International Journal of Clinical Obstetrics and Gynaecology

ISSN (P): 2522-6614
ISSN (E): 2522-6622
© Gynaecology Journal
www.gynaecologyjournal.com
2019; 3(1): 199-204
Received: 17-11-2018
Accepted: 23-12-2018

Dr. Roya Rozati
M.B.B.S., M.D., F.R.C.O.G., Professor and Head, Department of Obstetrics and Gynaecology, Owaisi Hospital and Research Centre; Medical and Research Director, M.H.R.T, Banjara Hills, Hyderabad, Telangana, India

Wajeeda Tabasum
Maternal Health and Research Trust, Telangana, India

Ayapati Gautam Mehdi
Maternal Health and Research Trust, Telangana, India

Ayapati Vikram Aiman
Maternal Health and Research Trust, Telangana, India

Dr. Avinash Bhadria
Owaisi Hospital and Research Centre, Telangana, India

Dr. Sumaya Fatima
Pharm-D, Department of Pharmacy Practice, Deccan School of Pharmacy, Owaisi Hospital and Research Centre, Hyderabad, Telangana, India

Correspondence
Dr. Roya Rozati
drroyarozati@gmail.com
M.B.B.S., M.D., F.R.C.O.G., Professor and Head, Department of Obstetrics and Gynaecology, Owaisi Hospital and Research Centre; Medical and Research Director, M.H.R.T, Banjara Hills, Hyderabad, Telangana, India

Identification of cells without colony-forming ability in Eutopic endometrial progenitor cells in infertile women with endometriosis in comparison with healthy fertile women in Indian perspective

Dr. Roya Rozati, Wajeeda Tabasum, Ayapati Gautam Mehdi, Ayapati Vikram Aiman, Dr. Avinash Bhadria and Dr. Sumaya Fatima

DOI: https://doi.org/10.33545/gynae.2019.v3.i1d.33

Abstract
Endometriosis is a chronic benign gynecological disease characterized by the presence of ectopic endometrial tissue outside of the uterus cavity. Human Endometrium is a highly regenerative tissue. The aim of our study is to screen potential stem cell markers for the prospective isolation of human endometrial stromal stem/progenitor cells. To determine their capacity to identify colony forming in eutopic endometrial progenitor Cells in infertile Women and healthy Women with Endometriosis in Indian perspective. This study identified CD90 marker of colony-forming human endometrial stromal cells are found to be positively stained.

Keywords: Endometrium, progenitor cells, endometriosis, clonogenicity, infertility

1. Introduction
Endometriosis is chronic benign gynecological disorder which is characterized by a growth of endometrial tissue outside the uterine cavity. Human endometrium undergoes cyclical processes of growth, differentiation, shedding, and regeneration as part of the menstrual cycle during the reproductive life of women (Maruyama T *et al*. 2008) [24]. Estimates shows that endometriosis affects 10-15% of all women population during reproductive age with pelvic pain and infertility (Sasson IE *et al*. 2008, Allaire C 2006) [32, 2] It is frequently associated with dysmenorrhea, menorrhagia and dyspareunia, leading to infertility (Surrey ES 2003, Oral E *et al*. 1997) [34, 29]
The pathological condition involves adhesion, proliferation, and development of the endometrial implants in ectopic regions such as the ovary and the peritoneal cavity. The pathogenesis of endometriosis is ascertained but there are 3 different entities which are involved in endometriosis are ovarian, peritoneal endometriosis and deep endometriotic nodules (Nisolle M *et al*. 1997) [28]. There are evidence which indicates that eutopic endometrium in women suffering from endometriosis is different from that of healthy controls. Apart from contributing factors like genetic predisposition, environmental factors, hormonal, alterations immune and endocrine functions plays a crucial role in the pathogenesis and etiology of endometriosis (Bondza P.K *et al*. 2009, Carvalho, L 2011 and Jensen, J.R 2010) [3, 4, 21]. The knowledge about the etiology and pathogenesis of this disease still remain uncertain, but there are a number of leading theories including retrograde menstruation, altered immunity, coelomic metaplasia, and metastatic spread.
Endometriosis is an estrogen-dependent benign inflammatory disease characterized by the presence of ectopic endometrium (Giudice *et al*. 2004) [18]. The role of eutopic endometrium in endometriosis-related infertility is still unclear due to a lack of understanding about the normal physiologic mechanisms. The eutopic endometrial glandular and Stromal cells may be functioning differently in women with endometriosis compared to normal women. These cells have characteristics which have favored the survival outside the uterine cavity and precede development of well-documented changes at the peritoneum and other ectopic sites (Akoum A *et al*. 2006) [1]. During menstruation, the endometrial cells in endometriosis patients could escape immune surveillance from the body and are less susceptible to apoptosis, resulting in an increase in viable cells. After overcoming a phase of immune tolerance, the next step in the development of early endometriosis is the adhesion of endometrial cells to mesothelium and

