protein (DAZL) expression [13]. Moreover, PGCs derived from mouse iPSCs that were reintegrated with ovarian somatic cells, develop into oocyte-like cells and produced live offspring. The embryonic ovarian stromal cells surrounding the iPSC-derived PGCs are enough to induce expression of early PGC markers like *Nanos3* and developmental pluripotency associated 3 (*Dppa3,* also known as *Stella*), late PGC markers like *DazL* and stimulated by retinoic acid 8 (*Stra8)* and behave like oocytes within a follicle [33].

In addition to the somatic cell function in driving the activation and growth of follicles, the mechanical environment, which controls mechanotransduction and physical forces, of the ovary is important to this process and can be engineered into the system using biomaterials [8, 36, 45]. Part of what creates the physically distinct areas of tissues is the extracellular matrix (ECM). The ECM is more dense and less vascularized in the rigid outer cortex of the ovary, where primordial follicles reside, than the less dense medulla, where the recruited follicles grow, differentiate and prepare for ovulation [46-49]. The ECM acts as a depository for growth factors and other ligands that induce cellular responses. The outer cortex provides an important environmental niche in which immature follicles can reside for decades of life. To phenocopy this part of the ovarian environment requires creating an artificial scaffold to simulate the ECM signaling factors and the mechanotransduction properties of the ovary. In the future iPSC-derived oocytes, surrounded by primary granulosa and theca cells, within a synthetic scaffold which recreates both cortex and medulla compartments could be imagined in order to retain the ordered activation of follicles and sequential development of mature gametes. A microfluidic system could provide the appropriate levels of FSH and LH to trigger the normal cyclical regulation of follicle development, something which is not possible in static culture. A functioning ovary mimic would then release the right hormones at the right time in the right amount to support endocrine function of reproductive and other target tissues.

The Fallopian Tubes: Ciliated Fimbria and Muscular Passages

The female reproductive tract organs, the fallopian tubes, uterus, cervix and vagina, develop from the Müllerian duct. The most anterior portion of the Müllerian duct develops into the fallopian tubes. These tubes are the site of fertilization, initial embryo development, and can be phenotypically and functionally divided into four segments. The segment closest to the ovary, the infundibulum, is shaped like the bell of a trumpet, consists of a fimbriated portion and is highly susceptible to hormonal changes [50]. The fimbriated portion is made of densely ciliated cells, which express acetylated tubulin. During ovulation, the tubal epithelial cells from the infundibulum respond to estrogen secretion from the ovary and come in contact with it. The ciliated cells express oviductal glycoprotein 1 (OVGP1) and guide the ovum through the ostium

or opening of the fallopian tube [51]. The ovum is received as a cumulus mass and remains that way unless it becomes fertilized. Fertilization occurs within the ampulla, which is highly ciliated and made of many folds. The most distal portion of the fallopian tube to the ovary is the isthmus, and is connected to the uterotubal junction or interstitial region. This portion of the fallopian tube contains more paired box 8 protein (Pax8)-positive secretory than ciliated cells and has fewer folds than the ampulla. The uterotubal junction provides tightly regulated movement of sperm from the uterus to the fallopian tube or embryo into the uterus. The opening and closing of this junction is regulated by steroid hormones and differs at points in the menstrual cycle. Prenatal exposure to a non-steroidal estrogen, diethylstilbestrol, or disruption of micro-RNA synthesis disrupts this junction in mice [52, 53]. The arrival of the embryo into the uterus must be precisely synced with uterine epithelial differentiation for proper implantation [9]. Failure of the embryo to be released into the uterus can result in implantation within the fallopian tube, which is known as an ectopic pregnancy and can be detrimental to the life of the mother.

A 3D microfluidic culture system is salient in maintaining the integrity of a fallopian tube mimic and ensuring a response to estrogen signals from the ovary [54]. As in most organs, the oviduct mesenchyme determines the adjacent epithelial cell fate. Undifferentiated epithelial cells adjacent to the ampulla will differentiate into more ciliated cells, while those adjacent to the isthmus mesenchyme will form more secretory cells [55]. With this in mind, region specific mesenchyme can be utilized to support and differentiate iPSCs into the appropriate epithelial cell type. Differentiation of the iPSCs into the desired epithelium can be monitored through expression of PAX8, forkhead box J1 (FOXJ1) and acetylated tubulin, and the proper response to paracrine signals from the ovary can be monitored through expression patterns and physiology mentioned above. The constructed organ pieces can then be integrated to form the entire fallopian tube and assembled within the microfluidic system.

