The role of stem cells in uterine involution

Madelyn K Spooner¹, Yasser Y Lenis¹,³, Rachel Watson², Daniela Jaimes⁴ and Amanda L Patterson¹,²

¹Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA, ²Department of Obstetrics, Gynecology and Women’s Health, University of Missouri, Columbia, Missouri, USA, ³Department of Animal Sciences, Faculty of Agricultural Sciences, National University of Colombia, Palmira, Colombia and ⁴Faculty of Agricultural Sciences, University of Applied and Environmental Sciences U.D.C.A, Bogota, Colombia

Correspondence should be addressed to A L Patterson; Email: Pattersonama@missouri.edu

Abstract

Uterine remodeling during pregnancy and repair postpartum are fundamental to the successful propagation of eutherian species. The most drastic remodeling occurs in species with invasively implanting embryos, including humans and mice. During embryo implantation, embryonic trophoblasts breach the epithelium, penetrating into the stroma. Stromal cell decidualization, which is critical for the establishment and maintenance of early pregnancy, occurs throughout the implantation site. Trophoblasts further invade into and remodel uterine spiral arteries, which is necessary for placental formation. The uterus increases in size up to 24-fold, which is largely attributed to myometrial expansion. Uterine changes that occur during pregnancy must then be resolved postpartum. Following parturition, the uterus repairs the remodeled tissue in the process of uterine involution. During involution, the majority of the endometrium is regenerated to replace the tissue that is shed postpartum. The myometrium returns to the pre-gravid state which is thought to occur through apoptosis and autophagy of smooth muscle cells. Although we understand the general process of postpartum uterine involution, the detailed mechanisms, particularly the role of putative stem cells, are poorly understood. This review discusses the evidence for the existence of epithelial, stromal and myometrial stem cells and their role in uterine involution. Gaps in knowledge and areas for future research are also considered. Studies of both postpartum and menstrual uterine repair, which likely involve similar mechanisms, are described under the broad definition of uterine involution. Although the primary focus of this review is human, mouse models are discussed to provide additional information.

Reproduction (2021) 161 R61–R77

Overview of uterine physiology

In the simplest of terms, pregnancy and postpartum involution can be likened to normal physiological damage and repair processes, respectively. During pregnancy, the uterus undergoes dramatic remodeling events including invasion by embryonic trophoblasts, stromal cell decidualization and myometrial hyperplasia and hypertrophy, among others. Although these events are physiologically normal and necessary for pregnancy, they result in physiological ‘damage’ and loss of many cell types that require regeneration and repair postpartum. Likewise, the menstrual cycle in women is a series of physiological remodeling and repair events, which likely shares similarities with those that occur during pregnancy and postpartum involution. It has long been proposed that due to the extensive remodeling that the uterus undergoes, it likely contains stem cells that function to repopulate the various cell types of the uterus (Gargett 2007). In this section, a brief description of the uterine histoarchitecture of the human and mouse is given. This is followed by discussion of the major events in the menstrual (and estrous) cycle, pregnancy and uterine involution (both menstrual and postpartum). The primary focus of this review is human uterine physiology, however comparisons with mice are included to provide additional insight into uterine remodeling and regeneration.

Uterine histoarchitecture

Uterine involution has mostly been studied either by histological evaluation of uterine tissue during menstrual repair in humans or using pregnancy and menses-like mouse models. Histologically, the uteri of humans and mice are similar being comprised of three distinguishable tissue layers: (1) the outer-most perimetrium, or serosa, which is contiguous with the broad ligament, (2) the smooth muscle layer, termed the myometrium and (3) the inner-most endometrium (Gargett 2007). In humans, the endometrium is subdivided into the transient functional layer (functionalis; inner two-thirds), which is shed and regenerated throughout the menstrual cycle and the germinal bas al layer (basalis; lower one-
Uterine remodeling during the menstrual and estrous cycles

The menstrual cycle is standardized to 28 days and proceeds in three phases: menses, proliferative and secretory. Ovulation occurs on day (D) 14 and demarcates the proliferative and secretory phases, which correspond to the estrogen (E_2) dominant follicular phase and progesterone (P_4) dominant luteal phase, respectively, of the ovarian cycle. The first day of menses (menstrual bleeding/shedding) marks the beginning (D1) of the menstrual cycle and occurs due to steroid hormone withdrawal following the demise of the corpus luteum (luteolysis). During menses, desquamation occurs: epithelial, stromal and endothelial cells of the functionalis undergo apoptosis leading to shedding and expulsion of the tissue (Jabbour et al. 2006). With loss of the functionalis, only a small proportion (~2 mm) of the endometrium, the basalis, remains. Regeneration of the functionalis occurs simultaneously with degeneration (Ferenczy 1976a, Ludwig & Spornitz 1991). Re-epithelialization begins on D2 during active menstruation and is complete by D5–6 (Ferenczy 1976a, Ludwig & Spornitz 1991). On D6–7 (proliferative phase), substantial stromal proliferation begins (Ludwig & Spornitz 1991), increasing the overall thickness of the endometrium from 1–4 mm to 11 mm just prior to ovulation (Nalaboff et al. 2001). In addition to stromal growth, both GE and LE proliferation continues along with angiogenesis under E_2 stimulation. On approximately D14, ovulation occurs initiating the secretory phase. Cellular changes in this stage under P_4 influence include epithelial differentiation, particularly GE, stromal cell decidualization and infiltration.

Figure 1 Uterine histoarchitecture and hypothesized mechanisms of uterine involution. (A) Human and mouse histology images show myometrial (Myo) and endometrial (Endo) layers of the uterus. The human endometrium is segmented into the functionalis and basalis, indicated by dashed lines in cartoon/histology images. Both mouse and human uteri contain luminal and glandular epithelial cells (LE and GE, respectively) stromal cells, and myometrial cells. (B) Key of cartoon cells depicted in A and C. (C) Proposed mechanisms of uterine involution include: 1) replication of residual glandular and luminal epithelial cells that escaped desquamation, 2) Endogenous epithelial, stromal and myometrial stem cells, 3) Mesenchymal-epithelial transition (MET) of stromal cells into epithelial cells, and 4) Exogenous bone marrow-derived cells.
of immune cells (late secretory). Of importance is decidualization of perivascular stromal cells. Decidualization is the morphological and functional differentiation of stromal cells into decidual cells to provide support for an embryo. The process begins with a brief increase in mitotic activity of perivascular stromal cells around D22–23 (Gellersen & Brosens 2014) resulting in minimal polyploidization (~1% of stromal cells) (Qi et al. 2015). This is followed by transition of stromal cells from elongated, fibroblast-like mesenchymal cells into rounded, secretory epithelial-like cells. Changes indicative of epithelial-like transformation include expression of E-cadherin and cytokeratin, increased cellular area and circularity, enlarged and rounded nuclei, dense membrane-bound secretory granules and cytoplasmic accumulation of glycogen and lipid droplets (Gellersen & Brosens 2014, Pan-Castillo et al. 2018). Decidualization occurs in preparation for an embryo and is critical for establishment and maintenance of early pregnancy. Finally, at the end of the secretory phase, in the absence of pregnancy, endometrial shedding ensues and the menstrual cycle repeats.