invasion of the extracellular matrix, since the eutopic endometrium of women with endometriosis are more adhesive and invasive normal endometrium. After the last step of angiogenesis, the endometrial cells establish a new blood supply for the survival of implants, continue to proliferate in ectopic sites, and finally results in endometriosis

In a regular event, in response to the mounting levels of estrogen, new functionalis layer begins to grow during Proliferative phase (Ferenczy A *et al.* 1979) [11]. Rising progesterone in secretory phase blocks epithelial mitosis, and cells undergo differentiation.

A striking feature of the human endometrium is spontaneous decidualization of the stromal compartment during the mid-luteal phase of each cycle, a process also responsible for the menstrual shedding of the endometrium in the absence of pregnancy (Lam EW *et al* 2012) [23]. The colony forming units have self-renewal capacity by undergoing cloning in vitro (Gargett, C.E 2007, Morrison, S.J 1997) [16, 27]. Adult stem cells in human endometrium are clonogenic which are idenitified from small population of colony forming units (CFU). The human endometrium contain small population of clonogenic epithelial (0.22%) and stromal cells (1.25%), exhibiting stem-cell function in vitro. (chan rw 2004) [6] The clonogeniety of cells from proliferative, secretory, and inactive endometrium was demonstrated too. (schwab ke 2005) [33]

The human endometrium contained small populations of epithelial progenitor cells and MSC-like cells (Gargett, C.E. 2009) [13, 14]. Cultured endometrial stromal cells also differentiate into mesodermal lineages and lineages of ectodermal and endodermal origin (Wolff, E. F *et al.*2007, Wolff E. F *et al.* 2010, Santamaria X *et al*. 2011, Dimitrov, R. *et al*. 2008) [37, 36, 31, 8] indicating that endometrial stromal cells have considerable plasticity.

The human endometrium exhibits remarkable regenerative capacity (Gargett CE *et al.* 2012) [12] which is rich in mesenchymal stem-like cells (eMSCs), and are immuno-privileged compared to other types of stem-like cells, rendering them a promising resource for cell-based therapies Santamaria, X *et al.* 2011, Wolff, E.F *et al.* 2011, and Ulrich D *et al.* 2013) [31, 35, 38]. In the absence of implantation, the functionalis relapses and sheds during menstruation, commencing a new cycle.

Recent studies have been demonstrated that stem- progenitor cells play an important role in the onset of gynecological diseases such as endometriosis (Gargett CE *et al*. 2008) [15]. In stem cells, the human endometrium contains a small population of endometrial epithelial and stromal cells with high proliferative potential (Chan RW *et al*. 2004, Gargett CE *et al.* 2005) [6, 17]

Mesenchymal or stromal stem cells are considered as separate stem cell population which (MSC) have several stroma-containing tissues, including bone marrow, synovial fluid, dental pulp, adipose tissue, cord blood and skeletal muscle Minguell JJ *et al.* 2001, Romanov YA *et al.* 2003) [26, 30]. MSC have the ability to differentiate into cells of a different phenotype than their tissue of origin (Herzog EL *et al*. 2003, Grove JE *et al.* 2004) [20, 19]. Various markers have been used to isolate MSC. Markers which have been used to partially purify MSC include CD34 and CD90. CD90 is an accepted marker of cultured MSC[27] and it has never been used to isolate MSC, but rather has been used in combination with other negative markers.

The aims of this study were to screen potential stem cell markers for the prospective isolation of human endometrial stromal /progenitor cells, to determine the capacity to identify colony Forming in eutopic Endometrial progenitor Cells in infertile Women and healthy Women with Endometriosis in Indian perspective and the location of cells expressing these markers in human Endometrium. This study used a colony-forming assay as a screening test for identifying potential markers of endometrial Stromal stem/progenitor cells.

