

Somasundaram Indumathi  
and Marappagounder Dhanasekaran

## Introduction

The uterus is by far the largest female organ of the body, playing an integral role in the reproductive life of every woman. It plays a pivotal role in implantation and in absence of pregnancy, menstruation. The innermost layer of the uterus is known as tunica mucosa, popularly termed as endometrium, opposed to the outer perimetrium and median myometrium. The uterus is the only organ whose lining is almost entirely expelled and reconstructed periodically, both phenomena taking place at each ovarian cycle. With the purpose of facilitating the periodic elimination of the endometrium that undergoes regression, shrinkage, and necrosis at end of each cycle, the uterus also exhibits the unique peculiarity of physiological bleeding. The endometrial histophysiology is entirely controlled by the ovarian hormones along the cycle. Of all tissues of the human body, the endometrium is the one that, throughout the ovarian cycle, most accurately reflects the levels of estrogen and progesterone. Estradiol, produced by the ovaries on approximately day 4 or 5 of the cycle, induces growth and proliferation of the endometrium. The levels of estrogen are normally elevated during the proliferative phase of the menstrual cycle as it serves to promote proliferation of the luminal and glandular epithelial cells associated with the thickening of the endometrial lining as well as vascularization. The cessation of endometrial growth occurs before estradiol levels reach their peak and prior to the onset of progesterone production, thereby indicating that nonsteroidal factors limit the growth of endo-

metrium. Progesterone is responsible for the secretory phase of the ovulatory cycle, and its action upon the endometrium serves two purposes. The first can be regarded as “medical.” It greatly reduces the proliferative activity of the endometrial glands, thereby preventing the appearance of endometrial hyperplastic alterations. The second is essentially “reproductive,” that is vital to create an ideal condition in the endometrium for the implantation and development of the egg [1–3].

Reproduction in the mammalian female thus requires a precisely timed and complex interaction between the hypothalamic-pituitary-ovarian (HPO) axis and the uterine endometrium. Abnormalities in HPO axis are being treated effectively with advancements in reproductive medicine. However, the abnormalities in endometrium function that serve as a major cause of poor implantation of the fetus in the uterine wall remain poorly understood. Abnormalities in endometrium also lead to female infertility and several pathological diseases, like endometrial cancer, endometriosis, endometrial hyperplasia, and endometritis, to name a few. Development of effective therapy for infertility or other diseases due to endometrial dysfunction requires enhanced understanding of the latest advance in uterine/endometrial cell biology, more precisely the discovery of endometrial stem cells.

This chapter provides a brief overview on the current understanding of the evidence supporting the existence of the uterine adult stem cells in the endometrial tissue and the role these cells likely play in normal adult uterine physiology. We describe the isolation of endometrial stem cell, their propagation, their biomarker expression, and their differentiation potential. In addition, we review the possible roles in gynecological disorders associated with abnormal endometrial proliferation and the potential use of endometrial stem cells in therapeutics.

## Endometrial Stem Cells: Research Background

Embryonic stem cells, the cells derived from the inner cell mass (ICM) of the developing embryo, are pluripotent and

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S. Indumathi, PhD  
Department of Stem cells, National Institute of Nutrition,  
Tarnaka, Secunderabad, Andhra Pradesh 500007, India

Lifeline RIGID Hospitals, Kilpauk, Chennai, Tamil Nadu 600010, India  
e-mail: [indu.stemcell@gmail.com](mailto:indu.stemcell@gmail.com)

M. Dhanasekaran, PhD (✉)  
Stemcell Banking and Therapeutics, Ree Laboratories Private  
Limited, Andheri West, Mumbai, Maharashtra 400053, India