The Uterus: Cycling Endometrium and Contractile Myometrium

The primary purpose of the uterus is to harbor and nurture the developing fetus throughout gestation. The dynamic and regenerating uterine endometrium potentially undergoes hundreds of cycles that involve differentiation, growth and shedding throughout a woman's reproductive lifespan [56]. The endometrium is composed of two layers, the functionalis layer, which contains glands with loose stroma, and basilis layer, which contains branching glands and dense stroma. Both layers receive steady support by the myometrium. Estrogen receptor-alpha (ESR1) and progesterone receptor (PR) expression increases in the functionalis endometrium upon increased estrogen secretion by the ovary. The endometrium thickens and luminal and glandular cells multiply during the proliferative phase. The induced proliferation of this layer gradually ceases upon ovulation and induction of progesterone. The uterus prepares for a potential blastocyst implantation by secreting glycogen and other histotrophic products [57]. The physiological changes that occur during the menstrual cycle are accompanied by changes in the gene expression profile, including upregulation of fucosyltransferase 4 (*Fut4*) during the early and mid-secretory stages and upregulation of transforming growth factor alpha (*Tgfα*) during the mid-secretory to menstrual stages [58]. Without implantation, the circulating estrogen and progesterone levels fall, and the functionalis layer is shed, leaving the basilis layer to remodel and rebuild the uterine lining. The regenerative capacity of the uterine tissue has been attributed to adult stem cells that reside in the basilis layer [59-61]. Basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) are secreted by the adjacent stroma to induce proliferation of the endometrial stroma, and hepatocyte growth factor (HGF) produced from these cells induces proliferation of the adjacent endometrial epithelium [56]. Inappropriate remodeling of this tissue can lead to miscarriage or infertility. However, little is known about implantation of the embryo due to lack of models that appropriately mimic the human menstrual cycle, implantation and pregnancy.

Human ESCs that were differentiated into embryoid bodies and cultured with neonatal mouse uterine mesenchyme differentiated into female reproductive tract-like cells that formed ductal glands, expressed PAX2 and homeobox A10 (HOXA10) and responded to cycling estrogen and progesterone by secreting glycodelin A [31]. Primary myometrial cells have been isolated from biopsy samples, cultured and are able to replicate the in vivo tissue [62-64]. A biological scaffold, such as a fibrin-alginate network, could be utilized to support mesenchymal cell expansion. It is clear that the neonatal mouse uterine mesenchyme produced specific factors not produced in other cell types that contributed to the differentiation of hESCs. However, the identity of these components remain unclear, but could include TGF-beta and wingless-type MMTV integration site family (WNT) morphogens [31]. While it would be ideal to create healthy and diseased uterine mimics from primary tissue biopsies, the types of tissue collected for research are mostly from older women undergoing hysterectomies or removal of leiomyomas. Myometrial cells may support iPSC differentiation in a similar manner to form a uterine mimic and provide a high throughput screen for drug testing and/or tissue replacement with patient-specific phenotypes and genotypes. Immunocytochemistry with an antibody against vimentin could identify stromal cell differentiation, while K18 and cancer antigen 125 (CA-125) staining could identify epithelial differentiation of the female reproductive tract. Interaction between cultured human uterine tissue and human cytotrophoblast cells induces a differentiation or decidualization response, including increased expression of insulin-like growth factor binding protein-1 (IGFBP-1) in the uterine stroma cells [65]. This technique could be used to determine functionality of the uterine tissue mimic and determine how diseases or potential treatments may affect implantation.

The Cervix and Vagina: Barrier and Passage

Together the cervix and the vagina act as a barrier from potential exterior pathogens that may affect the more cranial reproductive tract organs. While the endocervix epithelium remains columnar like the uterine epithelium, the ectocervix is phenotypically similar to the vagina. These tissues are comprised of mesenchyme and epithelium layers that lack glands. The epithelium consists of a basal layer and approximately 28 layers of stratified squamous cells that maintain the integrity of the barrier through mucous production and cornification. The epithelial layers can change in thickness and composition in response to circulating hormones [66-68]. Loss of ovarian function results in epithelium atrophy and a slowed mucous production in the vagina and ectocervix [69]. The proliferative effects of estradiol on this tissue require ESR1 expression in the stroma, much like in the uterine tissue [70-72]. Additionally, exposure to estradiol induces PR expression and induces stratification and cornification in mouse vaginal tissue [70, 73]. In order to establish a working vaginal mimic that can respond to hormones, it is important to establish an epithelium-stroma interaction that could be maintained within a biochemical scaffold.

