Endometrial reconstruction from stem cells

Caroline E. Gargett, Ph.D.,[a,b] and Louie Ye, Ph.D.[a,b]

[a] The Ritchie Centre, Monash Institute of Medical Research, and [b] Department of Obstetrics and Gynaecology, Monash University, Monash Medical Centre, Clayton, Victoria, Australia

Adult stem cells have been identified in the highly regenerative human endometrium on the basis of their functional attributes. They can reconstruct endometrial tissue in vivo suggesting their possible use in treating disorders associated with inadequate endometrium. The identification of specific markers for endometrial mesenchymal stem cells and candidate markers for epithelial progenitor cells enables the potential use of endometrial stem/progenitor cells in reconstructing endometrial tissue in Asherman syndrome and intrauterine adhesions. (Fertil Steril® 2012;98:11–20. ©2012 by American Society for Reproductive Medicine.)

Key Words: Endometrium, adult stem cells, mesenchymal stem cells, epithelial progenitor cells, endometrial regeneration, Asherman syndrome

Human endometrium is a highly regenerative tissue, undergoing more than 400 cycles of growth, differentiation, and shedding during a woman's reproductive years (1, 2). Under the influence of increasing levels of circulating estrogen (E) during the proliferative phase of the menstrual cycle, the endometrium grows 4–10 mm each cycle (3). Endometrial regeneration also occurs after parturition, endometrial resection, and in postmenopausal women taking E replacement therapy (2). This level of tissue regeneration is similar to that of hemopoietic tissue, intestinal epithelium, and the epidermis. In these continuously regenerating tissues, adult stem cells maintain cellular production. It was originally hypothesized that adult stem or progenitor cells are responsible for the cyclic regeneration of the endometrial functionalis layer each month that these adult stem cells reside in the basalis layer, and are present in the atrophic endometrium of postmenopausal women (2). However, as endometrial stem/progenitor cells are being identified with specific markers (4, 5), it has become apparent that they may reside in both functionalis and basalis layers (6). Endometrial stem/progenitor cells originating from the functionalis and shed in menstrual debris may also contribute to eutopic endometrial regeneration from those fragments remaining within the uterine cavity (7, 8).

ENDOMETRIAL STEM/PROGENITOR CELLS AND DISORDERS ASSOCIATED WITH INADEQUATE ENDOMETRIUM

If endometrial stem/progenitor cells are responsible for regenerating endometrium each menstrual cycle, then their diminished numbers and/or function should result in a thin (<7 mm), dysregulated endometrium, incapable of supporting embryo implantation (9). Similarly, in scarred endometrium or endometrium obliterated with adhesions, functional endometrial stem/progenitor cells likely are lacking, as scant functional endometrium is present in the uterine cavity. In Asherman syndrome there is complete obliteration of the uterine cavity with adhesions resulting in amenorrhea and infertility (10). Intrauterine adhesion (IUA) comprise a less severe condition involving partial replacement of the endometrial surfaces with fibrotic tissue (11). Symptoms relate to the degree and location of IUA, and include irregular bleeding ranging from hypomenorrhea to amenorrhea, infertility, and pregnancy loss. Between 2% and 22% of infertile women have Asherman syndrome and IUA (10, 11). Trauma to the endometrium, including the basalis and underlying myometrium may result from postpartum curettage, spontaneous miscarriage, termination of pregnancy, or endometrial ablation procedures (10–12). In the postpartum period, low E levels hinder endometrial regeneration, although endometrial stem/progenitor cells survive in the absence of E (13). Infection and inflammation may inhibit regeneration of traumatized endometrium through damage to the stem/progenitor cells by effector

Received March 2, 2012; revised May 2, 2012; accepted May 3, 2012; published online May 30, 2012.
C.E.G. has nothing to disclose. L.Y. has nothing to disclose.
Supported by Australian National Health and Medical Research Council grants (545992, 1021126, 1021127) and R.D. Wright Career Development Award 465121 (C.E.G.), Monash IVF Foundation (C.E.G.), the Victorian Government's Operational Infrastructure Support Program and an Australian Postgraduate Award (L.Y.).
Reprint requests: Caroline E. Gargett, Ph.D., The Ritchie Centre, Monash Institute for Medical Research, PO Box 5418, Clayton, Victoria 3168, Australia (E-mail: Caroline.Gargett@monash.edu).

Fertility and Sterility® Vol. 98, No. 1, July 2012 0015-0282/$36.00

doi:10.1016/j.fertnstert.2012.05.004

molecules, which also contribute to the deposition of fibrotic tissue.

After menstruation or parturition the endometrial surface epithelium repairs without scarring (14) in the absence of E (15). In other mucosal surfaces (e.g., intestine), regeneration of damaged mucosa occurs without scarring unless the injury involves the deep muscularis layers. Deep trauma involving the basalis and underlying myometrium may explain the failure of the endometrial functional layer to regenerate in Asherman syndrome and IUA (11, 12). Resident endometrial stem/progenitor cells may be damaged or lost, particularly if the basalis layer is involved. In these disorders, dense fibrotic areas of the uterine cavity show little or no endometrium (16). Where present, the endometrium is thin and atrophic with inactive glands and scant poorly vascularised stroma (10).

ADULT STEM CELLS IN HUMAN ENDOMETRIUM

Clonogenic Cells

The first evidence for adult stem cells in human endometrium came from cell cloning studies that identified small populations of colony-forming unit (CFU) activity in freshly isolated and purified epithelial ($EpCAM^+$) and stromal ($EpCAM^-$) cells (0.22% and 1.25%, respectively) (17, 18). Clones of different sizes were noted for both cell types. The single cell-derived large CFU have self-renewal activity, demonstrated by serial cloning in vitro, and high proliferative potential, undergoing 30–34 population doublings (19). Single large epithelial CFU differentiated into large $cytokeratin^+$ gland-like structures when cultured in Matrigel (Becton Dickinson). Large single stromal CFU were multipotent, differentiating into smooth muscle cells, adipocytes, osteoblasts, and chondrocytes when cultured in appropriate induction media. Large stromal CFU had a typical mesenchymal stem/stromal cell (MSC) phenotype (20). Small CFU had limited expansion capacity and their differentiation could not be examined. Self renewal, differentiation, and high proliferative capacity are key functional properties of adult stem cells (2, 21). It was concluded that human endometrium contained small populations of epithelial progenitor cells and MSC-like cells (19). Cultured endometrial stromal cells also differentiate into mesodermal lineages and lineages of ectodermal and endodermal origin (22–25), indicating that endometrial stromal cells have considerable plasticity.