2. Materials and Methods

Clinical samples of endometriotic tissues were collected from 30 patients from reproductive age group between 18-35 years were collected at the Maternal Health research Trust and Owaisi Hospital and Research Centre, Hyderabad, who underwent laparoscopic surgery between Aug 2015 – July2017. Informed written consent was obtained from each patient as a part of the study protocol.

Out of 30 patients 15 were diagnosed with stage1 endometriosis who had not taken exogenous hormones for 3 months prior to surgery were only included. Menstrual cycle stage, assessed by histological examination according to well-established criteria, was obtained from pathology reports.

3. Methodology

Endometrial tissue samples were collected in 1 X Phosphate buffered saline with 10% antibiotic anti-mycotic solution. Samples were maintained at 4 ºC AND processed. All samples were processed within 2 hrs from collection. The tissues were digested with collagenase III (HyClone Laboratories, USA) for 30 mins at 37 ºC. Samples were pipetted vigorously in between. The digestion was stopped by adding absolute media to the samples. All the samples were centrifuged at 100g X 10 mins and the pellet was suspended in 2 ml media. All the samples are cultured at 37 ºC with 5% CO_2 in a humidified incubator. After surgery, endometrial biopsies were fixed in formaldehyde fixed, and hematoxylin-stained cross sections were analyzed by experienced histopathologists for assessment of the grade of endometriosis (I–IV) and for determination of the stage of the menstrual cycle (proliferative or secretory), referring to established histological criteria

3.1 Cell culture

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) with 1% Antibioticantimycotic solution (Gibco), and 15% FBS (HyClone Laboratories). The culture medium was replaced every 48 h. For passaging, the cells were washed with 1X PBS (pH 7.2) and treated with 0.25% trypsin-EDTA (Gibco) for 15 min at 37 ºC, complete DMEM was added to stop the trypsinisation reaction. The cells were centrifuged at 100g X 10 mins and resuspended in culture medium. The cells were cultured till three passages (P3) before clonogenic assay.

3.2. Clonogenic assay

Cultures, out of passage 3 (P3), were plated at a density of 100 cells/cm^2 in DMEM (Gibco), along with 1% Antibiotic anti-mycotic solution (Gibco), and 15% FBS. Colony formation was monitored regularly. On day 14, cells were fixed with absolute methanol for 2 minutes and stained with 1% crystal violet aqueous solution for 5 minutes. (Clones or colony-forming units (CFUs) consisting of 50 cells were counted to determine the cloning efficiency (CE) percentage, which was the number of colonies formed per seeded cell multiplied by 100.) Colonies with more than 50 cells were counted for the assay. Each assay was repeated twice, and cloning efficiency [CE] was calculated as CE% = (n. clones/cells seeded) X 100.

4. Statistical analysis

Colony-forming capacity was performed using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA, USA). Unpaired *t*-Test was performed to evaluate the difference between clonal efficiency in women with and without endometriosis. Data is presented as Mean ± SEM. Variable difference with $P < 0.05$ is considered statistically significant. The sample size was determined by using the open EPi statistics and 95% of confidence was used to detect the results with 90% of sample power.

5. Results

Small populations of human endometrial stromal cells expressed each of the markers. CD90 was strongly expressed by functionalis stroma and perivascular cells, but only weakly expressed in the basalis stroma. This study identified CD90 as a candidate marker of colony-forming human endometrial stromal cells supporting the concept that human endometrium contains a population of stromal stem/progenitor cells.CD90 has been linked to spindle shape cells. Primarily both epithelial and Stromal cells were cultured. Epithelial cells died around 7-8 days of culture. After 10-14 days, the cultures consist of spindle like cells. With each passage, the cell population became more homogenous, and after third passage the spindle like cells were the only type detected in the culture. The results clearly demonstrate that endometrial cells are negative for CD34 and positively stained for CD90 (fig: 1)