The Müllerian duct epithelium differentiates into stratified squamous epithelium along the ectocervix and vagina in response to paracrine signals from the mesenchyme. The basal layer of vaginal epithelium expresses the delta-N isoform of the tumor protein 63 (TP63), much like the basal layer of skin [74, 75]. Because interaction with other undifferentiated cell types with the developing mesenchyme can induce the expression of deltaN-Trp63 in mice, the potential for the vaginal mesenchyme to induce a similar stratified squamous epithelium from iPSCs would be of interest [76]. The differentiated iPSC recombined with the vaginal mesenchyme could create the vaginal tissue mimic. Appropriate identification of these stratified squamous cell layers could be achieved by identifying expression of E-cadherin (CDH1) and K14.

Significance

The studies and concepts described here support the rationale for developing reproductive tract mimics. To create an ideal reproductive tract mimic each tissue niche needs

to be developed in order to support iPSC differentiation into the appropriate cell type. Given the hormonal response profile of these tissues, a microfluidic system is warranted. Establishing tissue banks of biopsies collected from both healthy and diseased patient tissues at various points in the menstrual cycle will provide a wide range of biological/fertility/infertility mimics.

The future of medical technology for the female reproductive tract will rely on the ability to accurately mimic these dynamic tissues in a system that can be adapted for genetic variations and diseased models, and replicated for high throughput screens. While this concept may seem futuristic, recent advances in iPSC and microfluidic technologies indicate that organ mimic development is on the horizon to satisfy the urgent unmet needs of patients.

The authors have nothing to declare. This article is part of a supplement for the **NIH Microtissue Initiative.** The published review (of 1500 words) can be found in *Stem Cell Research & Therapy*. Funding for this review, and the costs of its publication, is supported by UH2 ES022920.

References:
1. Achkar JM, Fries BC: **Candida Infections of the Genitourinary Tract**. *Clinical Microbiology Reviews* 2010, **23**:253–273.

2. King SM, Burdette JE: **Evaluating the progenitor cells of ovarian cancer: analysis of current animal models**. *BMB Reports* 2011, **44**:435–445.

3. Sandoz KM, Rockey DD: **Antibiotic resistance in Chlamydiae**. *Future Microbiology* 2010, **5**:1427–1442.

4. Liebrich C, Brummer O, Wasielewski Von R, Wegener G, Meijer C, Iftner T, Petry KU: **Primary cervical cancer truly negative for high-risk human papillomavirus is a rare but distinct entity that can affect virgins and young adolescents.** *Eur J Gynaecol Oncol* 2009, **30**:45–48.

5. Jeruss JS, Woodruff TK: **Preservation of fertility in patients with cancer**. *New England Journal of Medicine* 2009, **360**:902–911.

6. Kim AM, Tingen CM, Woodruff TK: **Sex bias in trials and treatment must end.** *Nature* 2010, **465**:688–689.

7. Tingen CM, Kim AM, Wu P-H, Woodruff TK: **Sex and sensitivity: the continued need for sex-based biomedical research and implementation**. *Women's Health* 2010, **6**:511–516.

8. Woodruff TK, Shea LD: **A new hypothesis regarding ovarian follicle development: ovarian rigidity as a regulator of selection and health**. *J Assist Reprod Genet* 2011, **28**:3–6.

9. Eddy CA, Pauerstein CJ: **Anatomy and physiology of the fallopian tube.** *Clin Obstet Gynecol* 1980, **23**:1177–1193.

10. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA: **Human Induced Pluripotent Stem Cells Free of Vector and Transgene Sequences**. *Science* 2009, **324**:797–801.

11. Kahler DJ, Ahmad FS, Ritz A, Hua H, Moroziewicz DN, Sproul AA, Dusenberry CR, Shang L, Paull D, Zimmer M, Weiss KA, Egli D, Noggle SA: **Improved Methods for Reprogramming Human Dermal Fibroblasts Using Fluorescence Activated Cell Sorting**. *PLoS ONE* 2013, **8**:e59867.

12. Rufaihah AJ, Huang NF, Kim J, Herold J, Volz KS, Park TS, Lee JC, Zambidis ET, Reijo-Pera R, Cooke JP: **Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity.** *Am J Transl Res* 2013, **5**:21–35.