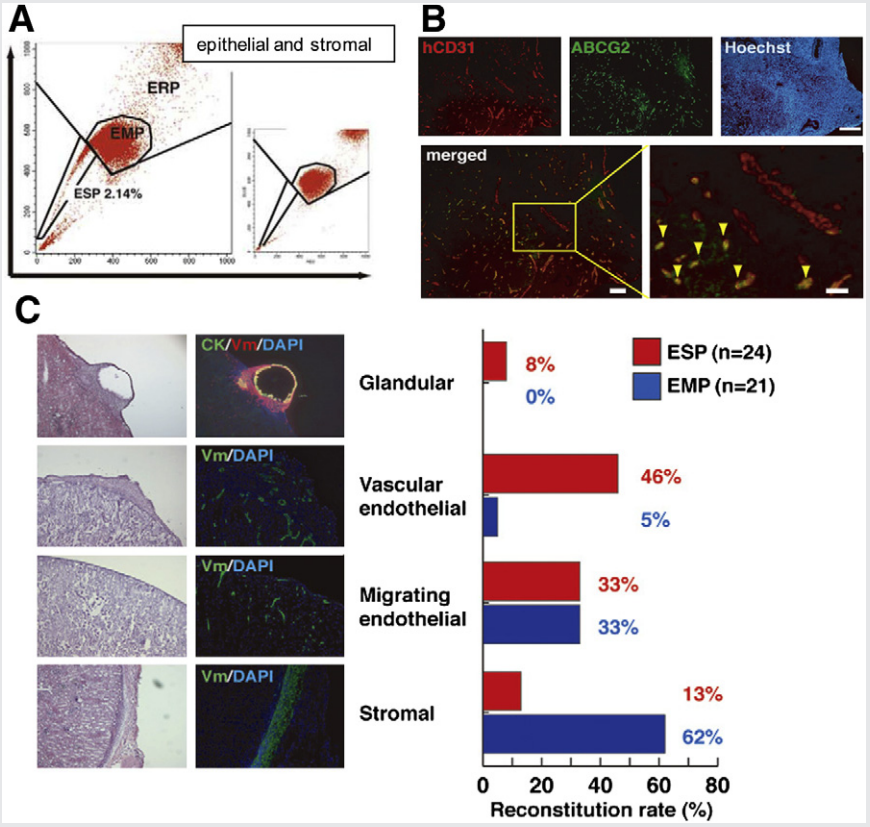
Side Population Cells

One way of identifying adult stem cell activity is to examine side population (SP) cells, which have capacity to rapidly efflux a vital DNA-binding dye, Hoechst 33342. The SP cells form a distinct side population using dual-color flow cytometry (26, 27). The SP cells (0–5%) were identified in freshly isolated (5, 28, 29) and short-term cultures (30) of human endometrial cells. The percentage of SP cells was highly variable between subjects, although higher numbers were found in menstrual (30) and proliferative (5, 29) stages. Freshly sorted human endometrial SP cells showed little clonogenic growth in culture as most were quiescent (G_0 phase of the cell cycle, 85%), a feature of adult stem cells. In contrast cultured SP cells were primarily in G_1 and G_1/M/S phases and showed enhanced clonogenicity (29). Intermediate levels of telomerase are expressed by freshly isolated endometrial SP cells (28). Human endometrial SP cells are a mixed population, comprising predominantly of endothelial cells (5), but also of epithelial and stromal cells (28, 29). Both epithelial and stromal SP cells have characteristics of MSC (28). Immunostaining with the SP marker ABCG2 revealed $ABCG2^+$ cells as $CD31^+$ endothelial cells lining blood vessels in both the functionalis and basalis layers (5, 29). The identification of endometrial SP cells provides further support for adult stem cell populations residing in human endometrium, but the exact nature of this population remains unclear.

Tissue-reconstituting Cells

Tissue reconstitution in vivo by xenografting candidate adult stem cell populations is the most rigorous proof of adult stem cell activity (2). Transplantation of fully dissociated unfractionated human endometrial epithelial and stromal cell suspensions directly beneath the kidney capsule of ovariectomized and E-supplemented NOD/Shi-*scid*/IL-2Rγnull (NOG) mice reconstructed well-organized endometrial layers of functional endometrium comprising $cytokeratin^+CD9^+$ glandular structures and $CD10^+CD13^+$ stroma. The reconstructed human endometrium responded to cyclic sex steroid hormones, forming glands, and decidualized stroma when E and P were administered, and large blood-filled cysts after hormonal withdrawal, reminiscent of menstruation (31). Freshly isolated human endometrial SP cells, but not non-SP cells, reconstituted endometrial tissue components and entire endometrium when transplanted under the kidney capsule of immunocompromised mice (5, 32). In one study, the composition of the endometrial constructs was predominantly vasculature and migrating endothelial cells (80%), which expressed E receptor-β (ERβ), with minor endometrial stromal (13%) and glandular (8%) components (Fig. 1) (5). In a second study, endometrial tissue was reconstructed from epithelial and stromal SP cells, which immunostained for stroma (vimentin) and epithelium (CD9), but not endothelium (CD31) (32). Organized endometrial glands were not readily apparent, possibly due to prolonged clonal culture of the SP cells before to xenografting. The xenografted tissue did not express ERs, although some cells expressed P receptors (32). These important studies indicate that human endometrial cell subpopulations can reconstruct endometrial tissue in vivo and could potentially initiate ectopic endometriosis lesions. The nature of the transplanted SP cells needs to be clarified with more robust markers. Administration of the SP population to patients with Asherman syndrome has the potential to reconstruct endometrium (Fig. 2). It is also possible that endogenous SP cells in inadequate endometrium could be stimulated to regenerate thick, hormone-responsive endometrium capable of supporting implantation, once more is known about activating this candidate adult stem cell population.

FIGURE 1



Side population cells isolated from human endometrial cell suspensions. (A) Flow cytometric analysis of endometrial cells stained with Hoechst 33342 showing endometrial side population (ESP) (*left*) using 50 μM reserpine (*right*) to define the electronic gates for the ESP fraction. ERP = endometrial replicative population; EMP = endometrial main population (nonside population cells). (B) Immunofluorescence images of human endometrium stained with antibodies against hCD31 and ABCG2. *Yellow arrowheads* indicate $hCD31^{+}ABCG2^{+}$ endothelial cells. (C) Hematoxylin and eosin (H & E)-stained and immunofluorescence images of ESP- or EMP-initiated xenografts stained with antibodies against CK (cytokeratin) and Vm (vimentin). The histogram shows the frequency of the ESP- or EMP-initiated xenografts in mice, which predominantly possessed human endometrium with glandular structures, vessel-like structures consisting of endothelial cells, migrating endothelial cells, or stromal cell components. Bars = 200 μm (B, *upper*), 100 μm (B, *lower left*), 50 μm (B, *lower right*). (Reproduced from Masuda et al. PLoS ONE 5 (4) e10387 (5).

