What Happens to Endometriosis During the Menstrual Cycle?

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ABSTRACT

Introduction: The objective of this study is to determine the structural changes in endometriosis throughout the menstrual cycle.

Materials and Methods: This retrospective comparative study was undertaken in a gynaecological unit of a university teaching hospital and looked at the immunohistochemical appearances of epithelial cells of the endometrium and endometriosis in 17 cases at various stages of the menstrual cycle, particularly during menstruation.

Results: The epithelium in endometriosis lesions undergoes the same cyclical morphologic changes that are observed in eutopic endometrium. In particular, each of the six cases of endometriosis observed during the active bleeding phase showed evidence of epithelial shedding of the terminally differentiated secretory-phase epithelial cells and their almost immediate replacement by small undifferentiated cells.

Conclusion: The cyclical shedding/regeneration of endometriotic epithelium during menstruation has not previously been recognised, and it may have significant implications for the understanding of the aetiology and best management of endometriosis.

INTRODUCTION

It is almost 100 years since Sampson published his landmark paper on the menstrual dissemination of endometrial tissue into the peritoneal cavity.[1] This theory of retrograde menstruation, as the cause of endometriosis, has persisted but never completely satisfied scientific investigation. Over the ensuing century, there have been ever-increasing efforts, using increasingly sophisticated technologies, to refine these concepts.[2] In our previous studies, using a combination of immuno-histochemistry, scanning electron microscopy and hysteroscopy, we investigated cellular changes within the endometrium during different phases of the menstrual cycle. We concluded that epithelial regeneration after menstruation was a very rapid, piecemeal process that occurred without cell division.[3] We also demonstrated that, contrary to accepted theory, epithelial cells of the unshed glands within the basalis do not persist but are shed into the gland cavities.[4] During these studies we observed, by chance, that the epithelium of a gland in some associated endometriosis also seemed to be shed during menstruation. We then undertook a literature review to discover what was known about this phenomenon and found, to our considerable surprise, that there was no literature at all about the effects of the menstrual cycle on the morphology of endometriosis. Therefore, collectively, we do not know what the subsequent fate is after an endometrial implant is established (by whatever mechanism). Do the epithelial cells making up the endometriosis lesions persist unchanged thereafter? Or do they undergo a cycle of menstrual-related morphological changes similar to those observed in eutopic endometrium? This study attempts to explore these issues using primarily immuno-histological observations.

MATERIALS AND METHODS

Study population

This study was approved by King Edward Memorial Hospital's ethics committee. Of 34 patients in the study group, 17 patients primarily had endometriosis and one adenomyosis. Six of these were in the proliferative phase, five in the secretory phase, and six in the active bleeding phase of the cycle.

Obtaining specimens from the menstrual phase of the cycle presents a number of specific practical and social problems, and such samples are rarely available for routine clinical evaluation. This seems particularly true of endometriosis.

The study was of a prospective observational design to observe the morphological features of epithelial cells at various times throughout the cycle. Endometrial samples were obtained either from timed hysterectomy or deep curettage samples. Samples of endometriosis were obtained from laparoscopically excised specimens obtained concurrently with endometrial sampling.

Samples were counterstained in standard manner with one or more primary antibodies of CD34, cKit, and Ki-67. For this paper, each antibody was selected solely because it provided the clearest images of the cellular morphology observed in a particular specimen at a particular moment in the cycle.

IMMUNOHISTOCHEMICAL LABELLING

This was a standard indirect immunoperoxidase procedure using mouse anti-rat Ab. Biotinylated latex sheep anti-mouse and streptavadin-horseradish peroxidase (S-HRP), as RG previously described, was used.[5] Whole-mount sections were incubated in a blocking solution for one hour at room temperature followed by primary antibody to either CD34 (DakoCytomation, Glostruo, Denmark) at a concentration of 0.1 ug/ml (Clone Q8 End 10) or antibody to c-Kit (DakoCytomation, Glostrup, Denmark or antibody to Ki-67 Invitrogen, Mount Waverley, Victoria, Australia Pty) with incubation times of 30–45 minutes at room temperature.