Lifeline RIGID Hospitals, Kilpauk, Chennai, Tamil Nadu 600010, India  
e-mail: [dhanasekarbio@gmail.com](mailto:dhanasekarbio@gmail.com)

were considered an ideal source of regenerative therapeutics for treatment of a wide range of diseases. But ethical issues and immune rejection limit their clinical applications. Moreover, the tumorigenicity of embryonic stem cells is a concern [4–6]. Hence, there occurred a successful search for stem cells from adult tissues and organs. Bone marrow and adipose tissue are promising sources of adult stem cells and are now being used in a wide range of therapies [7–13]. This revolutionary change has increased the demand of identification of stem cells from other tissues and body fluids such as tendon [14], periodontal ligament [15], synovial membrane [16], lung [17], liver [18], synovial fluid [19], amniotic fluid [20–22], as well as endometrial tissue [23] and menstrual blood [24, 25]. Despite the advances in recent years in the isolation and applicability of stem cells from many sources, there exist only limited studies that consider the availability and applicability of stem cells in female and male reproductive organs. Studies of adult stem cell biology in the uterus lag far behind other areas of stem cell research despite the fact that the uterus undergoes perhaps the most extensive proliferative changes and remodeling in adult mammals compared with other organs.

Human endometrium lines the uterine cavity as far the isthmus of the uterus, where it becomes continuous with the lining of the cervical canal. The endometrium begins to reach full development at puberty and thereafter exhibits dramatic changes during each menstrual cycle. It undergoes further changes before, during, and after pregnancy, during the menopause, and in old age. The endometrium is a simple columnar epithelium. It is divided into two zones, the inner functionalis which is adjacent to the uterine cavity and a deeper basalis layer which overlies the myometrium. The functionalis layer is shed each month with menstruation and is then regenerated from the basalis layer which is not shed. The functionalis, comprising the upper two-thirds of the endometrium, is divided into stratum compactum and stratum spongiosum. The stratum compactum is a superficial thin layer nearest to the uterine cavity and contains the lining cells, necks of the uterine gland, and relatively dense stroma. The stratum spongiosum is the deeper part of functionalis composed of main portions of the uterine glands and accompanying blood vessels; the stromal cells are more loosely arranged and larger than in the stratum compactum. The lower basalis contains the basal region of the uterine glands, dense stroma (that remains relatively unaltered during the menstrual cycle), large blood vessel remains and lymphoid aggregates. It serves as the germinal compartment for generating new functionalis each month [26–28]. It has been postulated that the niche of these adult stem or progenitor cells of the endometrium is the lower basalis. These stem or progenitor cells were also identified to be in the trophic endometrium of postmenopausal women [29, 30].

Accumulating evidence from the literature on the existence of epithelial and stromal/stem cells in endometrial

tissue [28–31] has substantiated that it possesses a remarkable capacity for regeneration. However, there is only limited information pertaining to these stem cells derived from endometrial tissue as obtaining these stem cells directly from the endometrial tissue (the inner lining of the uterus) requires an endometrial biopsy or uterine curettage. Endometrial biopsies are routinely performed for diagnosis of abnormal uterine bleeding and can be done easily in an office setting with a small curette or the self-contained small vacuum cannula (Pipelle™) which minimizes the patient's discomfort. Each menstrual cycle is associated with the vascular proliferation, glandular secretion, and the endometrial growth. Absence of progesterone, the demise of corpus luteum, and the subsequent fall in circulating progesterone lead to vasoconstriction, necrosis of the endometrium, and menstruation. Menstrual blood includes the apical portion of the endometrial stroma. Hence, menstrual blood has become the most convenient source in the search for endometrial stem cells because collecting menstrual blood is easy and noninvasive and endometrial stem/progenitor cells are shed in menstrual blood. For these reasons, reliable studies on menstrual blood-derived stem cells are in process [32–35]. Furthermore, menstrual blood-derived stem cells demonstrate great promise for use in tissue repair and treatment of diseases, due to the plasticity and longevity of the cells. This has been identified through the *in vitro* and *in vivo* studies of characterization, proliferation, and differentiation [23, 25, 34].