Gargett. Endometrial reconstruction from stem cells. Fertil Steril 2012.

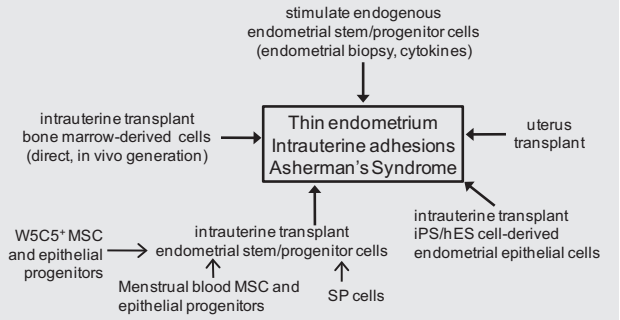
Menstrual Blood Stem/Progenitor Cells

Stromal cells have been cultured from menstrual blood in a manner similar to bone marrow-derived MSC (33–36), suggesting that endometrial MSC are shed during menstruation (6). Epithelial cells have not been detected or have been overgrown by stromal cells (37), suggesting that epithelial progenitors may not be shed during menstruation and more likely reside in the basalis. Cultured menstrual blood cells appear fibroblastic, have substantial proliferative capacity (34–36), yet maintaining a stable karyotype (35). They have telomerase activity and expressed human telomerase reverse transcriptase (hTERT), as well as similar phenotypic markers as endometrial MSC (33–36). They have broad differentiation capacity, producing all mesodermal lineages including skeletal and cardiac muscle cells (33, 34) and neural lineages (36). Menstrual blood MSC express major hisotcompatibility complex (MHC) class I but not class II molecules (33, 36), are of low immunogenicity when transplanted into immunocompetant mice, and may have immunomodulatory properties (38). Our own unpublished studies have also demonstrated that menstrual blood contains clonogenic, multipotent MSC (39). Shedding of endometrial MSC during menstruation suggests they may have a key role in initiating endometriotic lesions (2, 40, 41).

BONE MARROW-DERIVED STEM CELLS IN ENDOMETRIUM

Bone marrow-derived hemopoietic stem cells, MSC, and endothelial progenitor cells circulate in very low numbers, homing to sites of tissue damage. They incorporate into organs, contributing to angiogenesis, or transdifferentiate into the cells of the new tissue in which they reside (42, 43). Circulating myeloid cells also integrate into damaged tissues and transdifferentiate into host tissues, including endometrium (42, 44). Evidence from human and mouse

FIGURE 2



Schematic showing possible stem/progenitor cell-based therapies to reconstruct inadequate human endometrium. hES = human embryonic stem cell; iPS = induced pluripotent stem cell; MSC = mesenchymal stem/stromal cell; SP = side population.

Gargett. Endometrial reconstruction from stem cells. Fertil Steril 2012.

studies suggest that bone marrow-derived cells incorporate into the endometrium in low numbers and transdifferentiate into endometrial epithelial, stromal, and endothelial cells (45–50). In both single human leukocyte antigen (HLA-antigen) and gender-mismatched human bone marrow transplant recipients, 1%–52% of endometrial epithelial, stromal, or vascular cells were of donor origin (45–48). In the majority of cases the percentage of donor-derived endometrial cells was low (~1%–8%). Generally the incorporated bone marrow cells did not appear to undergo clonal expansion or contribute to new endometrial tissue in an ongoing manner, suggesting that the incorporating cells may be myeloid cells rather than bone marrow stem cells. This was confirmed in mouse bone marrow transplant studies (reviewed in Gargett and Masuda (6) and Du and Taylor (43)) and in a human study of female recipients of male donor bone marrow transplants, in which male XY^+ endometrial cells were identified in the non-SP rather than SP cells (47). However, human bone marrow-derived MSC can differentiate into decidualized endometrial stromal fibroblasts in vitro as shown by gene profiling (51).

Circulating Endothelial Progenitor Cells and Vascularization During Endometrial Reconstruction

Reconstruction of endometrial tissue during the menstrual cycle or after parturition requires angiogenesis to support the rapidly growing tissue. Endometrial neovascularization occurs by vessel elongation, intussusception, and incorporation of circulating endothelial progenitor cells (EPC) (52). Circulating EPC have been detected in women during the menstrual cycle by flow cytometry, peaking in the proliferative (53), ovulatory (54), or luteal (49) stages. These differences are likely due to methodology, particularly the combination of surface markers used in the identification of peripheral blood EPC. Human circulating EPC express both ERα and ERβ (54), whereas mouse EPC only express ERα (49). Estrogen promoted the migration of mouse circulating EPC (49). Gender mismatched bone marrow transplantation in mice and women has demonstrated that circulating EPC from male donors contributed to ~10% of endothelial cells in the endometrial vasculature (48, 49).

The vascular location of endometrial SP cells and perivascular endometrial MSC location (discussed later) suggests that the endometrial stem cell niche is associated with blood vessels, a convenient portal of entry for bone marrow-derived cells. The overall evidence indicates that resident adult stem cells in the human endometrium are likely responsible for endometrial reconstruction each month, and in regenerating atrophic endometrium in women who have undergone chemotherapy or radiotherapy, or are postmenopausal and taking hormones.

RECONSTRUCTING ENDOMETRIUM FROM ENDOMETRIAL STEM/PROGENITOR CELLS

The substantial evidence indicating that adult stem cell populations exist in human endometrium suggests that it should be possible to activate endogenous endometrial stem/progenitor cells in cases of thin dysfunctional or atrophic endometrium. It should also be possible to transplant endometrial stem/progenitor cells into the uterine cavity in Asherman syndrome or severe cases of IUA as a cell-based therapy for regenerating endometrium. Furthermore, endometrial stem/progenitor cells could be used for regenerative medicine applications other than endometrial reconstruction. To target endometrial stem/progenitor cells as cell-based therapies, it is necessary to identify them with cell-specific surface markers that allow their prospective isolation.

Markers of Human Endometrial Epithelial Progenitor Cells

Currently there are no publications identifying markers for human or mouse endometrial epithelial progenitor cells, although several candidates are under investigation in our and another laboratory. In these ongoing studies, stringent assays assessing key adult stem cell functions are being undertaken to verify these candidates.

