

Potential role of endometrial stem/progenitor cells in the pathogenesis of early-onset endometriosis

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Submitted on January 13, 2014; resubmitted on March 13, 2014; accepted on March 19, 2014

ABSTRACT: The pathogenesis of early-onset endometriosis has recently been revisited, sparked by the discovery of endometrial stem/progenitor cells and their possible role in endometriosis, and because maternal pregnancy hormone withdrawal following delivery induces uterine bleeding in the neonate. The neonatal uterus has a large cervix to corpus ratio which is functionally blocked with mucus, supporting the concept of retrograde shedding of neonatal endometrium. Only 5% show overt bleeding. Furthermore, the presence of endometriosis in pre-menarcheal girls and even in severe stage in adolescents supports the theory that early-onset endometriosis may originate from retrograde uterine bleeding soon after birth. Endometrial stem/progenitor cells have been identified in menstrual blood suggesting that they may also be shed during neonatal uterine bleeding. Thus, we hypothesized that stem/progenitor cells present in shedding endometrium may have a role in the pathogenesis of early-onset endometriosis through retrograde neonatal uterine bleeding. During the neonatal and pre-pubertal period, shed endometrial stem/progenitor cells are postulated to survive in the pelvic cavity in the absence of circulating estrogens supported by niche cells also shed during neonatal uterine bleeding. According to this hypothesis, during thelarche, under the influence of rising estrogen levels, endometrial stem/progenitor cells proliferate and establish ectopic endometrial lesions characteristic of endometriosis. This New Research Horizon review builds on recent discussions on the pathogenesis of early-onset endometriosis and raises new avenues for research into this costly condition.

Key words: endometrial stem / progenitor cells / endometriosis / neonatal uterine bleeding / pathogenesis / somatic stem cells

Introduction

A new theory has been recently outlined to explain early-onset endometriosis (Brosens and Benagiano, 2013), based on the finding that neonatal endometrial changes can induce uterine bleeding. A classic pathology study of 169 neonatal infants (Ober and Bernstein, 1955) showed decidualization of the endometrium or menstrual changes in 5%, secretion in 27% and proliferation in 68%. Interestingly, a clinical study of 75 neonates (Kaiser and Grassel, 1974) described grossly visible bleeding in 5.3% and occult bleeding, as detected by the presence of haemoglobin, in 61.3%. Unfortunately, there is no information on how the bleeding correlates with endometrial changes. The long cervical canal (cervix-corpora ratio of 2/1) that is plugged with thick mucus could promote the retrograde flux of menstrual debris containing endometrial cells (Fig. 1) (Terruhn, 1980). Indeed, a case of endometrial epithelium implantation on the bowel serosa in a newborn with hydrometrocolpos has been documented in the literature (Arcellana et al., 1996). The above-described situation,

together with the observation of a significantly increased risk of endometriosis in adolescents with cervical outflow obstruction and patent Fallopian tubes, supports the theory that endometriosis, especially in adolescents, may originate from retrograde uterine bleeding soon after birth (Fig. 1) (Brosens et al., 2013). In evaluating the pathogenesis of endometriosis, besides the classic mechanisms, recently a possible role of endometrial stem/progenitor cells (eSPC) has been hypothesized and discussed (Gargett, 2007b; Maruyama and Yoshimura, 2012). A review of existing knowledge on endometrial eSPC may shed light on the likelihood that they may be involved in early-onset, as well as adult endometriosis.

Endometrial stem/progenitor cells

The identification of the full potential of embryonic stem cells (ESC) found in the inner cell mass of the blastocyst capable of differentiating