However, putative adult stem or progenitor cells that are responsible for the cyclical regeneration of the endometrium functionalis, every month, are thought to reside in the basalis region of the endometrium [26–30]. Hence, the study of these stem cells from the basalis layer of the endometrial tissue is of utmost importance and is still in its infancy. Based on the dynamic tissue remodeling in all compartments of the uterus, during the menstrual cycle and pregnancy, it has been suggested that adult stem cells must play a role in uterine tissue maintenance and function. Hence, a thorough characterization of the uterine/endometrial stem cells derived from the endometrial tissue biopsy of the inner lining of the uterus or from the intact uterus surgically removed in the treatment is equally important as that of studies on menstrual blood stem cells. Once a mechanical or functional characteristic platform has been constructed, it then becomes easier to understand the complex mechanisms underlying the morphogenesis and physiological generation of the female reproductive tract, to improve the understanding of the pathophysiology of the gynecologic diseases such as endometrial cancer, fibroids, endometriosis, and pregnancy loss as well as determine the possible roles of endometrial stem/progenitor cells of the female reproductive tract to these gynecologic diseases, thereby considering them as a possible therapeutic target for treatment of wide horizon of diseases in regenerative medicine.

## Evidence for Endometrium Stem Cells

Adult stem cells in the endometrium are difficult to identify because they constitute very small populations of cells and because of the lack of precisely characterized cell surface markers specific for adult stem cells of the endometrium. Studies which provide indirect evidence for the existence of endometrial stem cells do so by characterizing cell populations in the endometrium which exhibit the functional properties of stem cells. These properties include clonogenicity, proliferative potential, and capacity for differentiation into one or more lineages [29]. Clonogenicity, defined as the ability of a single cell to produce a colony when seeded at very low densities, was demonstrated in human endometrium for the first time in 2004. The first published evidence on stem cells of human endometrium identifies two types of adult stem cells, clonogenic epithelial and stromal cells suggesting the presence of two types of adult stem/progenitor cells [36, 37]. Using a purified single cell suspension dispersed from hysterectomy specimens, Chan et al. identified a small population of stromal cells (1.25 %) and epithelial (0.22 %) cells in human endometrium that possessed clonogenic activity [37]. This rare population of epithelial and stromal colony-forming unit cells (CFUs) were found in normal cycling and inactive perimenopausal endometrium and in endometrium of women on oral contraceptives, suggesting that CFUs may be responsible for regenerating cyclic and atrophic endometrium [38, 39]. The findings, however, of clonogenic cells in inactive endometrium further supports the existence of an endometrium stem cell niche in the basalis, as this endometrium is predominantly basalis and lacks functionalis. Only large CFUs exhibit stem cell properties of self-renewal, differentiation, and highly proliferative potential, while small CFUs are transit-amplifying cells [24].

Another approach used by multiple investigators was to identify and characterize stem cells with the side population phenotype (SP). Side population cells are characterized by their ability to exclude the DNA binding dye Hoechst 33343 by expressing ATP-binding cassette transporter proteins. They exhibit the properties of adult stem cells including mature glandular epithelial, stromal, and endothelial cells in vitro. The SP phenotype cells have also been found to proliferate and differentiate into requisite cell types in vivo in the immunodeficient mice [40]. This method has been previously used to identify putative stem cell population in multiple tissues, including the bone marrow, liver, mammary gland, skin, and kidney, inclusive of endometrium. Several groups have identified a number of SP cells as candidate endometrial stem/progenitor cells. SP cells identified from human endometrium display long-term proliferative properties as well as differentiation into mature endometrial glandular, epithelial, stromal, and endothelial cells in vitro

[41–43]. Additional studies have reported the ability of endometrial SP to differentiate in vitro into adipocytes and chondrocytes, thus supporting a mesenchymal origin [44, 45]. These studies support the hypothesis that SP isolated from human endometrium are indeed adult stem cells. Other properties evaluated in characterization of an endometrial stem cell population include the capacity of multilineage differentiation. The differentiation potential of candidate stem cells is evaluated after culturing the cells in differentiation induction media. The endometrial stem cells were able to differentiate into muscle cells, adipocytes, osteoblast, and chondrocytes [44–48].