Markers of Human Endometrial MSC

Specific markers of human endometrial MSC have been identified. Coexpression of CD146 and platelet-derived growth factor receptor beta (PDGF-Rβ) isolates the majority of clonogenic stromal cells (CFU), enriching for endometrial MSC 8- to 10-fold versus unfractionated endometrial stromal cells (4). These $CD146^+PDGF\text{-}R\beta^+$ cells are multipotent, differentiating into typical mesodermal lineages (adipogenic, myogenic, osteogenic, chondrogenic), have a typical MSC surface phenotype, and are located perivascularly in both the functionalis and basalis layers, suggesting that they are pericytes (4). A separate study examining the gene profile of the same human endometrial populations ($CD146^+PDGF\text{-}R\beta^+$, endometrial MSC; $CD146^-PDGF\text{-}R\beta^+$, fibroblast; $CD146^-PDGF\text{-}R\beta^-$, endothelial cell) sorted by flow cytometry, confirmed that the double positive population were clonogenic, differentiated into adipocytes and were located perivascularly (55). The

gene profile of this CD146$^+$PDGF-Rβ^+ population indicated that these cells expressed pericyte markers, and genes associated with angiogenesis/vasculogenesis, steroid hormone/hypoxia responses, inflammation, immunomodulation, and signaling pathways associated with MSC self renewal and multipotency (Notch, transforming growth factor-β [TGF-β], insulin-like growth factor [IGF], Hedgehog, G protein-coupled receptors). Further analysis showed this double positive population clustered with endometrial fibroblasts and was distinct from endothelial cells, indicating that its genetic program was predictive of its differentiated lineage, the stromal fibroblast (55). The gene profile of human endometrial CD146$^+$PDGF-Rβ^+ cells suggest they have immunomodulatory potential, low immunogenicity (low MHC class I gene expression) similar to menstrual blood-derived MSC-like cells (38) and other sources of MSC (55). Their perivascular location in both functionalis and basalis indicates that endometrial MSC are shed in menstrual blood and may have a key role in establishing endometriosis implants, indicating their capacity to reconstruct endometrium in ectopic sites (6).

Screening human endometrial tissue and cells with perivascular markers has identified a single novel marker, W5C5, which purifies endometrial MSC, with all the in vitro properties of MSC (56). When transplanted under the kidney capsule into immunocompromised NOD/Scid/γ (NSG) mice W5C5$^+$ cells reconstruct human stromal tissue. The purification of human endometrial MSC has been simplified using this single W5C5 marker by allowing magnetic bead sorting, significantly increasing the yield (56). Human endometrium is the only source of tissue-derived MSC that can be isolated with minimal invasiveness without the need for an anesthetic. The W5C5$^+$ cells are located in the functionalis (and basalis) and can be retrieved by endometrial biopsy, as MSC-like cells have been demonstrated in endometrial biopsies (55, 57). In summary, W5C5$^+$ and CD146$^+$PDGF-Rβ^+ are essentially the same subpopulation of endometrial stromal cells (56) that have MSC properties and phenotype as defined by the International Society for Cellular Therapy (20). Both sets of markers contain all the clonogenic stromal cells including those initiating large stromal clones. Human W5C5$^+$ and SP cells reconstitute endometrial stromal tissue in vivo, although SP cells also produce endothelium and glands suggesting wider potential of a single adult stem cell population or that a mix of stem/progenitor cell types reconstruct these endometrial tissue components. Therefore the relationship between human endometrial SP cells and MSC-like cells defined by the markers has not yet been investigated and is currently unclear.

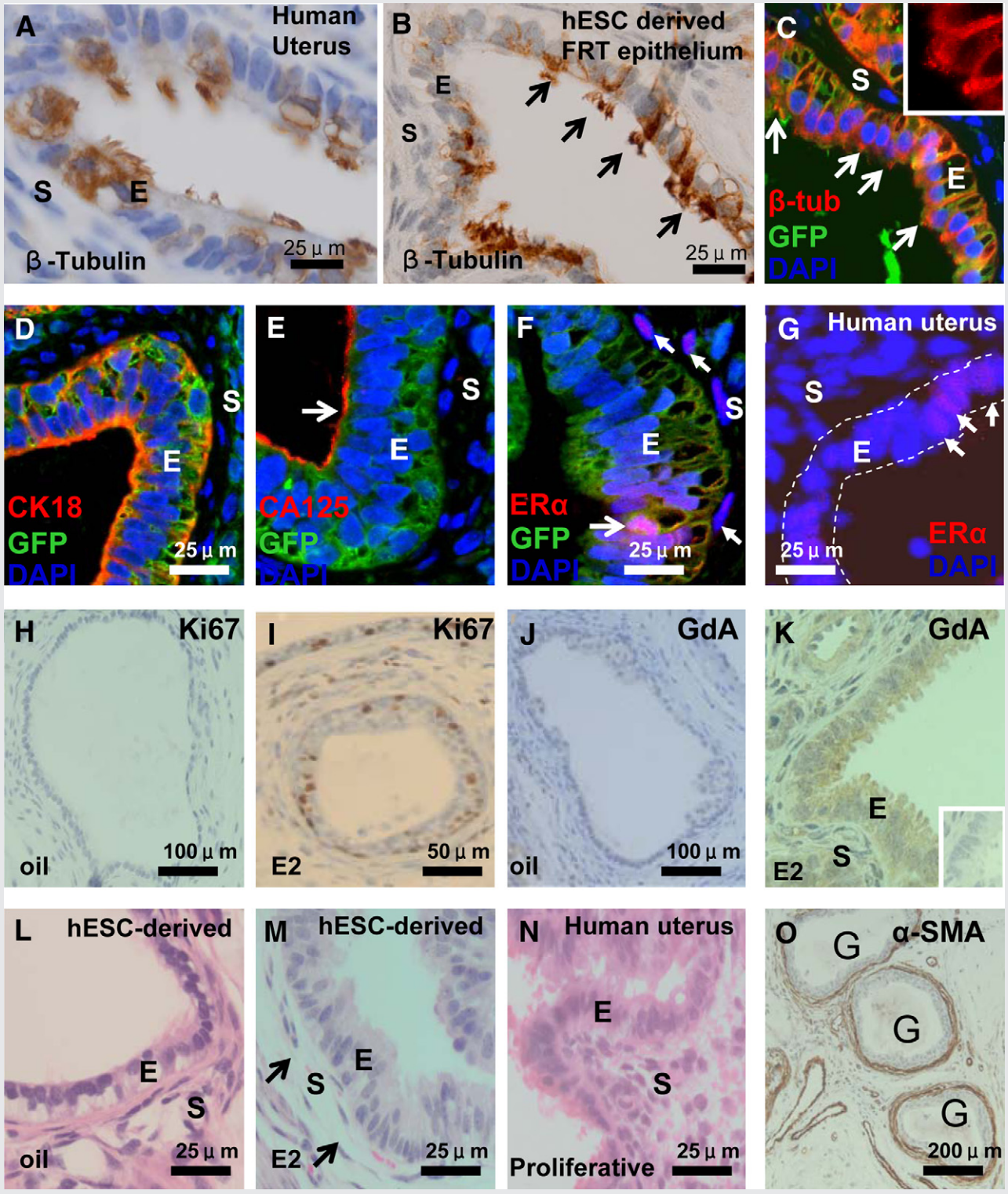
GENERATING HUMAN ENDOMETRIUM FROM HUMAN EMBRYONIC STEM CELLS

Embryonic stem cells are pluripotent stem cells that have potential to differentiate into any cell type in the body. Since isolation of human embryonic stem cells (ESCs) in 1998 (58), much research has focused on directing their differentiation in vitro into clinically relevant cell types for potential use in regenerative medicine (59). A major concern for transplantation of human ESC derivatives into the human body is that the transplanted cell population may include undifferentiated human ESCs that may differentiate uncontrollably into unwanted cell types, including tumors. Despite successful laboratory induction of human ESC toward lineage-specific cell types, there have only been two Food and Drug Administration-approved trials in the United States involving transplantation of human ESC-derived oligodendrocyte progenitor cells and human ESC-derived retinal pigment epithelial cells (60). The former has been halted due to financial reasons, whereas the latter showed engraftment and a promising safety profile after 4 months.