13. Panula S, Medrano JV, Kee K, Bergstrom R, Nguyen HN, Byers B, Wilson KD, Wu JC, Simon C, Hovatta O, Reijo Pera RA: **Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells**. *Hum Mol Genet* 2011, **20**:752–762.

14. Popescu IRI, Nicaise CC, Liu SS, Bisch GG, Knippenberg SS, Daubie VV, Bohl DD, Pochet RR: **Neural progenitors derived from human induced pluripotent stem cells survive and differentiate upon transplantation into a rat model of amyotrophic lateral sclerosis.** *Stem Cells Translational Medicine* 2013, **2**:167–174.

15. Donnez J, Silber S, Andersen CY, Demeestere I, Piver P, Meirow D, PELLICER A, Dolmans M-M: **Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births**. *Ann Med* 2011, **43**:437–450.

16. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, Schiff E, Dor J: **Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy.** *N Engl J Med* 2005, **353**:318–321.

17. Demeestere I, Simon P, Emiliani S, Delbaere A, Englert Y: **Orthotopic and heterotopic ovarian tissue transplantation**. *Hum Reprod Update* 2009, **15**:649–665.

18. Silber S, Kagawa N, Kuwayama M, Gosden R: **Duration of fertility after fresh and frozen ovary transplantation**. *Fertil Steril* 2010, **94**:2191–2196.

19. Silber S, Grudzinskas G, Gosden RG: **Successful pregnancy after microsurgical transplantation of an intact ovary.** *New England Journal of Medicine* 2008, **359**:2617–2618.

20. Andersen CY, Rosendahl M, Byskov AG, Loft A, Ottosen C, Dueholm M, Schmidt KLT, Andersen AN, Ernst E: **Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue**. *Human Reproduction* 2008, **23**:2266–2272.

21. Dolmans M-M, Marinescu C, Saussoy P, Van Langendonckt A, Amorim C, Donnez J: **Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe.** *Blood* 2010, **116**:2908–2914.

22. Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, Piver P: **Induction of puberty by autograft of cryopreserved ovarian tissue**. *Lancet* 2012, **379**:588.

23. Kisu L, Mihara M, Banno K, Hara H, Yamamoto T, Araki J, Iida T, Hayashi Y, Moriguchi H, Aoki D: **A new surgical technique of uterine auto-transplantation in cynomolgus monkey: preliminary report about two cases**. *Arch Gynecol Obstet* 2011, **285**:129–137.

24. Scott JR, Pitkins RM, Yannone ME: **Transplantation of the Primate Uterus**. *Surgery, Gynecology & Obstetrics* 1971, **133**:197–200.

25. Mihara M, Kisu I, Hara H, Iida T, Araki J, Shim T, Narushima M, Yamamoto T, Moriguchi H, Kato Y, Tonsho M, Banno K, Aoki D, Suganuma N, Kagawa N, Takehara Y, Kato O, Koshima I: **Uterine autotransplantation in cynomolgus macaques: the first case of pregnancy and delivery**. *Human Reproduction* 2012, **27**:2332–2340.

26. Mihara M, Kisu I, Hara H, Iida T, Yamamoto T, Araki J, Hayashi Y, Moriguchi H, Narushima M, Banno K, Suganuma N, Aoki D, Koshima I: **Uterus autotransplantation in cynomolgus macaques: intraoperative evaluation of uterine blood flow using indocyanine green**. *Human Reproduction* 2011, **26**:3019–3027.

27. Johannesson L, Enskog A, Molne J, Diaz-Garcia C, Hanafy A, Dahm-Kahler P, Tekin A, Tryphonopoulos P, Morales P, Rivas K, Ruiz P, Tzakis A, Olausson M, Brannstrom M: **Preclinical report on allogeneic uterus transplantation in non-human primates**. *Human Reproduction* 2012, **28**:189–198.

28. Johannesson L, Enskog A, Dahm-Kahler P, Hanafy A, Chai DC, Mwenda JM, Diaz-Garcia

C, Olausson M, Brannstrom M: **Uterus transplantation in a non-human primate: long-term follow-up after autologous transplantation**. *Human Reproduction* 2012, **27**:1640–1648.

29. Taylor RA, Cowin PA, Cunha GR, Pera M, Trounson AO, Pedersen J, Risbridger GP: **Formation of human prostate tissue from embryonic stem cells**. *Nat Meth* 2006, **3**:179–181.