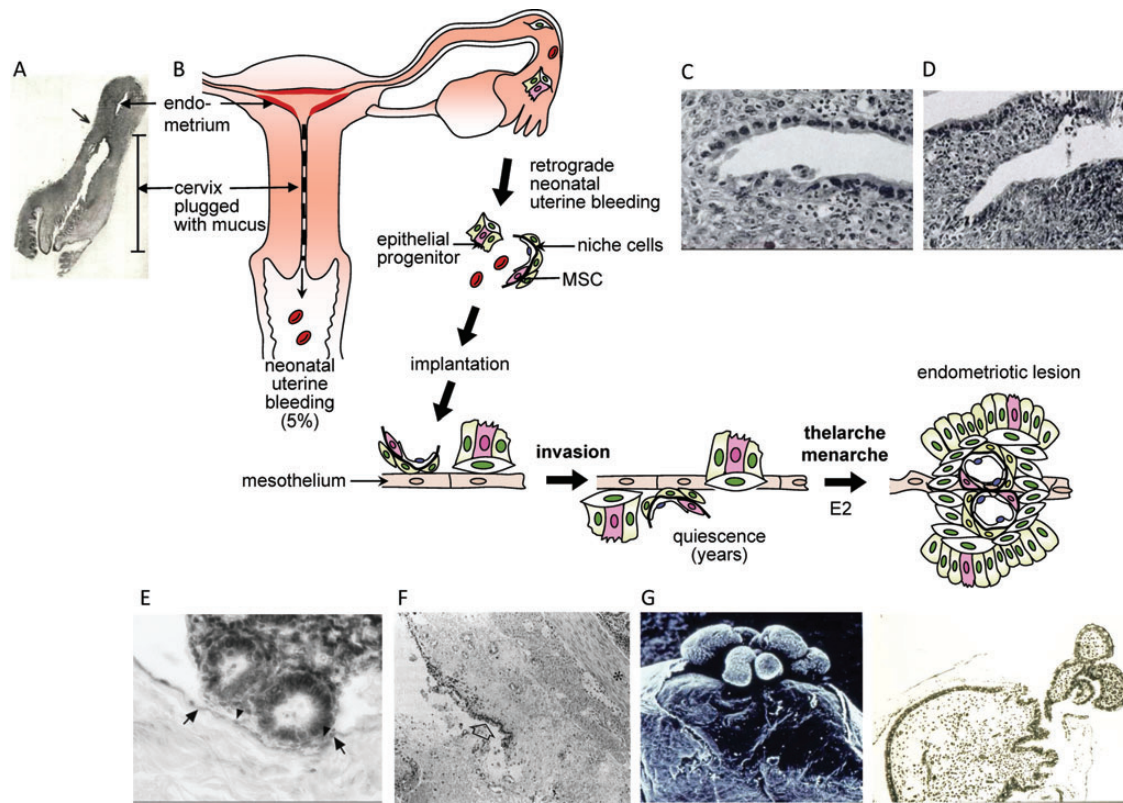


Figure 1 Schematic describing the hypothesis that endometrial stem/progenitor cells may play a role in early-onset endometriosis with supporting images from published works. **(A)** Neonatal uterus and vagina showing relatively long cervix in comparison to the uterine body. The arrow indicates the corpus-cervical junction. Mucus has been removed from the cervix. **(B)** Schematic showing neonatal uterine bleeding (which occurs in 5% of neonates) and hypothesized retrograde neonatal bleeding due to cervical obstruction by thick mucus in the long neonatal cervix. The fragments of shed endometrial tissue are postulated to contain an endometrial epithelial progenitor cell (pink) and a perivascular mesenchymal stem/stromal cell (MSC) (pink) together with niche cells. These rapidly adhere to the neonatal mesothelium, invade and/or become contiguous with the mesothelial lining where they remain in a quiescent state for ~10 years. Rising estrogen (E2) levels associated with thelarche and menarche reactivate the stem/progenitor cells to initiate the growth of endometriosis lesions on the surface of or below the peritoneal mesothelium. Neonatal **(C)** decidualized and **(D)** shedding endometrium. **(E)** Endometrial attachment to the mesothelium occurs within 1 h and **(F)** implantation by 18 h, with endometrial cells becoming contiguous with the mesothelium (arrow) before the onset of quiescence. **(G)** Scanning electron microscopy (left) and histological section (right) of a peritoneal endometriotic implant showing a polypoid lesion extending through the mesothelium in a young girl after a decade of quiescence. Images reprinted with permission from: Lippincott Williams and Wilkins/Wolters Kluwer Health, (A) Fig. 3 from [Fluhmann \(1960\)](#) and (G) Figs 1 and 2c from [Cornillie et al. \(1986\)](#); American Academy of Pediatrics, (C, D) Fig. 2 from [Ober and Bernstein \(1955\)](#); Elsevier, (E) Fig. 2B from [Witz et al. \(2001\)](#); Macmillan Publishers Ltd, (F) Fig. 1 from [Arcellana et al. \(1996\)](#).