For many vital organs in the human body, transplantation of human ESC-derived cells may be an option to regenerate damaged tissue, although the discovery of induced pluripotent stem (iPS) cells (61) will more likely be the source of patient-specific pluripotent cells for regenerative medicine. The use of iPS cells overcomes immunologic barriers posed by human ESC. The iPS cells generated from fibroblasts from women suffering with Asherman syndrome may provide an avenue for generating endometrial epithelial and stromal cells that could be transplanted into the uterine cavity for reconstructing their endometrium.

The critical first step in directing the differentiation of pluripotent cells (human ESC, iPS cells) to functional hormone-responsive endometrial epithelial cells is to emulate the developmental stages leading to Müllerian duct development during embryogenesis. In a proof-of-principle study, human endometrial-like epithelium was generated from undifferentiated pluripotent human ESCs using a two-step differentiation strategy that mimicked specific stages of embryogenesis and fetal development (62). This study capitalized on the power of tissue recombination to study the capacity of undifferentiated mouse neonatal uterine mesenchyme (63) to induce differentiation of human ESC into Müllerian duct epithelium. An advantage of using mouse rather than human uterine mesenchyme is that it can be readily harvested from neonatal mice, as unlike in humans, Müllerian duct differentiation is a postnatal rather than a fetal event. Mouse uterine mesenchyme signals to human endometrial epithelium in tissue recombinants (64) and species-specific markers can be exploited to determine the role of uterine mesenchyme-induced differentiation of the human ESC in this model (62, 65). An initial in vitro induction step that differentiates human ESCs using growth factors (Bone morphogenetic protein 4, BMP4 and activin A) and the inductive neonatal mouse uterine mesenchyme produced a homogenous population of Müllerian precursors (intermediate mesoderm) (62). These tissue recombinants were then xenografted in vivo for further differentiation. A comprehensive analysis of the human ESC-derived epithelium during graft development revealed human ESC-derived epithelial cells expressing characteristic Müllerian markers including LIM homeobox 1 (LIM1), Paired box gene 2 (PAX2), and Homeobox A10 (HOXA10), and colocalization of cytoskeletal markers, vimentin and cytokeratin. Over time the epithelial derivatives demonstrated characteristic features of mature human endometrial epithelium (CA-125, ERα expression, and the presence of β-tubulin$^+$ ciliated cells) (Fig. 3) (62, 65). Functional analysis showed that the human

FIGURE 3



Characterization of human embryonic stem cell (hESC)-derived endometrial-like epithelium. Recombinant (hESC/mouse neonatal uterine mesenchyme) 8-week xenograft showing β-tubulin expression in (A) ciliated columnar epithelia of human proliferative endometrium and (B) in hESC-derived endometrial-like ciliated, columnar epithelium (→, cilia). (C) The hESC-derived endometrial-like epithelium (Green fluorescent protein (GFP+), *green*) where β-tubulin (*yellow orange*) is coexpressed on cell surface (inset high power view of the cilia). Human cells are GFP^+ and derived from differentiated GFP-labeled hESC. Adjacent mouse uterine stromal cells are unstained (GFP^-). The hESC-derived endometrial-like epithelium (GFP^+) colocalized with (D) cytoplasmic CK18, (E) CA-125 on epithelial surface (open →), and (F) nuclear estrogen receptor-

Gargett. Endometrial reconstruction from stem cells. Fertil Steril 2012.

FIGURE 3 Continued

α (ERα) (*pink*). Weak ERα expression was present in mouse uterine stromal cells (filled →) and in (G) human proliferative endometrial gland (→), *dotted line* indicates epithelium. The Ki67 expression in hESC-derived endometrial-like epithelium (H) before and (I) after estrogen (E) injections showing E-induced epithelial cell proliferation. Cytoplasmic expression of glycodelin A (GdA) in hESC-derived endometrial-like epithelium (J) without and with (K) E injections showing E induction of GdA. Hematoxylin and eosin (H & E) stain of hESC-derived endometrial-like epithelium lined by simple columnar cells (L) without and (M) with E treatment, showing E-induced increase in epithelial height and stromal edema (→), classic hormonal responses of (N) endometrial epithelium. (O) Three hESC-derived endometrial epithelial-like structures surrounded by α-SMA positive cells. Inset in (K) is mouse IgG1 negative control. α-SMA = α-smooth muscle actin; CA-125 = cancer antigen 125; E = epithelium; E2 = estrogen; G = gland; GdA = glycodelin A; S = stroma. (Reproduced from Ye et al. PLoS ONE 6 (6) e21136 (62).

Gargett. Endometrial reconstruction from stem cells. Fertil Steril 2012.

ESC-derived epithelium proliferated and expressed glycodelin A in response to exogenous E (Fig. 3). Collectively, the human ESC-derived epithelium resembled adult human endometrial epithelium. These studies underscore the ability of mouse endometrial mesenchyme to guide the differentiation of human ESC. It is likely that patient-specific iPS cells could be differentiated into adult human endometrial epithelium in vitro using a similar strategy using a patient's own endometrial stromal cells to form Müllerian derivatives for transplantation into patients with Asherman syndrome or IUA (Fig. 2). However, it may be some time before pluripotent stem cell-derived endometrial epithelial precursors are available for clinical use.