30. Oottamasathien S, Wang Y, Williams K, Franco OE, Wills ML, Thomas JC, Saba K, Sharif-Afshar A-R, Makari JH, Bhowmick NA, DeMarco RT, Hipkens S, Magnuson M, Brock JW III, Hayward SW, Pope JC IV, Matusik RJ: **Directed differentiation of embryonic stem cells into bladder tissue**. *Dev Biol* 2007, **304**:556–566.

31. Ye L, Mayberry R, Lo CY, Britt KL, Stanley EG, Elefanty AG, Gargett CE: **Generation of Human Female Reproductive Tract Epithelium from Human Embryonic Stem Cells**. *PLoS ONE* 2011, **6**:e21136.

32. Cai H, Xia X, Wang L, Liu Y, He Z, Guo Q, Xu C: **In vitro and in vivo differentiation of induced pluripotent stem cells into male germ cells**. *Biochem Biophys Res Commun* 2013, **433**:286–291.

33. Hayashi K, Ogushi S, Kurimoto K, Shimamoto S, Ohta H, Saitou M: **Offspring from Oocytes Derived from in Vitro Primordial Germ Cell-like Cells in Mice**. *Science* 2012, **338**:971–975.

34. Murtagh J, McArdle E, Gilligan E, Thornton L, Furlong F, Martin F: **Organization of mammary epithelial cells into 3D acinar structures requires glucocorticoid and JNK signaling**. *J Cell Biol* 2004, **166**:133–143.

35. Hornick JE, Duncan FE, Shea LD, Woodruff TK: **Isolated primate primordial follicles require a rigid physical environment to survive and grow in vitro**. *Human Reproduction* 2012, **27**:1801–1810.

36. Xu M, West-Farrell ER, Stouffer RL, Shea LD, Woodruff TK, Zelinski MB: **Encapsulated Three-Dimensional Culture Supports Development of Nonhuman Primate Secondary Follicles**. *Biology of Reproduction* 2009, **81**:587–594.

37. Goodman NF, Cobin RH, Ginzburg SB, Katz IA, Woode DE, American Association of Clinical Endocrinologists: **American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the diagnosis and treatment of menopause: executive summary of recommendations.** *Endocr Pract* 2011:949–954.

38. Rocca WA, Grossardt BR, Miller VM, Shuster LT, Brown RD Jr: **Premature menopause or early menopause and risk of ischemic stroke**. *Menopause* 2012, **19**:272–277.

39. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E: **Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group.** *JAMA* 1998, **280**:605–613.

40. Ryan J, Carriere I, Scali J, Dartigues JF, Tzourio C, Poncet M, Ritchie K, Ancelin ML: **Characteristics of hormone therapy, cognitive function, and dementia: The prospective**

3C Study. *Neurology* 2009, **73**:1729–1737.

41. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zorn AM, Shroyer NF, Wells JM: **Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro**. *Nature* 2011, **469**:105–109.

42. Hayashi KK, Ohta HH, Kurimoto KK, Aramaki SS, Saitou MM: **Reconstitution of the Mouse Germ Cell Specification Pathway in Culture by Pluripotent Stem Cells**. *Cell* 2011, **146**:14–14.

43. Torres J, Prieto J, Durupt FC, Broad S, Watt FM: **Efficient Differentiation of Embryonic Stem Cells into Mesodermal Precursors by BMP, Retinoic Acid and Notch Signalling**. *PLoS ONE* 2012, **7**:e36405.

44. Fortune JE, Rivera GM, Yang MY: **Follicular development: the role of the follicular microenvironment in selection of the dominant follicle**. *Animal Reproduction Science* 2004, **82-83**:109–126.

45. Eppig J, Wigglesworth K: **Development of mouse and rat oocytes in chimeric reaggregated ovaries after interspecific exchange of somatic and germ cell components.** *Biology of Reproduction* 2000, **63**:1014–1023.

46. Woodruff TK, Shea LD: **The Role of the Extracellular Matrix in Ovarian Follicle Development**. *Reproductive Sciences* 2007, **14**:6–10.

47. Kidder GM, Mhawi AA: **Gap junctions and ovarian folliculogenesis.** *Reproduction* 2002, **123**:613–620.

48. Yamada S, Fujiwara H, Honda T, Higuchi T, Nakayama T, Inoue T, Maeda M, Fujii S: **Human granulosa cells express integrin alpha2 and collagen type IV: possible involvement of collagen type IV in granulosa cell luteinization.** *Molecular Human Reproduction* 1999, **5**:607–617.