into all cell types marked the beginning of a revolution not only in embryology, but in biology at large. Indeed, ESC are able to proliferate without limit and can form not only derivatives of the three embryonic germ layers, but also extra-embryonic tissues ([Xu et al., 2002](#); [Edwards, 2004](#)). As embryonic development proceeds, the potentiality of ESC progressively diminishes as they give rise to a variety of multipotent somatic stem cells (SSC) with the ability to replicate without differentiating (self-renew), which have now been found in almost every tissue and organ, including the endometrium. Indeed, human ESC have been induced to differentiate into endometrial gland-like cells in a xenograft model by growth factors and neonatal mouse uterine mesenchyme ([Ye et al., 2011](#)).

More than 30 years ago it was postulated that the endometrium, with its ability to cyclically regenerate each month, contained a population of SSC ([Pranishnikov, 1978](#)). However, only in 2007 was there enough direct and indirect evidence of the existence of endometrial stem/

progenitor cells for publication of the first major review on the subject ([Gargett, 2007b](#)). This review stressed the importance of verifying SSC activity using both *in vitro* (clonogenicity, proliferative potential, self-renewal and differentiation) and *in vivo* (tissue reconstitution and self-renewal) assays. One of the indirect pieces of evidence highlighted was that endometrial glands are monoclonal in origin, suggesting that they arise from a single epithelial progenitor cell. Since then, a potential role of endometrial stem/progenitor cells in cyclical endometrial regeneration and in the pathogenesis of gynaecological disorders associated with abnormal endometrial proliferation such as endometriosis has been postulated ([Gargett, 2007b](#); [Sasson and Taylor, 2008](#); [Oliveira et al., 2012](#)).

Over the last few years, research into the identification of endometrial stem/progenitor cells has made substantial progress, from the first cell cloning studies ([Chan et al., 2004](#); [Schwab et al., 2005](#)), to more sophisticated *in vivo* models, where the morphological and functional changes of the endometrium during the menstrual cycle were recapitulated in

xenografts derived from unfractionated single-cell suspensions of human endometrial cells implanted under the kidney capsule (Masuda *et al.*, 2007). Two stem/progenitor cell populations have been identified in the endometrium: epithelial progenitor cells (eEPC) and mesenchymal stem cells (eMSC). Both are clonogenic, have high proliferative potential, undergo self-renewal *in vitro*, differentiate into mature progeny and reconstitute tissue *in vivo* (Chan *et al.*, 2004; Masuda *et al.*, 2007; Gargett *et al.*, 2009). Further studies supporting the existence of adult endometrial stem/progenitor cells include those where cultured clonally derived endometrial stromal cells (Schwab *et al.*, 2005; Dimitrov *et al.*, 2008; Gargett *et al.*, 2009), or simple stromal cell cultures have been induced to differentiate into several mesodermal (Wolff *et al.*, 2007) and also ectodermal lineages (Wolff *et al.*, 2007, 2011).

Following the identification of stem/progenitor cell activity in human endometrium, researchers turned their attention to finding ways for identifying these SSC to enable their prospective isolation for further characterization and to examine their role in gynaecological disease. Surface markers enriching for eMSC have now been identified. When cultured these double expressing CD146 and Platelet-derived growth factor receptor β (PDGFR β) (Schwab and Gargett, 2007) or W5C5 expressing (Masuda *et al.*, 2012) cells have broad differentiation capacity with properties and phenotype similar to other MSC. W5C5⁺ cells can also reconstitute endometrial stroma under the kidney capsule in a xenograft model (Masuda *et al.*, 2012). These CD146⁺PDGFR β ⁺ or W5C5⁺ eMSC may be involved in the process of endometrial stromal vascular regeneration in each menstrual cycle.