RECONSTRUCTING OTHER TISSUES FROM ENDOMETRIAL AND MENSTRUAL BLOOD STEM/PROGENITOR CELLS

Bone marrow-derived MSC are an attractive source of cells for regenerative medicine (66). Intravenously infused MSC home to sites of tissue damage, secreting factors that promote tissue repair in a paracrine manner without engraftment (67). These factors are angiogenic, antifibrotic, antiapoptotic, immunosuppressive, and induce mitosis of resident progenitor cells promoting tissue repair (68). The regenerative potential of menstrual blood MSC has been demonstrated in an animal model of Duchenne muscular dystrophy where they incorporated into atrophied skeletal muscle fibers and contributed to muscle repair (33). Menstrual blood MSC have improved critical limb ischemia induced by femoral artery ligation in a mouse model (38). Clinical grade menstrual blood-derived endometrial regenerative cells were administered by IV and intrathecal routes in a small phase 1 clinical trial of four patients with multiple sclerosis (69). After 1 year no immunologic reactions or other adverse effects were documented. The first phase II double-blind clinical trial using menstrual blood-derived endometrial MSC-like cells has commenced for treating 60 patients with congestive heart failure (70). An alternative approach is to deliver tissue-specific stem cells in scaffolds for tissue engineering applications to repair tissues (66). The scaffolds provide structural support and cell adhesion substrates allowing large numbers of cells to be delivered directly to the damaged site as a living patch of tissue (71, 72). Using cell sheet technology, menstrual blood MSC grafted onto infarcted myocardium in nude rat model differentiated into striated cardiac muscle cells and significantly improved cardiac parameters (34). Menstrual blood MSC have potential as a possible cell-based therapeutic for cardiac disorders and perhaps for reconstructing endometrium if combined with endometrial epithelial progenitors (Fig. 2).

Another tissue engineering application for endometrial MSC is vaginal repair in women with pelvic organ prolapse (73), a common disorder resulting from vaginal birth injury. Current treatment for pelvic organ prolapse is reconstructive surgery required by 11%–19% of all women (74) with 15%–29% needing additional operations due to surgical failure or complications arising from the use of synthetic scaffolds (75). We are currently designing tissue engineering constructs incorporating human endometrial MSC ($W5C5^+$ cells) into novel scaffolds as an autologous cell-based therapy to regenerate the lost and damaged fascia of the vaginal wall and to support the pelvic organs. Initial studies have shown that $W5C5^+$ endometrial MSC reconstitute stromal tissue when transplanted into immunocompromised mice (56). It appears that human endometrial MSC, whether derived from menstrual blood or from biopsies, have significant potential in regenerative medicine applications.

RECONSTRUCTING ENDOMETRIUM FROM EXOGENOUS CELL AND ORGAN SOURCES

Innovative approaches have been used to reconstruct endometrial tissue in humans and animal models with the goal of overcoming uterine factor infertility. Recently, autologous bone marrow cells selected by magnetic bead sorting for a mix of fibroblast, MSC and EPCs were instilled into the uterus of a patient with Asherman syndrome and thin dysfunctional endometrium that failed to respond to E (76). The cells were implanted after curettage on day 2 of the patient's menstrual cycle, enabling access to endometrial stem cell niches through the damaged surface (9). After this treatment, high doses of E promoted endometrial regeneration within four artificial cycles and clinical pregnancy was achieved with IVF-ET (76). This case raises questions regarding the mechanisms involved in regenerating the woman's endometrium. It may have resulted from transdifferentiation of the bone marrow cells or from injury-induced stimulation of poorly functioning endogenous endometrial stem/progenitor cells, or a combination of both (9). The observation that curettage or serial biopsy in menstrual cycles preceding the IVF-ET cycle doubles the pregnancy rate (PR) and live birth rate, suggests that endometrial injury provokes an inflammatory and/or wound healing response with associated release of growth promoting cytokines inducing decidualization (77, 78).

Alternatively, tissue damage activates quiescent adult stem cells to initiate cellular replacement, fulfilling their role in restoring tissue homeostasis. After injury, adult stem cells often overproduce replacement cells. Maybe biopsy-induced endometrial injury generates a thicker endometrium that accounts for the increased PR and live birth rate observed in these biopsied patients undergoing IVF (9) (Fig. 2).

In an innovative rat model of uterine repair, the peritoneal cavity was used as a bioreactor to engineer tissue on the surface of a uterine-shaped object. Tissue is produced by encapsulating bone marrow-derived myeloid cells that differentiate into myofibroblasts (79). The encapsulating tissue was removed and grafted into lesioned uteri of autologous rats. As the uterus repaired, it was covered by a luminal-like epithelium from which simple glands generated, producing tissue resembling endometrium. Mated 12-week transplanted rats carried embryos close to term. This study raises the questions on whether the replenishing epithelial cells arose from transdifferentiation of the myofibroblast, or whether they migrated into the neotissue from niches in the undamaged part of the uterus, or from the bone marrow and transdifferentiated into endometrial epithelium. And would cycles of shedding and regeneration characteristic of human, but not rat, promote or hinder myofibroblast differentiation (79)?

One way of providing functional endometrium containing resident stem/progenitor cells for women with uterine factor infertility or without a functional uterus would be to transplant the entire uterus (Fig. 2) (80). Experimental models in animals have resulted in the development of protocols for uterus retrieval surgery, but work is still required on vascular anastomosis to overcome of thrombosis formation. There has been one case of a human uterine transplant that lasted 3 months (81). It is predicted that issues relating to ischemia-reperfusion and immunosuppression are solvable and that human uterine transplantation may become an experimental procedure, although the ethical issues will remain (80).

In conclusion, adult stem cells have been identified in human endometrium on the basis of their functional attributes. Their ability to reconstruct endometrial tissue in vivo suggests their potential use for treating disorders associated with inadequate endometrium. The identification of specific markers for human endometrial MSC has demonstrated their perivascular location in the basalis and functionalis. Once candidate markers for epithelial progenitors are verified the opportunity for using endometrial stem/progenitor cells in reconstructing endometrial tissue in Asherman syndrome and IUA will become feasible (Fig. 2). Similarly the role of endometrial stem/progenitor cells in generating thick functional endometrium can be investigated. New models for differentiating pluripotent stem cells (human ESC, iPS cells) and other sources of adult stem cells into human endometrial epithelium provide alternative avenues for reconstructing endometrium for potential therapeutic purposes. Hurdles to be overcome include culture expansion of adult stem cells without differentiation and potential immunosuppression for allogeneic transplantation. Endometrial stem cell research is gaining momentum and the knowledge generated may be translated into the clinic within the next decade.

Acknowledgment: This article was edited by Carlos Simon.