49. Iwahashi M, Muragaki Y, Ooshima A, Nakano R: **Type VI collagen expression during growth of human ovarian follicles.** *Fertil Steril* 2000, **74**:343–347.

50. Stewart CA, Behringer RR: **Mouse Oviduct Development**. In *Results and Problems in Cell Differentiation*. *Volume 55*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012:247–262. [*Results and Problems in Cell Differentiation*]

51. Arias EB, Verhage HG, Jaffe RC: **Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein.** *Biology of Reproduction* 1994, **51**:685–694.

52. Newbold RR, Tyrey S, Haney AF, McLachlan JA: **Developmentally arrested oviduct: a structural and functional defect in mice following prenatal exposure to diethylstilbestrol.** *Teratology* 1983, **27**:417–426.

53. Gonzalez G, Behringer RR: **Diceris required for female reproductive tract development and fertility in the mouse**. *Mol Reprod Dev* 2009, **76**:678–688.

54. King SM, Quartuccio S, Hilliard TS, Inoue K, Burdette JE: **Alginate hydrogels for three-dimensional organ culture of ovaries and oviducts.** *J Vis Exp* 2011.

55. Yamanouchi H, Umezu T, Tomooka Y: **Reconstruction of Oviduct and Demonstration of Epithelial Fate Determination in Mice**. *Biology of Reproduction* 2010, **82**:528–533.

56. Gargett CE, Chan RWS, Schwab KE: **Hormone and growth factor signaling in endometrial renewal: Role of stem/progenitor cells**. *Mol Cell Endocrinol* 2008, **288**:22–29.

57. Slayden ODO, Brenner RMR: **Hormonal regulation and localization of estrogen, progestin and androgen receptors in the endometrium of nonhuman primates: effects of progesterone receptor antagonists.** *Arch Histol Cytol* 2004, **67**:393–409.

58. Ponnampalam AP, Weston GC, Trajstman AC, Susil B, Rogers PAW: **Molecular classification of human endometrial cycle stages by transcriptional profiling**. *Molecular Human Reproduction* 2004, **10**:879–893.

59. Gargett CE, Schwab KE, Zillwood RM, Nguyen HPT, Wu D: **Isolation and Culture of Epithelial Progenitors and Mesenchymal Stem Cells from Human Endometrium**. *Biology of Reproduction* 2009, **80**:1136–1145.

60. Chan RWS, Gargett CE: **Identification of Label-Retaining Cells in Mouse Endometrium**. *STEM CELLS* 2006, **24**:1529–1538.

61. Chan RWS, Schwab KE, Gargett CE: **Clonogenicity of Human Endometrial Epithelial and Stromal Cells**. *Biology of Reproduction* 2004, **70**:1738–1750.

62. Rossi MJ, Chegini N, Masterson BJ: **Presence of epidermal growth factor, platelet-derived growth factor, and their receptors in human myometrial tissue and smooth muscle cells: their action in smooth muscle cells in vitro.** *Endocrinology* 1992, **130**:1716–1727.

63. Yin P, Roqueiro D, Huang L, Owen JK, Xie A, Navarro A, Monsivais D, Coon JS V, Kim JJ, Dai Y, Bulun SE: **Genome-Wide Progesterone Receptor Binding: Cell Type-Specific and Shared Mechanisms in T47D Breast Cancer Cells and Primary Leiomyoma Cells**. *PLoS ONE* 2012, **7**:e29021.

64. Ono M, Maruyama T, Masuda H, Kajitani T, Nagashima T, Arase T, Ito M, Ohta K, Uchida H, Asada H: **Side population in human uterine myometrium displays phenotypic and functional characteristics of myometrial stem cells**. *Proc Natl Acad Sci USA* 2007, **104**:18700–18705.

65. Jasinska A, Han V, Fazleabas AT, Kim J: **Induction of insulin-like growth factor binding protein-1 expression in baboon endometrial stromal cells by cells of trophoblast origin**. *Journal of the Society for Gynecologic Investigation* 2004, **11**:399–405.

66. Lehker MW, Sweeney D: **Trichomonad invasion of the mucous layer requires adhesins, mucinases, and motility.** *Sex Transm Infect* 1999, **75**:231–238.

67. Klumpp DJ, Forrestal SG, Karr JE, Mudge CS, Anderson BE, Schaeffer AJ: **Epithelial Differentiation Promotes the Adherence of Type 1–Piliated Escherichia coli to Human**

Vaginal Cells. *The Journal of Infectious Diseases* 2002, **186**:1631–1638.