The identification of markers for eEPC has been technically more difficult to achieve, but several recent studies have now found both markers (Valentijn *et al.*, 2013) and signalling pathways (Nguyen *et al.*, 2012) that distinguish basalis from functionalis epithelium, thus allowing their enrichment since it is hypothesized that eEPC are located in the basalis (Gargett, 2007b). At this stage therefore it is possible to examine the role of markers in endometrial regeneration and gynaecological diseases, such as endometriosis.

Another approach to purify endometrial stem/progenitor cells has made use of the Side Population (SP) phenotype, a well-known property of adult stem cells (Smalley and Clarke, 2005), where they can be sorted using fluorescence-activated cell sorting and characterized. Two per cent of all human endometrial cells were identified as SP cells (Masuda *et al.*, 2010) expressing endothelial cell markers and, compared with the main population of cells, they are capable of proliferating and differentiating into the various types of endometrial cells. Others have characterized endometrial SP cells and shown they express pluripotency markers, such as Octamer-binding transcription factor-4 (OCT-4) and have telomerase activity levels between embryonic stem cells and mature tissue cells (Cervello *et al.*, 2011). Together these studies suggest that the SP fraction contains endometrial stem/progenitor cell populations. When transplanted into severely immuno-deficient mice, endometrial SP cells predominantly reproduce endometrial stromal tissue with mature blood vessels, but also occasional glandular structures, suggesting that retrogradely shed endometrial SP cells possess the properties necessary to establish endometriotic lesions in the peritoneal cavity. Further, cells co-expressing the endometrial MSC markers CD146 and PDGFR β (Schwab and Gargett, 2007) but, interestingly, not the single marker W5C5 (Masuda *et al.*, 2012), were expressed on SP cells (Miyazaki *et al.*, 2012). However, it is still uncertain if there are one or more stem/progenitor subpopulations within the SP.

Source of endometrial stem/progenitor cells

Endometrial stem/progenitor cells may be derived from endogenous SSC laid down during embryogenesis; however, there is evidence that the bone marrow could also be a source of cells for regenerating endometrial tissue. Bone marrow-derived cells (BMDC) have been identified in the endometrium of single human leukocyte antigen mismatched female bone marrow transplant recipients, suggesting that non-uterine stem cells contribute to regeneration of endometrial tissue (Taylor, 2004). In a follow-up study, Du and Taylor (2007) showed in two separate mouse models that BMDCs migrated and differentiated into eutopic and ectopic endometrial cells, although the contribution was very modest. Another study using green fluorescent protein-tagged donor bone marrow cells showed significant contribution to the endometrium, from 3 to 12 months post-transplant, supporting the concept of bone marrow as one source of cells for monthly endometrial regeneration (Morelli *et al.*, 2013). However, examination of gender mismatched bone marrow transplant recipients (Cervello *et al.*, 2012) showed that BMDC did not contribute to the endometrial SP cell population, suggesting that the BMDC were more likely myeloid cells rather than bone marrow-derived stem cells.