The clonogenicity of endometrium from women with endometriosis (n = 15) and without endometriosis (n = 15) was compared. The total clonogenic efficiency of endometrial progenitor cells was significantly greater (0.25 ± 0.03%) in fertile women compare with endometriosis (0.13 ± 0.01%, $p < 0.05$). The categorization of colony size for endometrial progenitor cells was observed in of both the groups. Small CFUs were defined as comprising <4000 large loosely-packed cells and large CFUs as comprising > 4000 cells with a dense center of tightly packed cells. During the first week of culture, the growth rates for the two colony types were similar, with colonies generally comprising <100 cells after 7 days. Around day 10 to day 11, however, small CFUs stopped proliferation and maintained their size, but the growth of some colonies increased dramatically and formed large CFUs containing as many as 15,000 cells by day 14 (Figure 2a, 2b, 2c). There is no significant difference in the larger colonies between the woman without endometriosis and with endometriosis (0.11 ± 0.01% *vs* 0.05 ± 0.01 respectively). Smaller colonies also did not reveal any difference between the woman without endometriosis and women with endometriosis (0.14 ± 0.02% *vs* 0.06 ± 0.02% respectively) (Table 1).

Table 1: Colony Efficiency of Stromal Cells with and without Endometriosis

Samples	CE %	*P*- Value	Colonies	CE %	*P*- Value
With Endometriosis (n=15)	0.13 ± 0.01	P = 0.0007*	Large	0.05 ± 0.01	0.32**
			Small	0.06 ± 0.02	
Without Endometriosis (n=15)	0.25 ± 0.03		Large	0.11 ± 0.01	0.19**
			Small	0.14 ± 0.02	

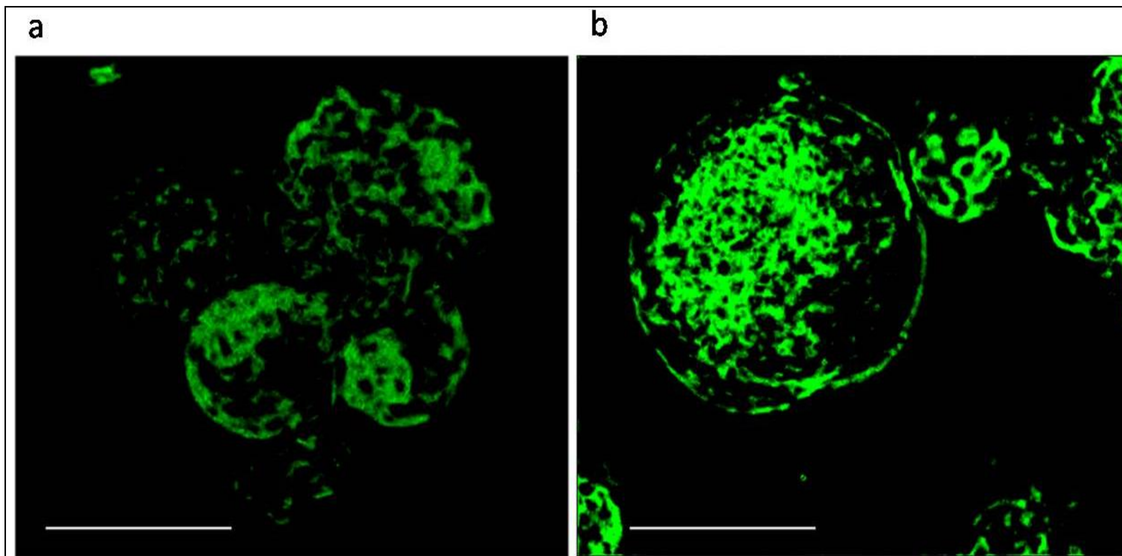
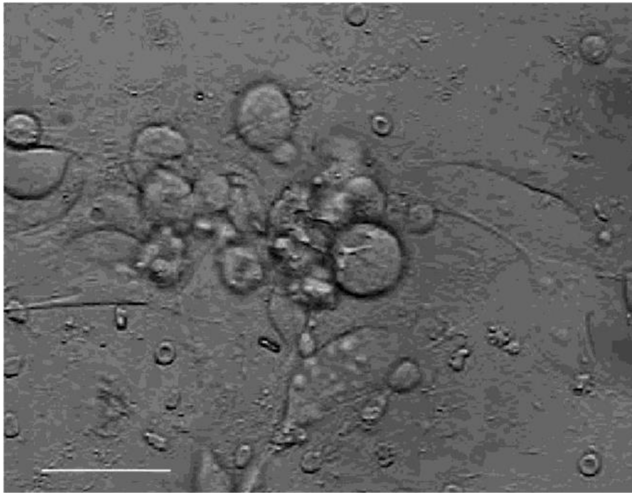
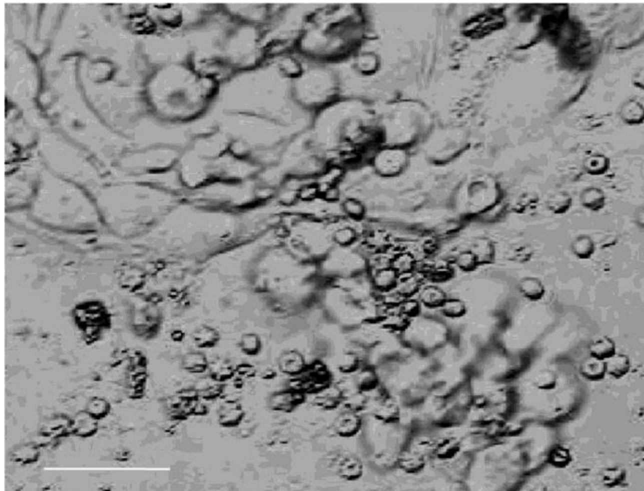