REFERENCES

1. Jabbour HN, Kelly RW, Fraser HM, Critchley HOD. Endocrine regulation of menstruation. Endocrine Rev 2006;27:17–46.
2. Gargett CE. Uterine stem cells: what is the evidence? Hum Reprod Update 2007;13:87–101.
3. Gargett CE, Chan RW, Schwab KE. Hormone and growth factor signaling in endometrial renewal: role of stem/progenitor cells. Mol Cell Endocrinol 2008;288:22–9.
4. Schwab KE, Gargett CE. Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Hum Reprod 2007;22:2903–11.
5. Masuda H, Matsuzaki Y, Hiratsu E, Ono M, Nagashima T, Kajitani T, et al. Stem cell-like properties of the endometrial side population: implication in endometrial regeneration. PLoS One 2010;5:e10387.
6. Gargett CE, Masuda H. Adult stem cells in the endometrium. Mol Hum Reprod 2010;16:818–34.
7. Gaide Chevronnay HP, Galant C, Lemoine P, Courtoy PJ, Marbaix E, Henriet P. Spatiotemporal coupling of focal extracellular matrix degradation and reconstruction in the menstrual human endometrium. Endocrinology 2009;150:5094–105.
8. Maruyama T, Masuda H, Ono M, Kajitani T, Yoshimura Y. Human uterine stem/progenitor cells: their possible role in uterine physiology and pathology. Reproduction 2010;140:11–22.
9. Gargett CE, Healy DL. Generating receptive endometrium in Asherman's syndrome. J Hum Reprod Sci 2011;4:49–52.
10. Yu D, Wong Y-M, Cheong Y, Xia E, Li T-C. Asherman's syndrome—one century later. Fertil Steril 2008;89:759–79.
11. Panayiotides I, Weyers S, Bosteels J, Van Herendael B. Intrauterine adhesions (IUA): has there been progress in understanding and treatment over the last 20 years? Gynecol Surg 2009;6:197–211.
12. Lo ST, Ramsay P, Pierson R, Manconi F, Munro MG, Fraser IS. Endometrial thickness measured by ultrasound scan in women with uterine outlet obstruction due to intrauterine or upper cervical adhesions. Hum Reprod 2008;23:306–9.
13. Chan RWS, Kaitu'u-Lino T, Gargett CE. Role of label-retaining cells in estrogen-induced endometrial regeneration. Reprod Sci 2012;19:102–14.
14. Salamonsen LA. Tissue injury and repair in the female human reproductive tract. Reproduction 2003;125:301–11.
15. Kaitu'u-Lino TJ, Morison NB, Salamonsen LA. Estrogen is not essential for full endometrial restoration after breakdown: lessons from a mouse model. Endocrinology 2007;148:5105–11.
16. Schenker JG, Margalioth EJ. Intrauterine adhesions: an updated appraisal. Fertil Steril 1982;37:593–610.
17. Chan RWS, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod 2004;70:1738–50.
18. Schwab KE, Chan RW, Gargett CE. Putative stem cell activity of human endometrial epithelial and stromal cells during the menstrual cycle. Fertil Steril 2005;84(Suppl 2):1124–30.
19. Gargett CE, Schwab KE, Zillwood RM, Nguyen HPT, Wu D. Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. Biol Reprod 2009;80:1136–45.
20. Dominici M, Le BK, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–7.
21. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell 1997;88:287–98.
22. Wolff EF, Wolff AB, Du H, Taylor HS. Demonstration of multipotent stem cells in the adult human endometrium by in vitro chondrogenesis. Reprod Sci 2007;14:524–33.
23. Wolff EF, Gao XB, Yao KV, Andrews ZB, Du H, Elsworth JD, et al. Endometrial stem cell transplantation restores dopamine production in a Parkinson's disease model. J Cell Mol Med 2010;15:747–55.