Endometrial stem cells in menstrual blood

The upper functionalis layer of the human endometrium sheds every month, leaving behind the basalis layer from which a new functionalis regenerates. Endometrial MSC co-expressing CD146 and PDGFR- β reside in both these layers (Schwab and Gargett, 2007). Therefore, it is likely that eMSC are shed each month during menstruation. Indeed menstrual blood has been cultured in the same manner as bone marrow aspirates and a population of adherent cells with fibroblastic appearance have been identified. These cells have been described as endometrial regenerative cells (ERC). ERC have a similar surface phenotype to endometrial and bone marrow MSC, expressing CD9, CD44, CD29, CD73, CD90 and CD105, while lacking haematopoietic markers, CD34, CD45 and CD133 (Meng *et al.*, 2007; Musina *et al.*, 2008). Previous studies have shown stem cell markers, OCT-4 and stem cell factor receptor (C-KIT) expression in eutopic stromal and epithelial endometrial cells, and in epithelial cells of endometriotic lesions (Matthai *et al.*, 2006; Pacchiarotti *et al.*, 2011). Similar to endometrial MSC, ERC express OCT-4, C-KIT and stage-specific embryonic antigen-4 (SSEA-4), but lack the STRO-1 marker (Meng *et al.*, 2007; Patel *et al.*, 2008). ERC undergo extensive proliferation, reaching 68 doublings *in vitro*, whilst maintaining karyotypic stability. ERC are capable of undergoing multilineage differentiation: adipogenic, osteogenic, chondrogenic, cardiogenic and neurogenic (Meng *et al.*, 2007; Musina *et al.*, 2008; Patel *et al.*, 2008). Higher concentrations of pro-angiogenic factors and MMPs are produced by ERC compared with other MSC sources (Meng *et al.*, 2007), as could be expected considering the original location of these cells in the highly vascularized endometrium. Cultured ERC grow and differentiate *in vivo* in several animal models used to regenerate damaged tissues in a variety of disease states. For example, ERC differentiated into cardiac troponin-I⁺, spontaneously beating cardiomyocytes *in vitro* (Hida *et al.*, 2008). When these ERC were engrafted onto

recipient rat hearts as a 3-dimensional cell sheet, they trans-differentiated into a cardiac tissue layer *in vivo*, significantly improving cardiac function and decreasing myocardial infarction in a nude rat model (Hida et al., 2008). Menstrual blood-derived ERC are also neuro-protective in an *in vivo* rat model of ischaemic stroke (Borlongan et al., 2010), and when administered intramuscularly improved Duchenne muscular dystrophy (Cui et al., 2007). These studies indicate the potential of menstruated ERC to generate tissue in several *in vivo* locations, thus suggesting they play a role in the initiation of growth of endometriosis should they reach the peritoneal cavity by retrograde menstruation.

To date, no studies have directly compared ERC with endometrial MSC, but rather they have been compared with umbilical cord or bone marrow MSC. It would be interesting to determine if there are differences in potential of these two stem/progenitor cell populations, since it is possible that in women with endometriosis the endometrial MSC in the functionalis are less differentiated than those in normal women. Studies investigating the difference between the CD146⁺PDGFRβ⁺ endometrial MSC located in the functionalis versus basalis might shed some light on why only some women are diagnosed with endometriosis when the majority regularly shed endometrial MSC every month.

Endometrial stem cells and the pathogenesis of endometriosis

A recently promulgated concept is the involvement of somatic stem cell populations in the initiation or progression of proliferative disorders and cancer in a range of tissues/tumours (Gargett, 2007a). Some existing evidence already supports the presence of endometrial stem/progenitor cells in endometriotic lesions, and thus their role in its pathogenesis. The monoclonality of epithelial cells in endometriotic lesions has been demonstrated using the human androgen receptor (HUMARA) assay or polymorphisms in the X-linked phosphoglycerate kinase gene, suggesting their possible initiation by an epithelial progenitor cell (Jimbo et al., 1997; Tamura et al., 1998; Wu et al., 2003). In addition, laser capture microdissection has been used to refute previous claims that lesions contained polyclonal epithelial cells, by demonstrating that each focus of a multifocal lesion is monoclonal (Wu et al., 2003). Cells with properties of endometrial MSC have also been identified in freshly isolated and cultured cells from endometriotic lesions (Chan et al., 2011; Kao et al., 2011). These ectopic endometrial MSC underwent multilineage differentiation into adipocytes, osteoblasts, chondrocytes and cardiomyocytes, and trans-differentiation into neural cells (Kao et al., 2011). Self-renewing epithelial and stromal colony forming units (CFU) were identified in ovarian endometriomas, further proof of the involvement of stem/progenitor cells in the pathogenesis and progression of endometriosis (Chan et al., 2011). Clonogenic cells were also identified in a long-term culture derived from a sample of endometriosis tissue (Tanaka et al., 2003). Eutopic and ectopic endometrial MSC are very similar, sharing OCT-4 mRNA and MSC surface marker expression, and deficiency of gap junction intercellular communication (Kao et al., 2011). DNA losses/genomic imbalances identified in endometriotic stroma and epithelia further support the clonal proliferation and involvement of stem/progenitor cells in the pathogenesis of endometriosis (Silveira et al., 2012).