The estrous cycle comprises four phases: proestrus, estrus, metestrus and diestrus. Proestrus and estrus correspond to the ovarian follicular phase and are dominated by E$_2$, whereas metestrus and diestrus are dominated by P$_4$ and correspond to the luteal phase. In mice the cycle duration is 4–5 days, and in contrast to the menstrual cycle, D1 begins on the day following ovulation which coincides with behavioral estrus (Bertolin & Murphy 2014). LE proliferation is relatively comparable across the cycle however, apoptosis is significantly higher in metestrus (Wood et al. 2007) resulting in a two- to four-fold higher cell number in proestrus and estrus (Evans et al. 1990). Stromal cell proliferation is highest in estrus, with a moderate amount in the other three stages, and apoptosis is highest in metestrus. Interestingly, changes in the GE do not follow the same pattern as the LE. GE proliferation is lowest in estrus and highest in proestrus and metestrus, with low levels of apoptosis throughout the cycle except in estrus. Overall uterine width is positively correlated with E$_2$ and negatively correlated with P$_4$ (Wood et al. 2007). In the absence of copulation, the ovarian corpora lutea regress rapidly and the next cycle begins.

The menstrual and estrous cycles are both characterized by stages of differential cellular activity including proliferation, differentiation, degeneration and regeneration which are regulated by ovarian derived E$_2$ and P$_4$. However, a major distinction between the menstrual and estrous cycles is the extent of endometrial remodeling that occurs. The greatest amount of remodeling occurs during menstruation in which the functionalis is shed and expelled from the body thus requiring extensive regeneration of the endometrium. However, in estrous cycling species such as mice, the endometrium is not shed, rather the tissue goes through cycles of mild resorption and growth (Wood et al. 2007).

Regarding the myometrium, much less is known about the cellular changes that occur throughout the menstrual cycle. Up until the early 2000s, the myometrium was considered a quiescent tissue outside of pregnancy, composed predominantly of differentiated smooth muscle cells (Teixeira et al. 2008). However, the myometrium is a dynamic tissue, responsive to various stimuli including E$_2$, P$_4$ and a milieu of paracrine factors (Burroughs et al. 2000, Taniguchi et al. 2001, Liu et al. 2013). During the menstrual cycle, myometrial proliferation is greatest in the secretory phase, whereas there are low levels of relatively consistent apoptosis throughout (Wu et al. 2000). This shows that, although less than the endometrium, there is in fact cellular turnover in the myometrium across the menstrual cycle.

**Pregnancy remodeling and postpartum repair (species comparison)**

During pregnancy, embryo implantation, stromal cell decidualization and placentation are critically important events in human (and murine) pregnancy that result in a substantial amount of uterine remodeling. In human implantation, embryonic trophoblasts invade the endometrium in between adjoining uterine LE cells (intrusion) with the goal of degrading the basement membrane and contacting the underlying stroma (Bischof & Campana 1996). Comparatively in mice, the LE is also displaced by intrusion, as well as protrusion and entosis by trophoblasts. Additionally, LE opposite the invading trophoblast undergoes apoptosis (Li et al. 2015). In women, stromal cell decidualization expands beyond the perivascular stromal cells that differentiated in the secretory phase of the menstrual cycle, to those adjacent to the embryo (reviewed in: Abrahamsohn & Zorn 1993, Ramathal et al. 2010). In mice, decidualization does not occur during the estrous cycle and is therefore only initiated by an implanting embryo. Trophoblast cells continue to invade the decidualized stroma to the myometrium and remodel the myometrial spiral arteries, which is critical for formation of the placenta. As pregnancy progresses, in women in particular, the uterus increases in weight approximately 24-fold, which is largely attributed to expansion of the myometrium by hyperplasia and hypertrophy (Johansson 1984). The human hemochorial placenta represents the most intimate association between the fetal membranes and maternal circulation and as such is also the most invasive form of placenta and involves substantial uterine remodeling (reviewed in: Rosenfeld 2007, Senger 2012). Mice also have a hemochorial placenta, however, major anatomical differences with the human placenta include: (1) trophoblast invasion stops at the myometrium in mice but continues into the muscle in women, (2) labyrinth (mouse) vs chorionic villi (human) structure and (3)
number of trophoblast cell layers: monochorial (human) vs trichorial (mouse) (Schmidt et al. 2015). Following parturition and expulsion of the placenta in both species, the uterus exists in a degenerated state and all cellular components of the endometrium and myometrium are remodeled and/or repaired. Similar to menstrual regeneration, re-epithelialization of the endometrium precedes stromal expansion. This is likely because in the degenerated state, the endometrium is essentially an extensive wound and rapid re-epithelialization is needed to prevent infection. Historical observations in women show that the epithelium covers the non-placental site of the endometrium by D7 postpartum and re-epithelialization of the entire endometrium is complete by approximately D14 (Sharman 1953). This is then followed by stromal expansion. In mice, the majority of information on endometrial repair comes from induced decidualization models (also termed menses-like models, described in detail subsequent). Similar to humans, epithelialization commences first within 24 h after the initiation of decidual breakdown/shedding and is proceeded by stromal expansion (Bastered et al. 2003). Repair is complete within 48 h. The myometrium having expanded several-fold due to cellular hyperplasia and hypertrophy, regresses to near its pre-gravid size, which is thought to include apoptosis, proliferation (Shynlova et al. 2009) and autophagy (Hsu et al. 2014) based on mouse studies. Uterine involution following parturition occurs relatively quickly allowing the uterus to be receptive to another embryo in 40–45 days in women and 3–4 days in mice.

Menstrual and postpartum uterine involution, specifically regeneration of lost or terminally differentiated cells (epithelial, stromal, myometrial), is highly dynamic and poorly understood. Proposed mechanisms of uterine regeneration include: (1) replication of residual GE and LE cells that escaped desquamation; (2) epithelial, stromal and myometrial stem cells; (3) mesenchymal-epithelial transition (MET) of stromal cells; and (4) infiltration and differentiation of bone marrow (BM)-derived cells into endometrial stromal and epithelial cells (Fig. 1C).

Evidence for uterine stem cells and their role in involution

Adult stem cells play an important role in tissue homeostasis and regeneration in many tissues (Blanpain & Fuchs 2009, Biteau et al. 2011). Stem cells have the unique ability to self-renew, generate lineage-specific daughter cells, maintain long-term proliferative capacity and restore function to damaged tissue. They are also relatively quiescent compared to more lineage committed cells. Common methods used to enrich putative stem cells (Fig. 2) include expression of stem cell markers (Table 1), Hoechst dye exclusion (side population (SP) analysis) and label retention (Bianco et al. 2008, Vemuri et al. 2011). These putative stem cells must then be tested in a series of in vitro (colony/spheroid formation, lineage differentiation, long-term proliferation) and in vivo (lineage tracing, label retention) experiments to assess their stem cell qualities, the ‘gold standard’ of which is engraftment into the tissue of origin (i.e. orthotopic transplantation) and restoration of function to damaged tissue (van Os et al. 2004, Garry et al. 2009). Subsequent are discussions of the current evidence in support of the presence of uterine stem cells in women (summarized in Table 1). Following that are sections that describe mouse models used to further assess putative stem cells in uterine involution.