24. Santamaria X, Massasa EE, Feng Y, Wolff E, Taylor HS. Derivation of insulin producing cells from human endometrial stromal stem cells and use in the treatment of murine diabetes. Mol Ther 2011;19:2065–71.
25. Dimitrov R, Timeva T, Kyurkchiev D, Stamenova M, Shterev A, Kostova P, et al. Characterisation of clonogenic stromal cells isolated from human endometrium. Reprod 2008;135:551–8.
26. Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria MA, Paradis G, et al. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. Nature Med 1997;12:1337–45.
27. Challen GA, Little MH. A side order of stem cells: The SP phenotype. Stem Cells 2006;24:3–12.
28. Cervello I, Gil-Sanchis C, Mas A, gado-Rosas F, Martinez-Conejero JA, Galan A, et al. Human endometrial side population cells exhibit genotypic, phenotypic and functional features of somatic stem cells. PLoS One 2010; 5:e10964.
29. Tsuji S, Yoshimoto M, Takahashi K, Noda Y, Nakahata T, Heike T. Side population cells contribute to the genesis of human endometrium. Fertil Steril 2008;90:1528–37.
30. Kato K, Yoshimoto M, Kato K, Adachi S, Yamayoshi A, Arima T, et al. Characterization of side-population cells in human normal endometrium. Hum Reprod 2007;22:1214–23.
31. Masuda H, Maruyama T, Hiratsu E, Yamane J, Iwanami A, Nagashima T, et al. Noninvasive and real-time assessment of reconstructed functional human endometrium in NOD/SCID/g_c^{null} immunodeficient mice. Proc Natl Acad Sci U S A 2007;104:1925–30.
32. Cervello I, Mas A, Gil-Sanchis C, Peris L, Faus A, Saunders PT, et al. Reconstruction of endometrium from human endometrial side population cell lines. Plos One 2011;6:e21221.
33. Cui CH, Uyama T, Miyado K, Terai M, Kyo S, Kiyono T, et al. Menstrual blood-derived cells confer human dystrophin expression in the murine model of Duchenne muscular dystrophy via cell fusion and myogenic transdifferentiation. Mol Biol Cell 2007;18:1586–94.
34. Hida N, Nishiyama N, Miyoshi S, Kira S, Segawa K, Uyama T, et al. Novel cardiac precursor-like cells from human menstrual blood-derived mesenchymal cells. Stem Cells 2008;26:1695–704.
35. Meng X, Ichim TE, Zhong J, Rogers A, Yin Z, Jackson J, et al. Endometrial regenerative cells: a novel stem cell population. J Transl Med 2007;5:57.
36. Patel AN, Park E, Kuzman M, Benetti F, Silva FJ, Allickson JG. Multipotent menstrual blood stromal stem cells: isolation, characterization, and differentiation. Cell Transplant 2008;17:303–11.
37. Musina RA, Belyavski AV, Tarusova OV, Solovyova EV, Sukhikh GT. Endometrial mesenchymal stem cells isolated from the menstrual blood. Bull Exp Biol Med 2008;145:539–43.
38. Murphy MP, Wang H, Patel AN, Kambhampati S, Angle N, Chan K, et al. Allogeneic endometrial regenerative cells: an "off the shelf solution" for critical limb ischemia? J Transl Med 2008;6:45.
39. Anwar S, Buhring HJ, Gargett CE. A single perivascular marker identifies MSC in human endometrium and menstrual blood. Aust Health & Medical Research Congress 2008;4:P700.
40. Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. Ann NY Acad Sci 2008;1127:106–15.
41. Starzinski-Powitz A, Zeitvogel A, Schreiner A, Baumann R. In search of pathogenic mechanisms in endometriosis: the challenge for molecular cell biology. Curr Mol Med 2001;1:655–64.
42. Korbling M, Estrov Z. Adult stem cells for tissue repair—a new therapeutic concept? N Engl J Med 2003;349:570–82.
43. Du HL, Taylor HS. Stem cells and reproduction. Curr Opin Obstet Gynecol 2010;22:235–41.
44. Bratincsak A, Brownstein MJ, Cassiani-Ingoni R, Pastorino S, Szalayova I, Toth ZE, et al. CD45-positive blood cells give rise to uterine epithelial cells in mice. Stem Cells 2007;25:2820–6.
45. Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. JAMA 2004;292:81–5.
46. Ikoma T, Kyo S, Maida Y, Ozaki S, Takakura M, Nakao S, et al. Bone marrow-derived cells from male donors can compose endometrial glands in female transplant recipients. Am J Obstet Gynecol 2009;201:608.e1–8.
47. Cervello I, Gil-Sanchis C, Mas A, Faus A, Sanz J, Moscardo F, et al. Bone marrow-derived cells from male donors do not contribute to the endometrial side population of the recipient. Plos One 2012;7:e30260.
48. Mints M, Jansson M, Sadeghi B, Westgren M, Uzunel M, Hassan M, et al. Endometrial endothelial cells are derived from donor stem cells in a bone marrow transplant recipient. Hum Reprod 2008;23:139–43.
49. Masuda H, Kalka C, Takahashi T, Yoshida M, Wada M, Kobori M, et al. Estrogen-mediated endothelial progenitor cell biology and kinetics for physiological postnatal vasculogenesis. Circ Res 2007;101:598–606.
50. Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. Stem Cells 2007;25:2082–6.
51. Aghajanova L, Horcajadas JA, Esteban FJ, Giudice LC. The bone marrow-derived human mesenchymal stem cell: potential progenitor of the endometrial stromal fibroblast. Biol Reprod 2010;82:1076–87.
52. Gargett CE, Rogers PAW. Human endometrial angiogenesis. Reproduction 2001;121:181–6.
53. Robb AO, Mills NL, Smith IB, Short A, Tura-Ceide O, Barclay GR, et al. Influence of menstrual cycle on circulating endothelial progenitor cells. Hum Reprod 2009;24:619–25.
54. Foresta C, De TL, Di MA, Ferlin A, Perilli L, Bertuzzi I, et al. Role of estrogen receptors in menstrual cycle-related neoangiogenesis and their influence on endothelial progenitor cell physiology. Fertil Steril 2010;93:220–8.
55. Spitzer TL, Rojas A, Zelenko Z, Aghajanova L, Erikson DW, Barragan F, et al. Perivascular human endometrial mesenchymal stem cells express pathways relevant to self-renewal, lineage specification, and functional phenotype. Biol Reprod 2012;86:58.
56. Masuda H, Anwar SS, Buhring HJ, Rao JR, Gargett CE. A novel marker of human endometrial mesenchymal stem-like cells. Cell Transplant. 2012. DOI: 10.3727/096368911X637362.
57. Schuring AN, Schulte N, Kelsch R, Ropke A, Kiesel L, Gotte M. Characterization of endometrial mesenchymal stem-like cells obtained by endometrial biopsy during routine diagnostics. Fertil Steril 2011;95:423–6.
58. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–7.
59. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell 2008;132:661–80.
60. Schwartz SD, Hubschman JP, Heilwell G, Franco-Cardenas V, Pan CK, Ostrick RM, et al. Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 2012;379:713–20.
61. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.
62. Ye L, Mayberry R, Lo CY, Britt KL, Stanley EG, Elefanty AG, et al. Generation of human female reproductive tract epithelium from human embryonic stem cells. Plos One 2011;6:e21136.
63. Kurita T. Normal and abnormal epithelial differentiation in the female reproductive tract. Differentiation 2011;82:117–26.
64. Kurita T, Medina R, Schabel AB, Young P, Gama P, Parekh TV, et al. The activation function-1 domain of estrogen receptor alpha in uterine stromal cells is required for mouse but not human uterine epithelial response to estrogen. Differentiation 2005;73:313–22.
65. Ye L, Evans J, Gargett CE. Lim1/LIM1 is expressed in developing and adult mouse and human endometrium. Histochem Cell Biol 2012;137:527–36.
66. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 2007;213:341–7.
67. Prockop DJ. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther 2009;17:939–46.
68. Caplan AI. Why are MSCs therapeutic? New data: new insight. J Pathol 2009;217:318–24.
69. Zhong Z, Patel AN, Ichim TE, Riordan NH, Wang H, Min WP, et al. Feasibility investigation of allogeneic endometrial regenerative cells. J Transl Med 2009;7:15.
70. Medistem. Medistem and ERCell initiate phase II RECOVER-ERC heart failure trial. http://medisteminc.com/2012/medistem-and-ercell-initiate-phase-ii-recover-erc-heart-failure-trial/. Last accessed May 24, 2012.
71. Atala A. Engineering organs. Curr Opin Biotechnol 2009;20:575–92.

72. Schmidt D, Mol A, Neuenschwander S, Breymann C, Gossi M, Zund G, et al. Living patches engineered from human umbilical cord derived fibroblasts and endothelial progenitor cells. Eur J Cardiothorac Surg 2005;27:795–800.
73. Gargett CE. Identification and characterisation of human endometrial stem/progenitor cells. Aust N Z J Obstet Gynaecol 2006;46:250–3.
74. Smith FJ, Holman CD, Moorin RE, Tsokos N. Lifetime risk of undergoing surgery for pelvic organ prolapse. Obstet Gynecol 2010;116:1096–100.
75. FDA Public Health Notification. Urogynecologic surgical mesh: update on the safety and effectiveness of transvaginal placement for pelvic organ prolapse. http://www.fda.gov/downloads/MedicalDevices/Safety/AlertsandNotices/UCM262760.pdf. Last accessed May 24, 2012.
76. Nagori CB, Panchal SY, Patel H. Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. J Hum Reprod Sci 2011;4:43–8.
77. Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I. Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization. Fertil Steril 2003;79:1317–22.
78. Zhou L, Li R, Wang R, Huang H, Zhong BA. Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. Fertil Steril 2008;89:1166–76.
79. Campbell GR, Turnbull G, Xiang L, Haines M, Armstrong S, Rolfe BE, et al. The peritoneal cavity as a bioreactor for tissue engineering visceral organs: bladder, uterus and vas deferens. J Tissue Eng Regen Med 2008;2:50–60.
80. Brannstrom M, Wranning CA, Altchek A. Experimental uterus transplantation. Hum Reprod Update 2010;16:329–45.
81. Fageeh W, Raffa H, Jabbad H, Marzouki A. Transplantation of the human uterus. Int J Gynaecol Obstet 2002;76:245–51.