Fig 1: Depicting that cells are positive for CD90 (Green in colour) which are loosely arranged and tightly arranged. But they are negative for CD34 because the cells are not stained with any colour.

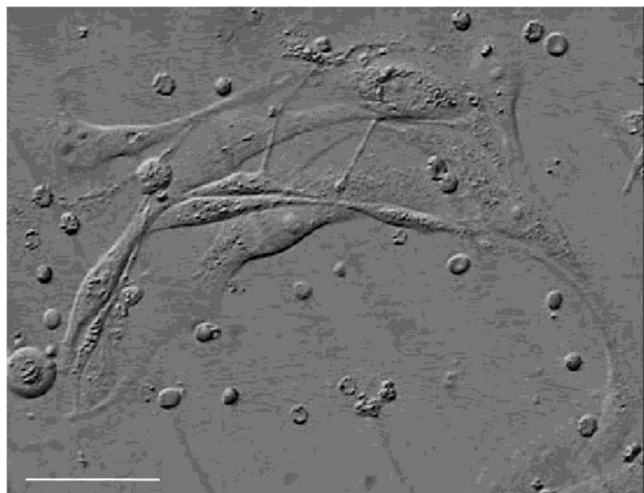
(a) Loosely arranged cells in colony showing positive expression for CD90-FITC and negative for CD34-PE with endometriosis **(b)** Tightly arranged cells in colony showing highly positive expression for CD90-FITC and negative for CD34-PE without endometriosis



(2a) Small loosely-arranged colonies



(2b) Large densely-packed colonies



(2C) Characteristic Spindle-like morphology of the cultured cells are positive for CD90

Fig 2: Adherent cells on day 4

6. Discussion

The biology of MSCs has been mainly studied due of its therapeutic potential.

Endometrial stromal cells are located not only in the basalis, but also in the functionalis, have the ability to reconstruct endometrial tissue *in vivo* suggests their potential use for treating disorders associated with inadequate endometrium. The identification of specific markers for human endometrial MSC has demonstrated their perivascular location in the basalis and functionalis.

Our study provides the evaluation of Cells without Colony-Forming in eutopic Endometrial progenitor Cells in infertile Women with Endometriosis compared to healthy fertile women in Indian perspective. The colony-forming ability of eutopic human endometrial samples with and without has been studied earlier without any significant difference in the total clonogeniety (Makarainen L 1988, Chan *et al*. 2011) [25, 5].

Our study shows the significant difference in the clonogenic progenitor cells with $p<0.05$ in infertile women on comparision with fertile women without endometriosis. Hence there is no significant difference in large and small colonies in both the groups. thus it can be assumed that the cells without clonogenic stem cell cells play an important role in the abnormal function of endometrium during implantation.

Therefore, we decided to study the function of CD90, one main immunophenotypical marker of MSCs.CD90 has been identified as a candidate marker for MSCs. Endometriosis seems to have a negative impact, on every part of the reproductive process subtly but significantly. Infertility associated with endometriosis can be even more puzzling, as not every patient experiences the same symptoms.