Schwab and Gargett demonstrated the existence of endometrial stem cell identification through the characterization of perivascular markers CD 146 and PDGF-R $\beta$ . They demonstrated that these perivascular markers enabled isolation of stromal cells from human endometrium which exhibit phenotypic and functional properties of MSC. The investigators then used immunohistochemistry to localize these cells to perivascular areas of the basalis and functionalis [48]. They hypothesized that these endometrial MSC-like cells may contribute to the cyclic regeneration of the endometrium and further postulated that they may play a role in pathogenesis of diseases such as endometriosis and adenomyosis. Despite the scanty citations on the expression profile of biomarkers of endometrium stem cells [49], the identification of other prospective markers to identify endometrial stem cells is underway.

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## Isolation and Propagation of Stem Cells Derived from Endometrial Tissue

Endometrium was collected from reproductively active women undergoing hysterectomy or endometrial biopsy for nonmalignant uterine tumors, fibroids, adenomyosis, and uterine prolapse. Endometrial tissue was collected from the different phases of endometrial cycle including menstrual, secretory, and proliferative phase. Sampling procedures varies with the aim of research, but samples were usually collected from women not under any kind of hormonal therapy [24, 49–51]. The endometrial biopsy samples containing the endometrial epithelial and stroma cells are collected in HEPES-buffered Dulbecco modified Eagle medium/Hams F-12 supplemented with antibiotic-antimycotic solution (final concentrations: 100 mg/ml penicillin G sodium, 100 mg/ml streptomycin sulfate, 0.25 mg/ml amphotericin B) and newborn calf serum [37] or Hanks balanced salt solution containing the antibiotics like streptomycin and penicillin [50].

Isolation of endometrium stem cells involves processing of the finely chopped tissue samples in phosphate-buffered saline devoid of calcium and magnesium ions. The mechanically minced tissue is further digested with collagenase type

III and deoxyribonuclease type I to prevent increase in viscosity of the cell suspension due to nucleic acid [37] or DMEM containing type Ia collagenase [50]. The stromal cell suspension is obtained and purified either by negative selection using magnetic Dynabeads coated with specific antibodies to remove epithelial cells (BerEP4) and leukocytes (CD45) [37] or by repeated centrifugation to obtain the epithelial cells and the stromal cells [50]. The stromal isolates are cultured and propagated in the complete culture medium.

Although the freshly isolated samples possess the heterogeneous mixture of both epithelial and stromal cell populations, a homogenous fibroblastic stromal population becomes prominent with a spindle-like cell morphology with centrally located nuclei as a monolayer upon culture [50]. The adult stem cell properties were also assessed by separating into EpCAM<sup>+</sup> epithelial cells and EpCAM<sup>-</sup> stromal cells. The self-renewal property of the adult stem cells was also monitored using a serial cloning strategy. Large and small clones were obtained wherein the large clones showed a better self-renewal capacity. Large clones of epithelial and stromal cells were capable of undergoing three rounds of serial cloning [24]. The percentages of colony-forming mesenchymal cells from endometrium were found to be higher when compared to bone marrow or dental pulp. Human endometrial stromal cells were found to have a colony-forming capacity of 1.2 % in comparison to the 0.1–0.01 % for dental pulp and bone marrow [51].

## Biomarker Expression

A great breakthrough has been achieved by the identification and isolation of the stem cells from endometrium. However, the search to identify the MSC population in human endometrium is still at its infancy [52]. Furthermore, there exist only scanty citations on identification of biomarker expression of the uterine/endometrial stem cells that can isolate/characterize specific cell population. A major advantage of being able to identify the cell surface markers of epithelial and stromal population of the endometrium is that their features can be characterized in noncultured cells and their utility in cell-based therapies for regenerative medicine evaluated in pre-clinical disease models. Furthermore, a detailed study of endometrial stem cell markers is necessary as pathology of several endometrial disorders is associated with these endometrial progenitor/stem cells.