The properties of eMSC from endometriotic lesions appear enhanced compared with eutopic eMSC. Ectopic clonally derived eMSC have

greater proliferative potential than eutopic eMSC, with significantly faster doubling times and larger cumulative proliferation doublings (Kao et al., 2011). The ectopic eMSC also had significantly greater invasiveness and migration ability than eutopic eMSC in both *in vitro* and *in vivo* assays, and were able to stimulate angiogenesis (Kao et al., 2011).

The classic theory on the aetiology of endometriosis is that viable endometrial cells reach the peritoneal cavity through retrograde menstruation (Sampson, 1927). However, 90% of women experience retrograde menstruation (Halme et al., 1984) and eMSC are present in most samples of menstrual blood. Yet only 6–10% of all women develop endometriosis. To address this disparity, one hypothesis suggests that more endometrial stem/progenitor cells are shed into the peritoneal cavity by retrograde menstruation in women with, but not in women without, endometriosis, where they implant and establish endometriotic growths (Starzinski-Powitz et al., 2001; Gargett, 2007b). In support of this hypothesis, we have presented pilot data indicating that W5C5⁺ cells are present in higher concentrations in the peritoneal fluid of menstruating women with, compared with women without, endometriosis (Gargett et al., 2011). This could be due to women with endometriosis having a larger volume of retrograde menstrual flow or experiencing retrograde menstruation more regularly, although this would be difficult to prove. However, in a baboon model of spontaneous endometriosis, it was observed that larger amounts of retrograde menstruation resulted in higher rates of endometriosis (D'Hooche and Debrock, 2002). In addition, women with endometriosis have shed fragments of basalis endometrium in menstrual blood more often than women without endometriosis, resulting in more stem/progenitor cells being retrogradely shed into the peritoneal cavity (Leyendecker et al., 2002). SSEA-1, a marker of basalis epithelial cells, was also present in endometriotic lesions (Valentijn et al., 2013), confirming that cells from the basalis contribute to the pathogenesis of endometriosis. More definitive markers of endometrial epithelial progenitor cells will assist in elucidating their role in the pathogenesis of endometriosis. Finally, acquired mutations in the endometrial stem/progenitor cells or alterations in the stem cell niche in women with endometriosis may result in greater shedding of endometrial stem/progenitor cells from the eutopic endometrium. It is also likely that altered immune clearance in the peritoneal cavity causes increased survival of endometrial stem/progenitor cells, thus directly or indirectly contributing to the pathogenesis of endometriosis (Gargett and Chan, 2006; Gargett, 2007a).

Another popular theory is based on the observation that eutopic endometrium in adult women with endometriosis is different in many ways from that of healthy subjects both in the proliferative and secretory phases, with heterogeneous responses (Brosens and Benagiano, 2013; Brosens et al., 2013). The key unanswered question, however, is how and when ectopic lesions cause the changes in the endometrium. Moreover, it remains to be shown that these endometrial changes are present in the adolescent. It is also not known if the stem/progenitor cells have altered expression of apoptotic molecules or steroidogenic enzymes or whether they differ in the niche cells that would accompany them, and would be essential for their survival and function in the peritoneal cavity. It is plausible that differences exist also in the endometrial stem/progenitor cells themselves and these anomalies may promote their survival and implantation in the peritoneal cavity.