Human endometrial mesenchymal stem cells (eMSCs)

The first report of putative eMSCs (i.e.stromal stem cells) was published in 2004 (Chan et al. 2004). Using in vitro colony formation, distinct small and large colonies were observed that formed from single human endometrial stromal cells plated at a density of 300 cells/cm² (Chan et al. 2004). An average of 1.25% of stromal cells formed colonies with significantly greater colony forming efficiency (CE) of small colonies (1.23%) compared to large (0.02%). Although more small colonies formed on initial seeding (Chan et al. 2004), large colonies had greater serial propagation/cloning potential (Gargett et al. 2009). This suggests that small colonies may represent more differentiated transit amplifying (TA) cells with limited propagation potential whereas large colonies may be enriched for stem/progenitor cells. Clonogenic stromal cells were further characterized and enriched for using surface markers including melanoma cell adhesion molecule (MCAM; CD146), platelet-derived growth factor receptor-beta (PDGFRβ; CD140b) (Schwab & Gargett 2007, Schwab et al. 2008) and sushio domain containing 2 (SUSD2; clone W5C5) (Masuda et al. 2012). The osteogenic, myogenic, chondrogenic and adipogenic lineage differentiation of colony forming CD146⁺CD140b⁺, and SUSD2⁺ cells was also shown (Schwab & Gargett 2007, Dimitrov et al. 2008, Schwab et al. 2008, Gargett et al. 2009, Schuring et al. 2011, Masuda et al. 2012, Spitzer et al. 2012, Fayazi et al. 2015). Further, CD146⁺CD140b⁺ and SUSD2⁺ cells were both located in the perivascular space (Cousins et al. 2018), which is a proposed MSC niche in multiple organs (Crisan et al. 2008). There was some overlap in expression of CD146/CD140b with SUSD2 (Masuda et al. 2012), however it is unclear if these markers identify the same or different populations of stem/progenitor-like cells.

Hoechst dye exclusion was used to obtain side population (SP) cells that were enriched for stem-like cells (Cervello et al. 2010). This cell-sorting procedure exploits the propensity of stem cells to efflux Hoechst dye because of relative overexpression of ATP transporters (Goodell et al. 1996). SP cells could form colonies...
and differentiate in vitro and expressed various stem cell markers. Unfortunately, this method poses some caveats. First, Hoechst dye is cytotoxic and can impact downstream assays making results difficult to interpret. Second, the SP, although enriched for stem-like cells, is a heterogeneous population. It may contain stromal, epithelial and endothelial stem-like cells. Therefore, purifying each cell type requires additional methods such as surface marker selection, which is often not performed.

In 2007, a mouse xenograft model was developed using human cells to study endometrial function (Masuda et al. 2007). Following injection of dissociated human endometrial cells under the renal capsule of immunodeficient mice and hormone treatment, endometrial-like tissue was formed that comprised of stroma and glands. Progesterone receptor (PGR) was upregulated upon E$_2$ treatment and the stroma expressed the decidual marker prolactin (PRL) with combination of E$_2$ and P$_4$ treatment. Moreover, menstrual-like changes could be induced with cyclic administration of E$_2$+P$_4$ followed by P$_4$ withdrawal. A hemorrhagic cyst was formed and there appeared to be epithelial and stromal cell shedding. This xenograft model has since been used as an in vivo stem cell assay to assess endometrial reconstitution by putative stem cells including SUSD2$^+$

**Figure 2** Diagram of common stem cell assays. Cells are typically isolated by various flow cytometry techniques to enrich for putative stem cells. The isolated cells are then tested for stem cell function in using in vitro and in vivo assays.
Table 1  Summary of proposed markers used to identify putative uterine stem cells in human and mouse.

<table>
<thead>
<tr>
<th>Stem cell/ proposed markers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td></td>
</tr>
<tr>
<td>eEpSCs</td>
<td></td>
</tr>
<tr>
<td>SSEA-1</td>
<td>Valentijn et al. (2013)</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>Nguyen et al. (2017)</td>
</tr>
<tr>
<td>β-catenin, SOX9</td>
<td>Nguyen et al. (2012, 2017), Tempest et al. (2018)</td>
</tr>
<tr>
<td>LGR5</td>
<td>Gil-Sanchis et al. (2013)</td>
</tr>
<tr>
<td>myoMSCs</td>
<td></td>
</tr>
<tr>
<td>OCT4</td>
<td>Ono et al. (2010)</td>
</tr>
<tr>
<td>CD44, Stro-1</td>
<td>Mas et al. (2015)</td>
</tr>
<tr>
<td>CD140b, CD146, SUSD2</td>
<td>Patterson et al. (2020)</td>
</tr>
<tr>
<td>CD34, CD49f</td>
<td>Ono et al. (2015)</td>
</tr>
<tr>
<td>eMSCs</td>
<td></td>
</tr>
<tr>
<td>MCAM (CD146), PDGFRβ (CD140b)</td>
<td>Schwab &amp; Gargett (2007), Schwab et al. (2008)</td>
</tr>
<tr>
<td>SUSD2 (clone W5C5)</td>
<td>Masuda et al. (2012)</td>
</tr>
<tr>
<td>MenSCs</td>
<td></td>
</tr>
<tr>
<td>OCT4</td>
<td>Meng et al. (2007), Nikoo et al. (2014), Patel et al. (2008)</td>
</tr>
<tr>
<td>SOX2, Nanog</td>
<td>Liu et al. (2018), Sun et al. (2019)</td>
</tr>
<tr>
<td>C-MyC</td>
<td>Liu et al. (2018)</td>
</tr>
<tr>
<td>SSEA-4</td>
<td>Liu et al. (2018), Patel et al. (2008), Rossignoli et al. (2013), Sun et al. (2019)</td>
</tr>
<tr>
<td><strong>Mouse</strong></td>
<td></td>
</tr>
<tr>
<td>eEpSCs</td>
<td></td>
</tr>
<tr>
<td>AXIN2</td>
<td>Syed et al. (2020)</td>
</tr>
<tr>
<td>CD44</td>
<td>Janzen et al. (2013)</td>
</tr>
<tr>
<td>mTert</td>
<td>Cousins et al. (2019), Deane et al. (2016)</td>
</tr>
<tr>
<td>LGR5</td>
<td>Seishima et al. (2019)</td>
</tr>
<tr>
<td>myoMSCs</td>
<td></td>
</tr>
<tr>
<td>OCT4,CD44</td>
<td>Brakta et al. (2018), Mas et al. (2017)*</td>
</tr>
<tr>
<td>Stro-1</td>
<td>Mas et al. (2017)*</td>
</tr>
<tr>
<td>CD140b, CD146</td>
<td>Patterson et al. (2018)</td>
</tr>
</tbody>
</table>

*Reference shows proposed markers also found in rat model.

eEpSCs, endometrial epithelial stem cells; eMSCs, endometrial mesenchymal stem cells; MenSCs, menstrual blood stem cells; myoMSCs, myometrial mesenchymal stem cells.

(Masuda et al. 2012) and SP enriched (Cervello et al. 2010, 2011, Masuda et al. 2010, Miyazaki et al. 2012) cells. However, it has not been used to study putative stem cells in menstrual repair. Although this renal-graft model can be used to provide valuable information about endometrial cell activity, to date, the ‘gold standard’ stem cell assay, which is transplantation into the tissue of origin (i.e. the endometrium), has not been performed. Transplantation of putative stem cells into the endometrium is necessary to determine their ability to contribute to endometrial function, including derivation of endometrial specific daughter cells that function in decidualization, embryo implantation and postpartum regeneration/repair.

Regarding the characteristics of putative eMSCs across the menstrual cycle, there do not appear to be significant differences in colony forming efficiency (Schwab et al. 2005, 2008) or expression of CD146 and CD140b (Schwab & Gargett 2007) based on cycle stage. These results are not surprising considering that putative eMSCs express relatively low levels of estrogen receptor alpha (ESR1) and progesterone receptor (PGR). Therefore, regulation of these cells is likely through paracrine signaling by the niche rather than direct hormone action. Interestingly, although E2 is required for epithelial proliferation during the proliferative phase of the cycle, re-epithelialization during menstruation occurs at a time of relatively low E2 levels (Ferenczy 1976a,b). Additionally, it was demonstrated that endometrial regeneration occurred following menstruation in human endometrial explants in ovariectomized mice in the absence of exogenous hormones (Matsuura-Sawada et al. 2005).