Negative controls were included in each run, substituting the primary antibody for an iso-type (IgGl, kappa) non-specific immunoglobulin at the same concentration (0./1 ug/ml) as the primary antibody.

Attempts to count cells were abandoned because of gross variations in individual specimens with shed, shedding, and regenerating specimens frequently co-existing in the same sample making any general count of the field not reflective of localised conditions.

RESULTS

The results of this study are the immuno-histochemical images at various stages of the menstrual cycle. Most of the images are counterstained with CD34 antibody which stains the endothelium of the blood vessels and in most cases stains the surfaces of positive cells brown.

Figure 1 shows two endometrial glands in the endometrium stained with CD34 antigen during the early proliferative phase. The glands are small and the cells are cuboidal in shape having tightly packed symmetrical nuclei that make up around 50% or more of the cell volume. The luminal surface of the cells are smooth and regular. Figure 2 shows glands at a similar stage of the proliferative phase of the cycle but stained with Ki67 antibody that is a marker of mitotic cellular proliferation. The expression of Ki-67 indicates actively dividing cells, in this case, during the early proliferative phase. As the cells divide, the glands increase in size and volume. Figure 3 shows glands counterstained with CD34 that shows the end stage of this growth, with the glands of a late secretory phase endometrium characterised by tall columnar epithelium. The nuclei occupy

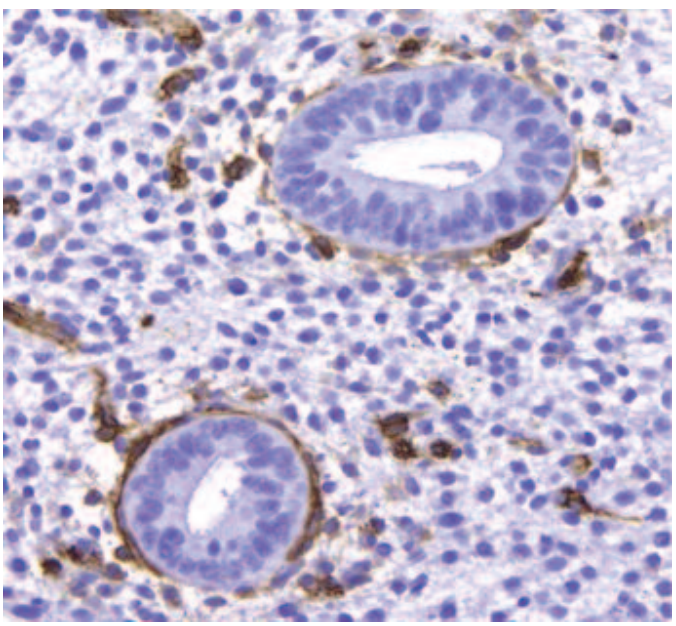

Figure 1. Eutopic endometrial glands from an early proliferative endometrium counterstained with CD34 antigen which is seen as brown staining. The CD34 is expressed in the endothelial cells of the small blood vessels and in the capsules of the stromal cells closely surrounding the two glands. These proliferative glands are characterised by cuboidal-shaped cells with a regular luminal outline and relatively large nuclei that occupy more than 50% of the cell volume.