The presence of mesenchymal stem cells can be confirmed by surface antigenic profiling. A positive expression of markers like CD105, CD73, and CD90 and the absence of CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR surface molecules define mesenchymal stem cells as proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy

(ISCT) [53]. These criteria help researchers to identify mesenchymal stem cells and progenitors derived from endometrium as well as their expression prevalence in endometrium associated diseases. Dimitrov and his co-workers [50] analyzed the cell surface markers for the cultured endometrial stem cells derived from both functionalis and basalis layer of the endometrium for phenotypic expression. Hematopoietic stem cell markers including CD 45, CD 14, CD 19, CD56/16, CD 34, and CD3 showed a negative expression. Whereas markers like CD 29, CD 73, and CD 90 were stained positive, strongly suggesting the mesenchymal nature of the cells. In addition, immunofluorescence staining of the stromal cells showed positivity for vimentin, prometastatinase-3 (proMMP 3), and endoglin (CD 105) showing their respective cellular localization.

Schwab and his co-workers identified the co-expression of perivascular cell markers like melanoma cell adhesion molecule (MCAM)/CD 146 and platelet-derived growth factor receptor  $\beta$  (PDGF-R $\beta$ ) using a fluorescence-activated cell sorter (FACS). The FACS-sorted CD146<sup>+</sup>PDGF-R $\beta$ <sup>+</sup>endometrial stromal cell population was found to have a positive phenotypic expression of mesenchyme-specific markers CD29, CD44, CD73, CD90, and CD105 and a negative expression of hematopoietic markers CD 34 and CD 45 as well as endothelial marker CD 31. This study also reveals that CD 146 and PDGF-R  $\beta$  cells were co-localized in both the functional and basal layer. Moreover, the conclusions imply that a few CD146<sup>+</sup>PDGF-R $\beta$ <sup>+</sup> cells may shed during menstrual cycle [48].

Schwab and his co-workers studied a sorting strategy to isolate human endometrial stromal stem cells. In this screening investigation, fresh endometrial stromal cell suspension was studied for the expression of the surface proteins, STRO-1, CD 133, CD 146, and CD 90, using immunohistochemistry, FACS sorting, and CFU assays [49]. Thy-1 (CD 90) was used in conjugation with CD 45 (negative marker) which led to the enrichment for CFU in CD90 stromal cell population. Along with CD 90, CD146 was also found to enrich human endometrial CFU, suggesting the perivascular location of these cells. This result was in accordance with the co-expression of CD 146 with platelet-derived growth factor receptor  $\beta$  (PDGF-R $\beta$ ) [48] and thus favors the isolation of mesenchymal stem cells from human endometrium. Even though the perivascular endometrial cells expressed STRO-1, these cells failed to enrich CFU thereby making them not useful for isolating human endometrial stem cells. Likewise, HSC marker CD 133 turned out to be a negative marker for stromal endometrium CFU. This study clearly suggests the utility of CD 146 and CD 90 as potential markers for the sorting strategy of endometrial CFU [49].

Octamer 4 (OCT-4) a highly expressed transcription factor in embryonic stem cells and in embryos at various stages of development was also studied by researchers on endometrium. OCT-4 expression was evident only in the endometrial

stromal cells and not in the myometrium. The study indicated that OCT-4 expression does vary with age, with phase of the menstrual cycle, or with the gynecologic disorders of the individual. The expression of OCT-4 suggests the existence of endometrial stem cells [54], lending further support to the hypothesis of endometrial regeneration by local stem cells in endometrial tissue. A similar study reported the expression of OCT-4 in the follicular and luteal phases of endometrium by Bentz and his co-workers [55], thereby suggesting the presence of pluripotent cells in the endometrium. On the other hand, as OCT-4 expression has been previously associated with germ cell tumors and embryonic carcinoma [56], a thorough study on OCT-4 expression of the endometrial stem cells is needed and may help us to understand the pathology of endometrial cancers and other disorders associated with abnormal proliferation of endometrium.

Overall, there are only limited studies on phenotypic biomarker characterization since endometrial stem/progenitor cells were only identified in the year 2004. Albeit these limited studies, endometrial tissue-derived stem cell had gained importance because of its ease of availability and distinct biomarker expression identified so far. However, much more needs to be explored in identifying the other cell surface markers that are unique to epithelial and stromal cell populations of the endometrium.