**Human menstrual blood stem cells (MenSC)**

Originally termed endometrial regenerative cells (ERC), putative menstrual blood-derived stem cells (MenSCs) were first observed in 2007 (Meng et al. 2007). Found to be derived from shed deciduous endometrial tissue (Rossignoli et al. 2013, Liu et al. 2018), these cells displayed the spindle-like morphology characteristic of fibroblasts (Mou et al. 2013, Rossignoli et al. 2013, Du et al. 2016, Faramarzi et al. 2016, Liu et al. 2018, Zheng et al. 2018). Through flow cytometry and immunofluorescence, it was demonstrated that putative MenSCs expresses various MSC markers but do not express hematopoietic, endothelial or immune activation markers (Patel et al. 2008, Mou et al. 2013, Rossignoli et al. 2013, Nikoo et al. 2014, Du et al. 2016, Faramarzi 2011).

Putative MenSCs have high proliferative capacity with no abnormalities identified by karyotyping following long-term passaging, thus indicating low mutagenesis (Meng et al. 2007, Patel et al. 2008, Rossignoli et al. 2013, Liu et al. 2018, Zheng et al. 2018). Interestingly, putative MenSCs have a higher CE (−14%) (Rossignoli et al. 2013) compared to reports of putative eMSCs. This may indicate that menstrual blood contains a mixture of stem, progenitor and transit amplifying cells which are all capable of forming primary colonies. Serial cloning may refine this population and give a more accurate estimation of stem cells. Many studies have demonstrated the in vitro capacity of putative MenSCs to differentiate into a variety of cell lineages: adipogenic, osteogenic, chondrogenic, neurogenic, cardiogenic, hepatogenic, pancreaticogenic, myogenic, endothelial and pulmonary epithelia (Meng et al. 2007, Patel et al. 2008, Mou et al. 2013, Rossignoli et al. 2013, Du et al. 2016, Ren et al. 2016, Liu et al. 2018, Sun et al. 2019). Putative MenSCs also reform endometrial-like tissue after s.c. grafting in immunocompromised mice and responded to hormone treatment as seen by expression of ESR1 and PGR (Zheng et al. 2018). When injected in the tail vein of immunocompromised mice, putative MenSCs tended to localize to the liver, lung and spleen and were not tumorigenic (Liu et al. 2018). Because MenSCs can reside in the liver following tail vein injection, evaluating their ability to regenerate hepatic tissue using a hepatic lineage seems promising. Indeed, transplantation of MenSC-derived hepatocyte-like cells following 2/3 partial hepatectomy in mice showed partial restoration of liver function (Mou et al. 2013). Therefore, providing the ease of collection and multi-lineage differentiation, researchers are beginning to realize the capabilities and uses of this putative stem cell population in regenerative medicine. Although these cells show promising therapeutic potential, more research is needed to fully characterize them and assess their regenerative capabilities.

**Human endometrial epithelial stem cells (eEpSC)**

Studies suggest the existence of endometrial epithelial stem cells (eEpSC) on the basis of in vitro colony formation, differentiation and long-term proliferation (Chan et al. 2004, Kato et al. 2007, Gargett et al. 2009, Nguyen et al. 2017) and in vivo reconstitution of endometrial epithelial-like tissue using the mouse renal-graft model (Masuda et al. 2007, Cervello et al. 2010, Miyazaki et al. 2012, Janzen et al. 2013). Uniquely, epithelial cells are capable of forming 3D colonies, termed spheroids or organoids. Organoids can be generated from individual epithelial cells and differentiate into the two primary endometrial cell types, ciliated and secretory (Boretto et al. 2017, Turco et al. 2017). Single-cell sequencing of endometrial epithelial organoids revealed a putative stem cell population (Fitzgerald et al. 2019). This population was the most predominant of all cell types when cultured in the absence of hormones. Upon hormone treatment, the presumptive stem-like population decreased and differentiated cell types increased. This may reveal a stem cell maintenance condition in hormone-free medium and differentiation of stem-like cells upon hormone induction. Whether this is an effect of culture or mimics in vivo regulation, remains to be determined. Interestingly, the proportion of cells expressing putative stem cell markers, in vivo, appeared to be higher in the hormone-deplete, post-menopausal endometrium compared to pre-menopausal (Valentijn et al. 2013, Nguyen et al. 2017). Together, these data may indicate that putative eEpSCs are more abundant in the absence of ovarian hormones and differentiate with hormone stimulus.

To date, there is no consensus on markers of putative eEpSCs, which has hindered further characterization and functional assessment of these cells. Proposed markers include the surface proteins SSEA-1 (Valentijn et al. 2013), N-cadherin (Nguyen et al. 2017), and LGR5 (Gil-Sanchis et al. 2013, Cervello et al. 2017, Tempest et al. 2018) and the transcription factors β-catenin and SOX9 (Nguyen et al. 2012, Valentijn et al. 2013, Nguyen et al. 2017, Tempest et al. 2018). N-Cadherin+ and SSEA-1+ cells were predominantly located in the basalis layer and showed higher in vitro CE and spheroid formation, respectively, compared to negative cells (Valentijn et al. 2013, Nguyen et al. 2017). SSEA1+ cells had reduced expression of ESR1 and PGR mRNA compared to SSEA1− cells, whereas most N-cadherin+ cells appeared to express ESR1 by immunofluorescence. SSEA1+ cells expressed SOX9 but N-cadherin+ cells rarely did and co-expression of SSEA1 and N-cadherin was infrequent. These reports suggest two distinct populations of cells with in vitro stem/progenitor activity that may be indicative of cells at different stages of differentiation. LGR5, a known intestinal epithelial stem cell marker, was also investigated as an eEpSC marker. By IHC, LGR5 was expressed in random epithelial, stromal and perivascular cells (Gil-Sanchis et al. 2013) but by in situ hybridization was localized exclusively to the epithelium in a cycle-dependent manner (Tempest et al. 2018). When isolated and characterized, LGR5+ cells did not reconstruct endometrial-like tissue but rather
had a phenotype more consistent with hematopoietic-derived cells (Cervello et al. 2017). Therefore, LGR5 is unlikely to be a useful eEPSC marker in women. Telomerase activity (Valentijn et al. 2015) and SP analysis (Cervello et al. 2010, Masuda et al. 2010) also revealed putative eEPSCs based on CE and spheroid formation, and in vivo epithelium reconstruction in mouse renal or s.c. xenografts were used to assess stem-like activity in endometrial epithelium. However, as with putative eMSCs, epithelial cells have not been transplanted into the endometrium to more definitively assess stem cell characteristics and function.