68. Patton DL, Thwin SS, Meier A, Hooton TM, Stapleton AE, Eschenbach DA: **Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle**. *Am J Obstet Gynecol* 2000, **183**:967–973.

69. Castelo-Branco C, Cancelo MJ, Villero J, Nohales F, Juliá MD: **Management of post-menopausal vaginal atrophy and atrophic vaginitis**. *Maturitas* 2005, **52**:46–52.

70. Buchanan DL, Kurita T, Taylor JA, Lubahn DB, Cunha GR, Cooke PS: **Role of stromal and epithelial estrogen receptors in vaginal epithelial proliferation, stratification, and cornification.** *Endocrinology* 1998, **139**:4345–4352.

71. Cooke PS, Buchanan DL, Young P, Setiawan T, Brody J, Korach KS, Taylor J, Lubahn DB, Cunha GR: **Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium.** *Proc Natl Acad Sci USA* 1997, **94**:6535–6540.

72. Winuthayanon W, Hewitt SC, Orvis GD, Behringer RR, Korach KS: **Uterine epithelial estrogen receptor α is dispensable for proliferation but essential for complete biological and biochemical responses**. *Proc Natl Acad Sci USA* 2010, **107**:19272–19277.

73. Kurita T, Lee KJ, Cooke PS, Taylor JA, Lubahn DB, Cunha GR: **Paracrine regulation of epithelial progesterone receptor by estradiol in the mouse female reproductive tract**. *Biology of Reproduction* 2000, **62**:821–830.

74. Kurita T, Cunha GR, Robboy SJ, Mills AA, Medina RT: **Differential expression of p63 isoforms in female reproductive organs**. *Mech Dev* 2005, **122**:1043–1055.

75. Yang A, Kaghad M, Wang Y, Gillett E, Fleming M, Dotsch V, Andrews N, Caput D, McKeon F: **p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities**. *Mol Cell* 1998, **2**:305–316.

76. Kurita T, Cooke PS, Cunha GR: **Epithelial-stromal tissue interaction in paramesonephric (Müllerian) epithelial differentiation**. *Dev Biol* 2001, **240**:194–211.

Figure 1: **Changes in the reproductive tract throughout menstrual cycle**. Follicle stimulating hormone (FSH) from the pituitary promotes follicle growth in the ovary. These follicles produce estrogen (E). In response to E, the functionalis layer of the uterine epithelium, and the stratified layer of the vaginal epithelium thickens as the infindibulum of the fallopian tube comes in contact with the ovary. The luteinizing hormone (LH) surge from the pituitary causes the dominant follicle to ovulate. The remaining follicular cells develop into a corpus luteum that produces E and progesterone (P). P in turn promotes more proliferation within the uterus and vagina, and cornification in the vagina. The uterotubal junction of the fallopian tube widens to allow the passage of the ovulated oocyte or fertilized embryo. Reduced E and P levels induce atrophy of vaginal epithelium and menstruation of the uterine functionalis layer.

Figure 2: **Example of iPSC-derived tissues for use in tissue repair or drug discovery.** (A) In this example uterine tissue is transplanted to remedy frequent miscarriages due to uterine endometrium malformation and (B) a similar uterine tissue mimic is used to screen potential drugs for treatment of frequent miscarriages.