The human endometrium exhibits 0.15% of clonogenic epithelial and 1.3% stromal cell populations (Chan, R.W *et al* 2004, Schwab, K.E *et al* 2005) [6, 33]. Clonogenicity studies of human stromal stem/progenitor cells have been studied d by *in vitro in various* differentiation assays

CD90 is a potential marker for human endometrial stromal Colony Forming Units that distinguishes basalis and functionalis stroma (Koumas L *et al* 2011). This study used CD90 in combination with CD34 as a negative marker (multipotent) and showed a trend to enrichment for CFU in the CD90 stromal cell population. Thus, the combination of CD90 with other markers may increase its value for identifying and isolating purer populations of endometrial stromal stem/progenitor cells. CD90 in MSCs represents a promising alternative strategy and an efficient approach to increase MSC differentiation efficiency in vitro; it may, therefore, be used in the future to improve MSC differentiation yields in cellular therapy.

In conclusion, we have evaluated that CD34, CD90 as hematopoietic markers of human endometrial stromal CFU. Increasing number of studies has shown that MSCs from different sources display significantly diverse properties and characteristics that may impact on their future therapeutic applications. The capacity of differentiation may vary according to the cell source (Schwab, K.E *et al*. 2005) [33]. CD90 may play an important role in maintaining the undifferentiated state of MSCs our findings indicate that a small population of endometriotic cells exhibits colony-forming activity, self-renewal capacity, and multi potency. To determine any difference between cells in endometriosis and those from endometrium, we compared the colony-forming activity of the endometrium and of the same patient, to avoid possible variation due to individual difference in genetic background. Significantly more clonogenic cells were detected from the endometrium in our conclusion the study suggests that there is a significant difference in the colony forming ability of the eutopic endometrium cells in women with endometriosis in comparison with the healthy fertile women in the Indian perspective.

The eutopic endometrium from women with endometriosis shares certain alterations with ectopic lesions that are not

observed in the endometrium from healthy fertile women. It also provides an understanding of not only the physiology of endometrium, but also the path physiology of endometrial endometriosis. The molecular and cellular mechanisms which are involved in the regulation of progenitor cells in the eutopic endometrium in women with endometriosis leads to better understanding of Endometrial stem cell research is gaining momentum and the knowledge generated may be translated into the clinic within the next decade

7. Authors Contribution

All authors contributed equally to this work. Dr. Roya Rozati along with other authors discussed the methodology and results and also helped in preparing the manuscript at all stages.