**Human myometrial mesenchymal stem cells (myoMSC)**

The majority of studies on putative myoMSCs were conducted to identify stem/progenitor cells in fibroids, benign uterine smooth muscle tumors, compared with normal myometrium. Investigators have identified putative myoMSCs using many of the aforementioned methods including surface markers (Mas et al. 2015, Ono et al. 2015, Patterson et al. 2020) and SP analysis (Ono et al. 2007, Chang et al. 2010) followed by in vitro and in vivo stem cell assays. In the first report, SP analysis was used to enrich cells with osteogenic and adipogenic differentiation potential. Similar to eMSCs, these cells expressed low levels of ESR1 and PGR (Ono et al. 2007). More importantly, SP cells were engrafted into the myometrium of immunocompromised mice and appeared to differentiate into α-SMA expressing myocytes. Although main population (MP) control cells were also able to engraft, they rarely expressed alpha-smooth muscle actin (α-SMA), suggesting they did not contain lineage-differentiating stem/progenitor cells. To date, there is no consensus on markers to isolate putative myoMSCs. OCT4 (POUF51 gene) was expressed at relatively low levels in myometrium but was enriched for in the SP compared to the MP (Ono et al. 2010). Known as a pluripotency marker and expressed in embryonic stem cells, OCT4 may serve as a candidate marker of human MyoMSCs, however, this has yet to be tested. Other candidate markers include CD34/CD49f (Ono et al. 2015), CD44/Stro-1 (Mas et al. 2015) and CD140b/CD146 or SUSD2 (Patterson et al. 2020). Compared to control cells (CD34/CD49f), CD34+/CD49f+ myometrial cells were enriched for SP cells, formed more colonies and had greater osteogenic and adipogenic differentiation potential in vitro (Ono et al. 2015). Of note, CD34+/CD49f+ cells showed higher myometrial engraftment in immunocompromised mice, which was further enhanced with pregnancy. Stro1+/CD44+ cells expressed stem cell markers (e.g. OCT4 and NANOG) and showed adipogenic, osteogenic and chondrogenic differentiation compared to non-inducing conditions, however, they did not show significantly higher CE in vitro (Mas et al. 2015). Stro1+/CD44+ cells were engrafted under the renal capsule of immunocompromised mice and appeared to reconstitute some myometrial-like tissue based on actin and collagen expression, however, the contribution of human-derived cells was difficult to ascertain based on the cell tracker method used (Molday Ion Rhodamine B). Additionally, Stro1+CD44+ cells were not compared with control cells to determine differences in engraftment potential (Mas et al. 2015). Lastly, the markers used to enrich for putative eMSCs, CD146/CD140b co-expression or SUSD2, were shown to enrich for putative myoMSCs (Patterson et al. 2020). CD146+CD140b+ and SUSD2+ cells were located in the perivascular region similar to their location in the endometrium and had higher CE compared to control cells (CD146+CD140b- or SUSD2-). Interestingly, SUSD2+ cells not only showed osteogenic and adipogenic differentiation but also appeared to undergo decidualization in vitro similar to endometrial stromal cells. This study suggests a possible common MSC for the endometrial stroma and myometrium (Patterson et al. 2020). However, further research is needed to assess the in vivo potential of SUSD2+ myometrial-derived cells to decidualize and contribute to uterine function in a stem/progenitor cell capacity. Although there is compelling evidence for human myometrial stem/progenitor cells, there is inconsistency in isolation methods and a lack of in-depth studies to assess in vivo function of these cells.

**Murine models of uterine stem cells**

The literature discussed previously provides a solid foundation for the existence of uterine stem cells in women, however, technical and ethical constraints prevent in vivo studies of these cells in uterine involution. However, through the use of mouse models invaluable insight into uterine repair mechanisms has been garnered. Mice and humans share many similarities in pregnancy and postpartum uterine involution. In both species, embryo implantation is invasive, breeching the luminal epithelium, the stroma decidualizes and a hemochorial placenta is formed. Postpartum, the uterus undergoes extensive remodeling including stromal and epithelial regeneration and myometrial reduction. Mice do not menstruate; however, a menses-like mouse model was developed, which mimics the events of human menstruation (Finn & Pope 1984, Brasted et al. 2003). Women menstruate, in part, because stromal cells near the spiral arteries decidualize in the late secretory phase of the menstrual cycle in preparation for pregnancy (Gellersen & Brosens 2014). In mice, decidualization is induced by an implanting embryo. However, it can be induced by scratching the LE or infusing oil into the uterine lumen in a hormonally primed mouse. The scratch/oil causes epithelial cell death which initiates the decidual cascade in stromal cells. Upon removal of P4 stimulus, decidual tissue will breakdown which is manifested as menstrual-like shedding. Comconitant with decidual...
degeneration is endometrial repair/regeneration akin to menstrual shedding and regeneration. Using mouse models, intricate studies can be performed that provide more in-depth understanding of uterine involution in vivo. This includes additional stem cell assays such as label retention and lineage tracing, which are discussed subsequently and summarized in Fig. 2.

**Label-retention**

Stem cells divide relatively infrequently and therefore are proposed to retain label, such as bromodeoxyuridine (BrdU), while other more frequently dividing cells will lose label over time. Label-retaining cells (LRC) are then identified and assessed for additional stem cell properties. BrdU label retention has been used to identify slow cycling LRCs as potential stem/progenitor cells in mouse endometrium (Chan & Gargett 2006, Cervello et al. 2007, Kaitu'u-Lino et al. 2010, 2012, Chan et al. 2012, Cao et al. 2015) and myometrium (Szotek et al. 2007). Stromal LRCs were localized primarily to the sub-luminal stroma, near blood vessels (perivascular) and at the stromal-myometrial border (analogous to the human basalis). This pattern remained consistent following pregnancy and postpartum uterine involution, although the percentage of LRCs was greatly reduced (Cao et al. 2015). Similarly, stromal LRCs were mostly perivascular and at the stromal-myometrial border during menses-like endometrial regeneration (Kaitu'u-Lino et al. 2012). The perivascular location of murine stromal LRCs corroborates data in human that putative eMSCs are also perivascular (Spitzer et al. 2012). Epithelial LRCs were predominantly found in the LE and to a lesser extent in the GE in nulliparous mice (Chan & Gargett 2006b), however, label was rapidly diluted in the LE but retained in the GE during menses-like regeneration (Kaitu'u-Lino et al. 2010).

Using a transgenic mouse LRC model (H2Bj-GFP), LRCs were identified in the distal oviduct epithelium but not in the endometrium after labeling cells in adult mice for 7 days (Wang et al. 2012). The oviduct LRCs formed spheroids and based on differentiation in vitro, were proposed to be the stem/progenitor cells for the endometrial epithelium. In another study, LRCs were also found in the distal oviduct epithelium as well as the endocervical transition zone after embryonic thru prepubertal labeling (Patterson & Pru 2013). However, these cells did not appear to participate in menses-like endometrial regeneration. Rather, when the label period was adjusted to the peripubertal period, long-term LRCs were found in the endometrial GE. These studies clearly illustrate how labeling under different physiological circumstances impacts which cells retain label. Recently, myometrial LRCs were characterized using the H2Bj-GFP transgenic labeling model (Patterson et al. 2018). The brightest LRCs expressed the putative human stromal and myometrial MSC markers, CD146 and CD144, were perivascular, and preferentially proliferated during menses-like uterine involution. Similar to putative human uterine stem cells, mouse uterine LRCs expressed little-to-no ESR1 and PGR (Chan & Gargett 2006b, Chan et al. 2012, Wang et al. 2012, Patterson & Pru 2013, Patterson et al. 2018).

An important caveat of LRC models is that cells undergoing terminal differentiation can also become labeled and if long-lived will be identified as LRCs. Therefore, follow up assays are critical to determine stem-cell activity of LRCs. To date, only one study has assessed the stem-like activity of epithelial LRCs by sphere formation (Wang et al. 2012). Stem cell activity of myometrial and stromal LRCs has yet to be assessed in vitro and no uterine LRCs have been transplanted to reconstitute uterine tissue in vivo.