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## Differentiation Potency

One of the unique characteristic features of stem cells is their ability to undergo multilineage differentiation. The mesenchymal and tissue stem cell committee of the International Society for Cellular Therapy (ISCT) has put forward certain minimal criteria to define and identify the MSC population [53]. One of the criteria is that the cells must be able to differentiate to mesodermal lineages of osteoblasts and adipocytes to demonstrate bone and fat phenotypes, respectively, under standard *in vitro* differentiating conditions [57]. Evidence on the existence of endometrial stem cells was derived from the phenotypic, functional, and proliferative studies. As per the criteria put forth by ISCT, it is imperative to understand whether the stem cells of endometrium have the ability to differentiate into multiple lineages as described for other stem cell types. Indeed several researchers demonstrated the ability of CD146<sup>+</sup>PDGFRb<sup>+</sup> MSC-like cells [48] or clonogenic human endometrial stromal cells [24, 50] to differentiate into mesodermal origin such as adipocytes, osteocytes, smooth muscle cells, and chondrocytes. Masuda et al. demonstrated that the endometrial tissue-reconstituting cells also possess the ability to differentiate into endothelial cells [58]. It has been demonstrated that not only the endometrial stem cells but also the SP cells of the endometrium have the ability to differentiate into endothelial and smooth

muscle cells [41–43]. Although accumulating evidence supports the ability of endometrial stem cells to differentiate into cells of mesodermal origin, the ability to transdifferentiate is yet to be defined. The possibility of these endometrial stem cells to differentiate into neuronal-like cells has been evaluated [59]. However, study of the transdifferentiation potential of these stem cells in near future might unearth the prospects of curative therapeutics.

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## Clinical Correlations and Regenerative Applications

Several gynecological conditions are associated with abnormal endometrial proliferation, and it is possible that putative endometrial stem/progenitor cells may play a role in the pathophysiology of diseases such as endometriosis, endometrial hyperplasia, endometrial cancer, and adenomyosis. Alterations in the number, function, regulation, and location of epithelial/stromal endometrial stem/progenitor cells may be responsible for any one of these endometrial diseases. Furthermore, study of the clinical correlations of endometrial stem cells with gynecological diseases may unravel several unresolved barriers and lead to the use of endometrial stem cells as an ideal alternative source of curative therapeutics.

## Endometrial Stem/Progenitor Cells in Endometriosis

Endometriosis, defined as the growth of endometrium outside the uterine cavity, is a common gynecological disorder affecting 6–10 % of women [60]. It is a major clinical problem causing inflammation, pain, and infertility. Despite its common occurrence, the pathogenesis of endometriosis is poorly understood [61, 62]. The most widely accepted mechanism is Sampson's retrograde menstruation theory where viable endometrial fragments reflux through the Fallopian tubes into the pelvic cavity and attach to and invade the peritoneal mesothelium to establish ectopic growth of endometrial tissue. Several other theories have been suggested, including abnormal endometrium, genetic factors, altered peritoneal environment, reduced immune surveillance, and increased angiogenic capacity. It is proposed that endometriosis results when endometrial stem/progenitor cells are inappropriately shed during menstruation and reach the peritoneal cavity where they adhere and establish endometriotic implants [63]. Although no direct evidence exist on the role of endometrial stem cells in pathogenesis of endometriosis, numerous studies demonstrate that unfractionated human endometrial cells establish ectopic endometrial growth in the many experimental models [58, 64]. Despite the existence of

several other preliminary studies *in vitro*, the exact role of endometrial stem/progenitor cells in the development of endometriosis will require more elucidation.