A third hypothesis is that normal endometrial stem/progenitor cells may be refluxed into an environment of altered immune clearance in the peritoneal cavity resulting in increased survival of refluxed

endometrial fragments. Higher levels of endometrial vascular endothelial growth factor (VEGF) were found in women with endometriosis during the late secretory phase (Donnez *et al.*, 1998), possibly creating a pro-implantation and pro-angiogenic state. In addition, endometrial stromal cultures supplemented with VEGF inhibitor, Sorafenib, showed a reduction in proliferation, migration and invasion of ectopic stromal cells to levels of eutopic stromal cells (Moggio *et al.*, 2012), suggesting an anti-angiogenic approach to the treatment of endometriosis (Pittatore *et al.*, 2013). Alternatively, the milieu of cytokines and hormones may alter the expression patterns of stemness-related genes and markers, such as Musashi-1, observed in endometriosis tissue compared with eutopic endometrium (Gotte *et al.*, 2008; Forte *et al.*, 2009). It has been hypothesized that endometrial menstrual debris (including those of retrograde menstruation) represents a mixture of endometrial cells at different developmental stages (Starzinski-Powitz *et al.*, 2003). As an example, cytokeratin-positive/E-cadherin-negative cells were considered less differentiated than cytokeratin-positive/E-cadherin-positive epithelial cells. Menstrual debris containing undifferentiated stem-like cells capable of self-renewal may act as the initiating cells for the creation of primary endometriotic lesions. In turn these cells, if present in the lesions, may contribute to the evolution of endometriosis, allowing the formation of secondary foci. Therefore it has been postulated that the severity or grade of endometriosis may be related to how primitive the cells were that initiated the lesion, with a stem/progenitor cell initiating a more severe lesion than a more differentiated transit amplifying cell or mature cell (Gargett, 2007b).

Finally, if neonatal bleeding is proved to play a role in early-onset endometriosis, the presence of an increased risk should be restricted to the 5% new-borns with fully developed endometrium and shedding in the neonatal period similar to menstruation (Ober and Bernstein, 1955).

Early-onset endometriosis

Endometrial stem/progenitor cells may be involved in the pathogenesis of pre-menarcheal and adolescent endometriosis through retrograde neonatal bleeding when the endometrium is fully developed similar to the adult (Brosens and Benagiano, 2013; Brosens *et al.*, 2013). We propose that in this group endometrial stem/progenitor cells, possibly together with niche cells, are retrogradely shed into the peritoneal cavity where they survive and given the right pro-implantation environment they may play a role in the pathogenesis of pre-menarcheal or adolescent endometriosis in those with susceptibility to endometriosis. Perhaps those that develop early-onset endometriosis may have had larger volume bleeds, with more endometrial stem/progenitor cells finding their way into the peritoneal cavity. Or, the fundamental differences in the endometrial stem/progenitor cell populations that predispose for endometriosis, such as carrying one of the susceptibility alleles recently identified for endometriosis risk (Nyholt *et al.*, 2012), may favour their survival by altering their clearance in the peritoneal cavity.

The ability of endometrial stem/progenitor cells to survive in the absence of estrogen for a decade is one of their hallmark features. This ability to remain 'dormant' is apparent in inactive post-menopausal endometrium, which regenerates on exposure to exogenous estrogen years after menopause and is capable of supporting embryo implantation and pregnancy to full term (Antinori *et al.*, 2003). Indeed, clonogenic epithelial and stromal cells have been identified in post-menopausal

endometrium (Schwab *et al.*, 2005) and more recently also an eMSC population in estrogen-treated post-menopausal endometrium was identified (Ulrich *et al.*, 2013). Interestingly, W5C5⁺ eMSC (unpublished observations) and eSPC do not express steroid receptors (Cervello *et al.*, 2011; Schuring *et al.*, 2011), which means they require paracrine stimulation from estrogen-responsive niche cells for their proliferation. Therefore, neonatal endometrial stem/progenitor cells arriving in the pelvic cavity following neonatal uterine bleeding are likely to survive for many years in the absence of circulating estrogen, providing niche cells are also present and immune clearance is avoided due to the immature immune system of the newborn (Fig. 1). These may remain relatively dormant until hormonal or other conditions favour their activation through niche cells. With puberty onset and increasing ovarian activity these dormant stem/progenitor cells are postulated to generate ectopic endometrial growth akin to regenerated post-menopausal eutopic endometrium.