**Lineage tracing and marker expression**

Using real-time, lineage tracing and ablation techniques, LGR5 was investigated as an eEpSC marker in mice (Seishima et al. 2019). LGR5 was expressed in the LE and budding GE during adenogenesis and became increasingly more restricted to the glands in prepubertal mice. In the adult, LGR5 was expressed sporadically in the LE and GE and almost exclusively during diestrus. The stem-like activity of LGR5+ cells was examined using a lineage tracing model (Lgr5-2A-CreERT2; R26-tdTomato) to indelibly label Lgr5 expressing cells and their progeny. When tracing was initiated during adenogenesis, labeling both LE and budding GE cells, LGR5+ cells contributed to growth and maintenance of the LE and GE in the adult. However, when tracing began after adenogenesis, labeling predominantly GE, LGR5+ cells only contributed to the GE in the adult but not the LE. Finally, if LGR5+ cells were traced beginning in the adult during diestrus, label was diminished by 2 weeks and undetectable after 1 year. These results suggest that prior to adenogenesis, LGR5 marks stem-like cells for both the LE and GE but shortly after, a delineation occurs and LGR5 only marks GE stem-like cells and in the adult does not serve as a marker for either. Importantly, ablation of LGR5+ cells during adenogenesis resulted in a significant reduction in the number of glands formed and present in the adult, indicating their requirement for adenogenesis (Seishima et al. 2019). Because of the dynamic expression of LGR5 during postnatal adenogenesis, it would be interesting to see if LGR5 is upregulated during postpartum adenogenesis and re-epithelialization and could serve as a marker of stem cells in adult epithelial regeneration.

Two independent groups used an inducible, clonal, lineage-tracing model to evaluate the presence and activity of putative eEpSCs. By using an epithelial specific promoter, Krt19 (Jin 2019) or Pax8 (Syed et al. 2020), and low dose of the inducing agent, small
subsets of LE and GE cells were labeled (fluorescent or LacZ) and their lineages traced over time. In both models, there was a progressive increase in the number of labeled cells throughout the epithelium suggesting they originated from individually labeled stem-like cells (Jin 2019, Syed et al. 2020). In a similar study, Confetti mice that expressed multiple fluorescent proteins stochastically were used to identify clones of epithelial cells that likely originated from individually labeled Pax8+ cells (Fu et al. 2020). These epithelial markers, however, are not restricted to putative stem cells but are also expressed in differentiated cells and in fact, abundantly throughout the endometrial epithelium (Fu et al. 2020, Syed et al. 2020). Therefore, more specific markers were used to assess clonal expansion of labeled cells as an indicator of stem cell activity. It has long been proposed that eEpSCs reside in endometrial glands (Gargett 2007). FOXA2 is a well-accepted GE marker (Jeong et al. 2010, Filant & Spencer 2013) and AXIN2 (Syed et al. 2020) was shown to be restricted to the GE in the adult predominantly at the base of the glands nearest the myometrium. Again, using a low dose to induce label, a small proportion of GE cells expressing AXIN2 became labeled, allowing subsequent daughter cells to be tracked (Syed et al. 2020). The label appeared to spread from the base of the glands toward the lumen and after 3 months nearly all GE cells were labeled suggesting that individual AXIN2+ stem-like cells were responsible for GE expansion during the estrous cycle. However, the extent of contribution of AXIN2+ cells to the LE is unclear. Even though the LE proliferated every 3 days, labeled LE cells were not observed until after 3 months (Syed et al. 2020). One explanation could be that the low initial labeling of cells requires more time for labeled cells to reach the LE from the base of the GE and replace non-labeled cells. However, even with extensive initial labeling and after approximately 17 estrous cycles (2.5 months), there appeared to be little-to-no contribution to the LE. Additionally, following six pregnancies, there appeared to be only a few patches of LE cells derived from the labeled AXIN2+ GE cells. In agreement with these results are those obtained from FOXA2-inducible lineage tracing, where FOXA2+ GE were reported to not contribute to the LE (Jin 2019), however, this model was not tested during postpartum repair. Although AXIN2+ GE cells may represent the stem/progenitor cell population for the GE, the identity of eEpSCs responsible for LE regeneration remains to be determined.

CD44 (Janzen et al. 2013) and mTert (mouse telomerase) (Deane et al. 2016, Cousins et al. 2019) have also been investigated as possible markers of endometrial stem/progenitor cells in mouse models. Using an in vivo renal-graft mouse model, hormonally deprived epithelial cells were serially transplanted in limiting dilution and as few as 100 transplanted epithelial cells were able to form gland-like structures (Janzen et al. 2013). These cells had increased expression of Cd44 and Axin2. CD44 and ITGA6, which marks the epithelial basement membrane, were used to isolate CD44+ITGA6+ epithelial cells. Using serial xenografting, 0.92% of CD44+ITGA6+ cells had regeneration potential compared to 0.03% of control cells (Janzen et al. 2013). In other studies, real-time expression of mTert was evaluated in the endometrium using mTert-GFP mice (Deane et al. 2016, Cousins et al. 2019). Expression was observed at very low percentages in several cell types within the endometrium, including immune, stromal, epithelial and endothelial (Deane et al. 2016). Consistent with other reports of putative stem/progenitor cells, no difference was found across the estrous cycle. Although some mTert-GFP+ GE cells co-stained for CD44, some stromal and immune cells also expressed CD44, indicating lack of specificity. mTert-GFP+ cells did not retain BrdU label or proliferate during endometrial maintenance (e.g. estrous turnover) (Deane et al. 2016). During menses-like repair in mice, the majority of mTert-GFP+ cells were immune cells and <0.1% of epithelial cells were mTert-GFP+ (Cousins et al. 2019). Very rare mTert-GFP+ cells co-localized with the proliferation marker, Ki-67. It was suggested that mTert may mark highly quiescent stem-like cells that upon activation undergo asymmetric cell division to generate highly proliferative transit amplifying cells (Cousins et al. 2019). It still remains to be determined to what extent mTert+ endometrial cells contribute to postpartum uterine involution. CD44, in combination with Str1 (CD44+Str1+), was also suggested as a marker for putative myoMSCs in rodent models. Rat CD44+Str1+ myometrial cells expressed the pluripotency marker Oct4 and low levels of ESR1 and PGR suggesting their undifferentiated state (Mas et al. 2017). Similarly, uteri from Oct4-GFP mice contained GFP myometrial cells that co-expressed CD44 (Brakta et al. 2018). Rodent cells expressing CD44/Str1/OCT4 require further testing as putative myoMSCs including analysis of stem cell activity (e.g. colony formation and lineage differentiation in vitro and in vivo, etc.) and function in uterine involution.

The aforementioned studies (summarized in Table 1) have provided valuable insight into uterine repair using mouse models. But it is important to note some of the caveats. Label retention in itself does not definitively delineate stem cells from non-stem cells, and thus far, the stem cell activity of uterine LRCs has not been validated. Also, it is difficult to extrapolate the information learned from LRC studies to humans because there is no comparable assay that can be performed in women. Therefore, the LRC technique should be used as a steppingstone to further characterize uterine stem cells, such as a means to identify surface markers that may be useful in humans. However, it is cautioned that mouse and human markers may not correlate. LRG5 is a good example of a putative neonatal stem/progenitor marker in mice (Seishima et al. 2019) but not in adult women (Cervello et al. 2017).
Bone marrow-derived cells

There are two potential sources of stem cells for the uterus: intra-uterine (endogenous uterine stem cells) and extra-uterine (Fig. 1). The previous sections were devoted to discussion of the evidence for endogenous uterine stem/progenitor cells in women and mice. In this section, a possible exogenous source will be discussed for both species.