### Cancer Stem Cells in Endometrial Cancer

It is currently uncertain whether cancer stem cells have a role in endometrial cancers or in endometrial hyperplasia. Recent reports suggest a role of cancer stem cell on endometrial cancer. In a comprehensive study from both types and all grades of endometrial cancer, a small population of less than 1 % of clonogenic tumor cells were identified that could be serially cloned *in vitro*, indicating self-renewal capacity [65]. Freshly isolated endometrial cancer cells transplanted in limiting dilution into immunocompromised NOD/SCID mice induced tumors recapitulating parent tumor histoarchitecture and marker expression with as few as 1/10,000 in 50 % of transplants, indicating that a small population of tumor-initiating cells or cancer stem cells differentiated *in vivo*. SP cells have been identified in several low and high-grade endometrioid endometrial cancer samples and several endometrial cancer cell lines [66]. Interestingly, the Hecl SP cells produced tumors comprising epithelial tumor cells with the positive expression of human vimentin and alpha smooth muscle actin, indicating an occurrence of epithelial to mesenchymal transition cancer progression of the SP cells in the tumors *in vivo*. Furthermore, the role of cancer stem cells in endometrial cancers has also been proposed with the identification of other cancer stem cell markers responsible for endometrial cancer. The first of the surface markers used to identify the CSC in human endometrial cancer is the CD 133/1 epitope. In a larger study group of endometrial cancer, CD 133<sup>+</sup> cells have higher cloning efficiency and proliferated at a faster rate when compared to the CD 133<sup>-</sup> population [67]. It was hypothesized that the SP phenotype might also play a role in identification of CSC phenotype. Further studies of the CSC-specific marker in endometrium unravel its role in endometrium associated disorders.

### Endometrial Stem Cells and Adenomyosis

Adenomyosis, a condition affecting 1 % of women, is characterized by the benign invasion of basal endometrial glands and stroma deep into the myometrium and is associated with smooth muscle hyperplasia. Little is known of the pathophysiology of adenomyosis. Adult stem cells are frequently activated in tissue injury, and it is possible that these have a role in establishing the ectopic lesions and their abnormal differentiation may be responsible for the smooth muscle hyperplasia [68]. Alterations in the orientation of endometrial stem/progenitor cells or their niche may be a major

cause of the abnormal behavior in adenomyosis. The cells undergo differentiation toward the myometrium rather than toward the functionalis, producing pockets of endometrial tissue deep within the myometrium. It was recently shown that stromal cells cultured from adenomyotic tissue undergo multilineage mesodermal differentiation and express MSC surface phenotypic markers [57]. It is not known if these adenomyotic stromal cells are clonogenic, self-renew, or contain a population of CD 146<sup>+</sup>PDGFRb<sup>+</sup> cells. This supports the idea that endometrial stem cells might be the major cause of adenomyosis. However, more research is required to establish this.

### Unresolved Barriers and Concluding Remarks

Regardless of the strong evidence on the existence of adult stem cells in endometrium, the endometrial stem cell research is at its infancy. Major advances have been made to identify the populations of epithelial and stromal cells with stem/progenitor activity in human endometrium that is responsible for its remarkable regenerative capacity. Despite the current advancements, there still remain several unresolved barriers that hinder the potential applications of endometrial-derived stem cells in regenerative medicine. Whether these endometrial stem cells possess MSC activity *in vivo* and whether the cultures of menstrual blood/endometrial tissue-derived stem cells are equal has yet to be determined. Although certain markers of endometrial stem cells have been identified, there still remains a need for definitive markers of both endometrial stem/progenitor cells for more selective isolation, enrichment, and possible use in therapeutic approaches. Besides, complete phenotypic and functional characterization of uterine stem cells, inclusive of the menstrual blood and endometrial tissue-derived stem cells, will improve our understanding of the mechanism supporting physiological regeneration of the female reproductive tract. As endometrial stem cells become further better characterized, their role in gynecological disorders associated with abnormal endometrial proliferation can be assessed. Furthermore, these efforts have the potential to change the way these diseases may be treated in the future, particularly as therapeutic agents that target key stem cell functions becomes available.

Undoubtedly, it can be concluded that endometrial stem cells may become key players in regenerative medicine because of their noninvasive mode of collection, ease of isolation, its enhanced proliferative ability, and multiple differentiation potentials. Furthermore, the easy access to the endometrial stem cells through the menstrual cell mass and their potential for storage may allow greater applicability for cell therapeutics than other stem cells that are more difficult to obtain. Together, augmented studies of the characteristics

of putative uterine stem cells of endometrial tissue-derived and menstrual blood-derived stem cell population might determine how faulty adult stem cells of the uterus contribute to gynecological disorders as well as explore its applicability in cell-based therapies for treating a wide range of diseases.

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