8. References

1. Akoum A, Metz CN, Al-Akoum M, Kats R. Macrophage migration inhibitory factor expression in the intrauterine endometrium of women with endometriosis varies with disease stage, infertility status and pelvic pain. Fertil Steril, 2006; 85:1379-85
2. Allaire C. Endometriosis and infertility: A review. J Reprod Med. 2006; 51:164-168
3. Bondza PK, Maheux R, Akoum A. Insights into endometriosis- associated endometrial dysfunctions: a review. Front. Biosci, 2009.
4. Carvalho L, Podgaec S, Bellodi-Privato M, Falcone T, Abrao MS. Role of eutopic endometrium in pelvic endometriosis. J Minim. Invasive Gynecol. 2011; 18:419-427.
5. Chan RW, Ng EH, Yeung WS. identification of cells with colony forming activity, self renewal capacity and multipotency in ovarian endometriosis. Med Sci Monit. 2011; 17:92-99
6. Chan RW, Schwab KE, Gargett CE. Clonogenicity of human endometrial epithelial and stromal cells. Biol Reprod. 2004; 70:1738-50.
7. Divya MS, Roshin GE, Divya TS, Rasheed VA, Santhoshkumar TR, Elizabeth KE *et al*. Umbilical cord blood-derived mesenchymal stem cells consist of a unique population of progenitors co-expressing mesenchymal stem cell and neuronal markers capable of instantaneous neuronal differentiation Stem Cell Res Ther. 2012; 3(6):57
8. Dimitrov R, Timeva T, Kyurkchiev D, Stamenova M, Shterev A, Kostova P *et al*. Characterisation of clonogenic stromal cells isolated from human endometrium. Reprod. 2008; 135:551-558
9. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006; 8:315-317.
10. Feng J, Mantesso A, De Bari C, Nishiyama A, Sharpe PT. Dual origin of mesenchymal stem cells contributing to organ growth and repair. Proc Natl Acad Sci USA. 2011; 108:6503-8. doi:10.1073/pnas.1015449108
11. Ferenczy A, Bertrand G, Gelfand MM. Proliferation kinetics of human endometrium during the normal menstrual cycle. Am J Obstet Gynecol 1979; 133:859-867
12. Gargett CE, Nguyen HP, Ye L. Endometrial regeneration and endometrial stem/progenitor cells. Rev Endocr Metab Disord. 2012; 13:235-251.
13. Gargett CE, Schwab KE, Zillwood RM, Nguyen HPT, Wu D. Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. Biol Reprod. 2009; 80:1136-1145
14. Gargett CE, Schwab KE, Zillwood RM, Nguyen HPT, Wu D. Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. *Biol Reprod*. 2009; 80:1136-1145.
15. Gargett CE, Chan RW, Schwab KE. Hormone and growth factor signaling in endometrial renewal: role of stem/progenitor cells. Mol Cell Endocrinol. 2008; 288:22-29.
16. Gargett CE. Uterine stem cells: what is the evidence?. Hum Reprod Update. 2007, 13.
17. Gargett CE, Zillwood R, Schwab KE. Characterising the stem cell activity of human endometrial epithelial and stromal cells. Reprod Fertil Dev 2005, 17
18. Giudice LC, Kao LC. Endometriosis. Lancet. 2004; 364(9447):1789-1799. Epub 2004/11/16
19. Grove JE, Bruscia E, Krause DS. Plasticity of bone marrow-derived stem cells. Stem Cells. 2004; 22:487-500
20. Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. Blood. 2003; 102:3483-3493.
21. Jensen JR, Coddington 3rd CC. Evolving spectrum: the pathogenesis of endometriosis. Clin. Obstet. Gynecol. 2010; 53:379-388.
22. Koumas L, King AE, Critchley HO, Kelly RW, Phipps RP. Fibroblast heterogeneity: existence of functionally distinct Thy 1(þ) and Thy 1(-) human female reproductive tract fibroblasts. Am J Pathol. 2001; 159:925-935.
23. Lam EW, Shah K, Brosens J. The diversity of sex steroid action: the role of micro-RNAs and FOXO transcription factors in cycling endometrium and cancer. J Endocrinol. 2012; 212:13-25.
24. Maruyama T, Yoshimura Y. Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. Endocr. J. 2008; 55:795-810.
25. Mäkäräinen L. Uterine contractions in endometriosis: effects of operative and danazol treatment. J Obst Gyna. 1988; 9:134-138.
26. Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. Exp Biol Med (Maywood). 2001; 226:507-520
27. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell. 1997; 88:287-298.
28. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. Fertil Steril. 1997; 68:585-596
29. Oral E, Arici A. Pathogenesis of endometriosis Obstet Gynecol Clin North Am. 1997; 24:219-33.
30. Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. Stem Cells. 2003; 21:105-110
31. Santamaria X, Massasa EE, Feng Y, Wolff E, Taylor HS. Derivation of insulin producing cells from human endometrial stromal stem cells and use in the treatment of murine diabetes. Mol Ther. 2011; 19:2065-2071.
32. Sasson IE, Taylor HS. Stem cells and the pathogenesis of endometriosis. Ann N Y Acad Sci. 2008; 1127:106-115.
33. Schwab KE, Chan RW, Gargett CE. Putative stem cell activity of human endometrial epithelial and stromal cells during the menstrual cycle Fertil Steril. 2005; 84(2):1124-1130
34. Surrey ES, Schoolcraft WB. Management of endometriosis-associated infertility. Obstet Gynecol Clin North Am. 2003;

30:193-208.
35. Wolff EF, Gao XB, Yao KV, Andrews ZB, Du H *et al*. Endometrial stem cell transplantation restores dopamine production in a Parkinson's disease model. J Cell Mol Med. 2011; 15:747-755.
36. Wolff EF, Gao XB, Yao KV, Andrews ZB, Du H, Elsworth JD *et al*. Endometrial stem cell transplantation restores dopamine production in a Parkinson's disease model. J Cell Mol Med. 2010; 15:747-755
37. Wolff EF, Wolff AB, Du H, Taylor HS. Demonstration of multipotent stem cells in the adult human endometrium by in vitro chondrogenesis. Reprod Sci. 2007; 14:524-533
38. Ulrich D, Muralitharan R, Gargett CE. Toward the use of endometrial and menstrual blood mesenchymal stem cells for cell-based therapies. Expert Opin Biol Ther. 2013; 13:1387-1400.