An important feature of early lesions is the presence of angiogenesis at the site of subtle endometriotic foci. VEGF expression by endometrial cells varies with the phase of the menstrual cycle and in peritoneal fluid is higher in the proliferative than in the secretory phase (McLaren *et al.*, 1996; Gargett *et al.*, 1999). Thus, in rare, selected cases, the estrogenic effect of maternal steroids or pre-pubertal estrogen secretion might be sufficient to stimulate angiogenesis in endometrial debris reaching the peritoneal cavity during the early neonatal period allowing them to develop into a premature type of subtle endometriosis with extensive angiogenesis and risk of bleeding, as suggested by the red peritoneal lesions, haemosiderin deposits and even endometrioma formation in early cases of endometriosis. Sampson's theory of retrograde menstruation and implantation cannot explain the presence of pre-menarcheal lesions, since these girls have not yet had menarche, but neonate uterine bleeding may cause the initial retrograde flux. Thus, we propose to extend Sampson's theory to include not only shedding of endometrial stem/progenitor cells in retrograde menstruation, but also a possible reflux of neonatal uterine bleeding capable of explaining pre-menarcheal endometriosis.

Conclusion

In conclusion, there are several elements which support our hypothesis that endometrial stem/progenitor cells may play a role in early-onset endometriosis. First, at the end of pregnancy the fetal endometrium can be transformed into a decidualized layer that is desquamated after birth, as manifested by overt vaginal bleeding in 5% of neonates. Secondly, regurgitation of endometrial shedding into the peritoneal cavity is likely to be favoured by a functional obstruction in the endocervical canal present in neonates. Finally, the pattern of pre-menarcheal endometriosis is similar to that of adolescent and adult endometriosis, and the lesions in pre-menarcheal disease with scanty glandular development seem to reflect a premature stage of the condition. For these reasons, there seems to be no need to assume a different origin and pathogenesis for pre-menarcheal endometriosis, but rather the regurgitation, implantation and long-term survival of endometrial stem/progenitor cells.

It is well appreciated that endometriosis may be present for a long time before a diagnosis can be posed. Recent studies have estimated the length of diagnostic delay in surgically treated endometriosis patients to be up to a decade (Arruda *et al.*, 2003). It would not be surprising that, taking into account the post-natal incubation time, the interval

between the primary endometrial shedding and the development of active lesions may involve an even longer delay.

A fundamental question remains: does the presence of overt neonatal uterine bleeding indicate a warning sign of the future development of endometriosis and increase the awareness of a devastating disease in the young woman (Brosens and Benagiano, 2013)? Unfortunately, to the best of our knowledge no systematic recording of the presence of uterine bleeding in the neonate exists from which to perform prospective studies. In order to identify whether overt neonatal uterine bleeding is indicative of early-onset endometriosis, it would be necessary to collect shed neonatal uterine blood and examine it for presence of eSPC using known markers (e.g. W5C5/SUSD2). It would be a challenging study to collect the small amounts of neonatal uterine blood, if any is present both technically for medical staff and psychologically for new mothers, although a vaginoscope could be used. Also, most neonates are discharged from hospital before neonatal endometrial shedding is evident. For this research to go forward, a paediatrician or paediatric gynaecologist with an interest in endometriosis who has access to neonates in the hospital and has the skills to collect the shed neonatal uterine blood is required. A registry of these patients is also required for follow-up until puberty and beyond to determine if they develop endometriosis.

Acknowledgement

The authors are grateful to Sue Panckridge, medical artist, MIMR-PHI Institute of Medical Research, for assisting in drawing of Figure 1.

Authors' roles

All authors contributed to the conception and design of this review, drafted and revised the article for intellectual content, and gave final approval of the published version.

Funding

This work was supported by the Australian National Health and Medical Research Council Senior Research Fellowship (1042298) to C.E.G., the Royal Australian and New Zealand College of Obstetrics and Gynaecology (RANZCOG) Ella Macknight Memorial Scholarship to K.E.S., and the Victorian Government's Operational Infrastructure Support Program. J.J.B. received support from Biomedical Research Unit in Reproductive Health, a joint initiative between the University Hospitals Coventry and Warwickshire National Health Service Trust and Warwick Medical School.

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