Figure 1

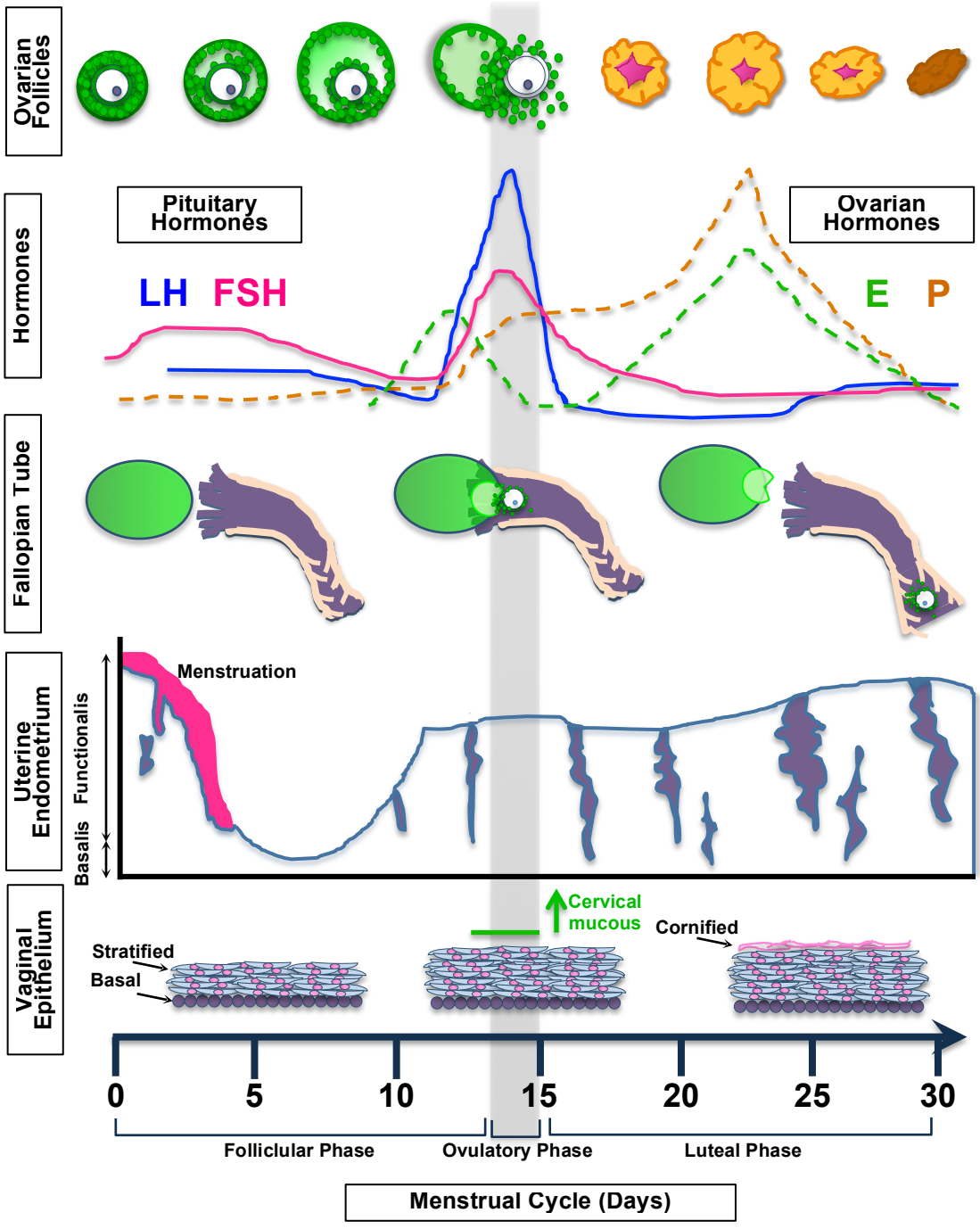
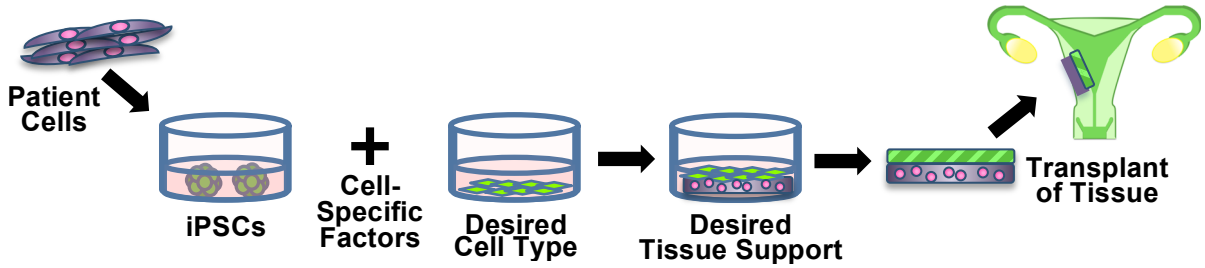

Ovarian Follicles
Hormones
Pituitary Hormones
Ovarian Hormones
LH FSH
E P
Fallopian Tube
Uterine Endometrium
Menstruation
Basalis Functionalis
Vaginal Epithelium
Cervical mucous
Cornified
Stratified
Basal
0 5 10 15 20 25 30
Follicular Phase
Ovulatory Phase
Luteal Phase
Menstrual Cycle (Days)

Figure 2

A. Restore uterine function.



B. Test drug efficacy.