In humans and mice, the presence of extra-uterine, bone marrow (BM)-derived cells was reported in the endometrium of transplant recipients (Taylor 2004, Du & Taylor 2007, Ikoma et al. 2009, Morelli et al. 2013, Gil-Sanchis et al. 2015, Tal et al. 2019). It is well-known that immune cells, which are derived from the BM, reside within all compartments of the uterus (Zhou et al. 2018, Lee et al. 2015), however, it is controversial whether these cells give rise to functional endometrial cells (Ong et al. 2018) or rather provide support to resident endometrial cells. Some reports suggest that non-immune BM-derived cells, presumably MSCs, engrat in the endometrium and become stromal, GE and LE cells by transdifferentiation (Taylor 2004, Du & Taylor 2007, Ikoma et al. 2009, Cervelló et al. 2012, Morelli et al. 2013, Gil-Sanchis et al. 2015, Tal et al. 2019). This theory is controversial and it is disputed if, and how, these BM-derived cells contribute to the endometrium in regards to regeneration, maintenance and function (Cervelló et al. 2012, Wolff et al. 2013, Ong et al. 2018).

The first report of BM-derived endometrial cells was conducted in 2004 (Taylor 2004). Uterine tissues were obtained from women who had previously received BM transplants, following radiation and chemotherapy (Taylor 2004). Donor-derived cells were identified in the endometrium of recipients and composed 0.2–48% of the epithelium and 0.3–52% of the stroma (Taylor 2004). In a similar study, 0.6–8.4% of epithelial cells and 8.2–9.8% of stromal cells were derived from donor BM (Ikoma et al. 2009). It is thought that BM-MSCs are the cells that engrat in the endometrium. However, Cervelló et al. (2012) concluded that engrafted BM-derived cells do not contribute to the endometrial SP and therefore are unlikely to contribute to the tissue in a stem cell capacity. Furthermore, clonal expansion of BM-derived cells, a hallmark of stem cell engratment, has only been documented in the original study (Taylor 2004) and has not been replicated. In both women and mouse models, only sporadic BM-derived cells have been verified to express epithelial or stromal cell markers while also being negative for the pan-leukocyte marker, CD45 (Taylor 2004, Du & Taylor 2007, Ikoma et al. 2009, Cervelló et al. 2012, Morelli et al. 2013, Gil-Sanchis et al. 2015). Thus, these studies concluded that although most BM-derived cells were immune cells, a few became epithelial or stromal cells. In contrast, another study used refined immunofluorescent and flow cytometry techniques in mice and reported that all engrafted BM-derived cells were immune cells based on marker expression and were therefore not epithelial or stromal cells (Ong et al. 2018). Another recent study used an inducible saturation labeling strategy in mice to investigate intra- vs extra-uterine sources of cells responsible for epithelial maintenance and regeneration postpartum. By labeling the majority of the epithelium (~90%), the contribution of non-labeled extra-uterine cells was evaluated by calculating the percentage of labeled epithelial cells over time. The percentage of labeled epithelial cells remained relatively unchanged, suggesting that an un-labeled extra-uterine cell (e.g. BM) did not produce endometrial epithelial cells (Syed et al. 2020).

Studies on the function of purported BM-derived endometrial epithelial or stromal cells during pregnancy and uterine involution are lacking. BM-derived cells improved fertility in a mouse model of Asherman’s syndrome (intra-uterine adhesions) (Alawadhi et al. 2014), but only rare BM-derived epithelial- and stromal-like cells were identified (0.14 and 0.55%, respectively). Although pregnancy rates were improved, their direct function in pregnancy was not assessed (Alawadhi et al. 2014). The only study that has investigated the role of BM-derived cells in pregnancy used tail vein BM transplantation following chemotherapy treatment (Tal et al. 2019). Depending on the day of pregnancy, the percentage of BM-derived cells in the endometrium ranged from 10 to 24%, with 72–95% being CD45+ leukocytes. Although other immune cell markers were used to assess different leukocyte populations that were CD45−, they were not used in combination. Therefore, the population of immune cells may have been underrepresented. Moreover, no BM-derived epithelial cells were identified. Putative BM-derived stromal cells were assessed based on expression of PGR and DPRP (decidual-prolactin related protein). However, PGR may be expressed by various types of leukocytes (Shah et al. 2019), and BM-derived cells were concentrated in the mesometrial pole of the uterus whereas DPRP is typically concentrated in the anti-mesometrial side (Rasmussen et al. 1997, Candeloro & Zorn 2007). The clonal expansion, longevity and contribution to subsequent pregnancies of these BM-derived putative stromal cells were not evaluated (Tal et al. 2019).

The evidence surrounding BM-derived cells in endometrial function is thus far circumstantial. BM-derived cells are being investigated for their ability to treat endometrial conditions including Asherman’s syndrome in women (Santamaria et al. 2016, Singh et al. 2014). It will therefore be important to gain a more complete understanding of the existence and function of non-immune, BM-derived cells in the uterus.

Mesenchymal–epithelial transition

In the past decade, mesenchymal–epithelial transition (MET) has become an emerging hypothesis as a
mechanism of endometrial re-epithelialization. Broadly, cellular transdifferentiation is the conversion of cells from one differentiated cell type to another, examples being MET or the reciprocal EMT (Lamouille et al. 2014). Of note, the occurrence of cellular transdifferentiation in the uterus was first described in embryonic development 60 years ago (Gruenwald 1959) and the MET hypothesis in adult re-epithelialization was first proposed in 1967 (Baggish et al. 1967). More recently using electron microscopy, the cellular and morphological changes during menstruation were evaluated (Garry et al. 2009). In the early stages of repair, single and small islands of new epithelial cells were observed that were frequently isolated from, and did not appear to arise from, protruding glandular stumps (Garry et al. 2009). These new cells were morphologically distinct from the more mature glandular cells; they appeared low and cuboidal in shape, smooth and lacked microvilli. As repair progressed, the new cells eventually fused with the more mature gland cells to form a continuous epithelial lining. The new epithelial cells, which formed in isolated singlets or small clusters, were suggested to originate from the stroma presumably by MET. Studies in mouse models also support the mechanism of MET in endometrial re-epithelialization (Huang et al. 2012, Patterson et al. 2013, Cousins et al. 2014, Yin et al. 2019). Mesenchyme-specific Cre driving promoters were used to induce reporter expression (e.g. EYFP or LacZ) to indelibly mark mesenchymal cells and their progeny. With this approach, reporter-positive epithelial cells were identified following postpartum and menes-like endometrial re-epithelialization suggesting that stromal cells underwent MET to produce epithelial cells (Huang et al. 2012, Patterson et al. 2013, Yin et al. 2019). Interestingly, presumptive mesenchymal/epithelial transitional cells (vimentin/cytokeratin co-expressing) were initially located at the stromal-myometrial boarder, a proposed mesenchymal stem cell niche for the stroma (Chan & Gargett 2006a, Cervello et al. 2007) and moved toward the lumen as regeneration progressed (Patterson et al. 2013). This may suggest a role for stromal stem/progenitor cells in MET. In support of this, mouse CD34+KLF4+ putative eMSCs enhanced epithelial regeneration presumably through MET (Yin et al. 2019), and human CD146+ putative eMSCs spontaneously differentiated into epithelial-like cells when cultured in a 3D-scaffold on top of myometrial cells (Fayazi et al. 2017). Collectively these studies provide evidence for MET, possibly by eMSCs, as a mechanism of endometrial re-epithelialization. However, this mechanism was recently challenged. Discussed previously, a saturation labeling technique was used in mice to investigate extratumor BM cell contribution to endometrial epithelial turnover/regeneration. Because the percentage of labeled epithelial cells remained relatively unchanged, this not only suggested that unlabeled BM-derived cells did not contribute to the endometrial epithelium but also that unlabeled stromal cells also did not (Syed et al. 2020). Furthermore, MET was refuted on the suggestion that the Cre reporter mouse models used previously were not mesenchyme-specific (Ghosh et al. 2020). Clearly, more studies need to be conducted to confirm MET as a mechanism of re-epithelialization and determine if eMSCs are the source. It also remains to be determined if MET-derived (i.e. stromal-derived) epithelial cells are functional endometrial epithelial cells.

Conclusions and future perspectives

The uterus in women is a highly dynamic organ that withstands repeated physiological damage and repair events during the menstrual cycle and pregnancy/postpartum. The extensive regeneration and rapid repair achieved suggests a role for stem/progenitor cells. The evidence for the existence of stem/progenitor cells is compelling however, the true identity, characteristics and functions of these putative cells have yet to be fully elucidated. This is due in part to the technical and ethical constraints surrounding human research. Although mouse models have provided invaluable clues into human uterine involution, caution must be placed on translation from mouse to human. Further, a suitable in vitro model that recapitulates the events of menstrual and postpartum involution, particularly cellular regeneration, has not been established. A recent review nicely highlights the advances in development of in vitro models of the human endometrium (Fitzgerald et al. 2020). Readers are directed to this review for a detailed discussion; however, some important points are described here. First, improvements have been made in endometrial epithelial cell culture to more accurately mimic in vivo histoarchitecture. Of note, is incorporation of extracellular matrix components, typically using Matrigel, treatment with chemically defined media and formation of 3D organoids. These organoids are genetically stable, can be propagated long-term, express molecular signatures of LE and GE and are hormonally responsive (Boretto et al. 2017, Turco et al. 2017). Moreover, organoids can be derived from single cells suggesting the presence of stem cells, which is further supported by scRNA-Seq data revealing a potential stem/progenitor cell population (Fitzgerald et al. 2019). Beyond epithelial culture alone, attempts have been made to incorporate stromal cells, which is critical for recapitulating the in vivo endometrial environment. Advancements have been made using 3D porous scaffolds (Abbas et al. 2020), PEG hydrogels (Cook et al. 2017, Valdez et al. 2017) and scaffold-free, self-assembling environments (Wiwatpanit et al. 2020), to co-culture stromal and epithelial cells with varying degrees of success. Considerations include scaffold or gel components, matrix stiffness, ability of cells to properly orient and media conditions to support both

Conclusions and future perspectives

The uterus in women is a highly dynamic organ that withstands repeated physiological damage and repair events during the menstrual cycle and pregnancy/postpartum. The extensive regeneration and rapid repair achieved suggests a role for stem/progenitor cells. The evidence for the existence of stem/progenitor cells is compelling however, the true identity, characteristics and functions of these putative cells have yet to be fully elucidated. This is due in part to the technical and ethical constraints surrounding human research. Although mouse models have provided invaluable clues into human uterine involution, caution must be placed on translation from mouse to human. Further, a suitable in vitro model that recapitulates the events of menstrual and postpartum involution, particularly cellular regeneration, has not been established. A recent review nicely highlights the advances in development of in vitro models of the human endometrium (Fitzgerald et al. 2020). Readers are directed to this review for a detailed discussion; however, some important points are described here. First, improvements have been made in endometrial epithelial cell culture to more accurately mimic in vivo histoarchitecture. Of note, is incorporation of extracellular matrix components, typically using Matrigel, treatment with chemically defined media and formation of 3D organoids. These organoids are genetically stable, can be propagated long-term, express molecular signatures of LE and GE and are hormonally responsive (Boretto et al. 2017, Turco et al. 2017). Moreover, organoids can be derived from single cells suggesting the presence of stem cells, which is further supported by scRNA-Seq data revealing a potential stem/progenitor cell population (Fitzgerald et al. 2019). Beyond epithelial culture alone, attempts have been made to incorporate stromal cells, which is critical for recapitulating the in vivo endometrial environment. Advancements have been made using 3D porous scaffolds (Abbas et al. 2020), PEG hydrogels (Cook et al. 2017, Valdez et al. 2017) and scaffold-free, self-assembling environments (Wiwatpanit et al. 2020), to co-culture stromal and epithelial cells with varying degrees of success. Considerations include scaffold or gel components, matrix stiffness, ability of cells to properly orient and media conditions to support both

Conclusions and future perspectives

The uterus in women is a highly dynamic organ that withstands repeated physiological damage and repair events during the menstrual cycle and pregnancy/postpartum. The extensive regeneration and rapid repair achieved suggests a role for stem/progenitor cells. The evidence for the existence of stem/progenitor cells is compelling however, the true identity, characteristics and functions of these putative cells have yet to be fully elucidated. This is due in part to the technical and ethical constraints surrounding human research. Although mouse models have provided invaluable clues into human uterine involution, caution must be placed on translation from mouse to human. Further, a suitable in vitro model that recapitulates the events of menstrual and postpartum involution, particularly cellular regeneration, has not been established. A recent review nicely highlights the advances in development of in vitro models of the human endometrium (Fitzgerald et al. 2020). Readers are directed to this review for a detailed discussion; however, some important points are described here. First, improvements have been made in endometrial epithelial cell culture to more accurately mimic in vivo histoarchitecture. Of note, is incorporation of extracellular matrix components, typically using Matrigel, treatment with chemically defined media and formation of 3D organoids. These organoids are genetically stable, can be propagated long-term, express molecular signatures of LE and GE and are hormonally responsive (Boretto et al. 2017, Turco et al. 2017). Moreover, organoids can be derived from single cells suggesting the presence of stem cells, which is further supported by scRNA-Seq data revealing a potential stem/progenitor cell population (Fitzgerald et al. 2019). Beyond epithelial culture alone, attempts have been made to incorporate stromal cells, which is critical for recapitulating the in vivo endometrial environment. Advancements have been made using 3D porous scaffolds (Abbas et al. 2020), PEG hydrogels (Cook et al. 2017, Valdez et al. 2017) and scaffold-free, self-assembling environments (Wiwatpanit et al. 2020), to co-culture stromal and epithelial cells with varying degrees of success. Considerations include scaffold or gel components, matrix stiffness, ability of cells to properly orient and media conditions to support both
Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement

A P conceptualized the manuscript. Y L S, M S, R W, D J and A P all contributed to composition of the manuscript.

References


Cervello I, Mas A, Gil-Sanchis C, Peris L, Faus A, Saunders PT, Critchley HO & Simon C 2011 Reconstruction of endometrium from human endometrial side population cell lines. PLOS ONE 6 e21221. (https://doi.org/10.1371/journal.pone.0021221)


Cousins FL, O DF & Gargent CE 2018 Endometrial stem/progenitor cells and their role in the pathogenesis of endometriosis. Best Practice &


Patterson IA, Pru JK & Zarnani AH 2018 Label-retaining, putative mesenchymal stem cells localize to distinct regions within the female reproductive epithelium. *Cell Cycle* 17 2888–2898. (https://doi.org/10.4161/cc.25917)


Reproductive (2021) 161 R61–R77


Uterine stem cells in involution


Received 31 July 2020
First decision 10 September 2020
Revised manuscript received 14 December 2020
Accepted 12 January 2021