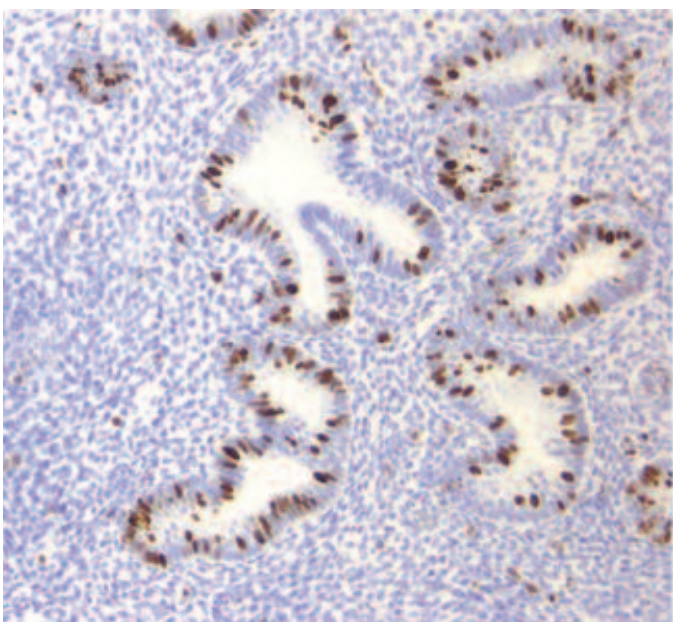

Figure 2. Eutopic endometrial glands counterstained with the proliferation marker Ki-67. This early proliferative phase endometrium again shows small-size epithelial cells with relatively large nuclei, many of which are stained brown indicating extensive mitotic cell division.

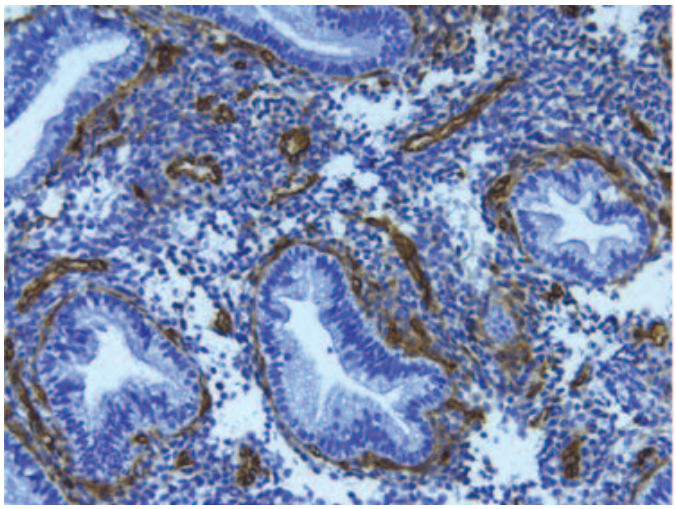

Figure 3. A sample of a late secretory eutopic endometrium counterstained with CD34. At this stage of the cycle, the cells are of larger volume and the cytoplasm of each cell is distended by obvious secretory vacuoles. The nuclei are arranged towards the periphery of each gland and occupy much less of the total cellular volume. CD34+ cells are again shown surrounding each gland.

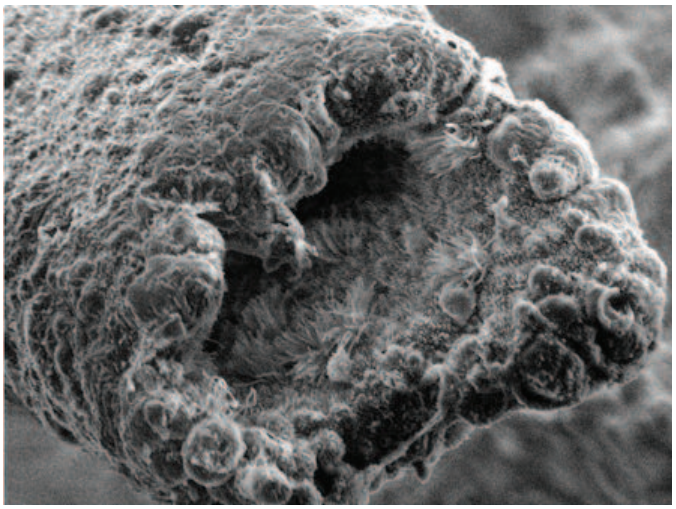

Figure 4. A scanning electron micrograph of the distal part of a gland left exposed after the shedding of the endometrial luminal epithelium and most of the functional stroma during the menstrual phase of the cycle. This image demonstrates the single layer of columnar epithelial cells making up a gland at this stage of the cycle, with the distorted luminal surface demonstrating its 'terminally differentiated' morphology with numerous microvilli and cilia protruding into the glandular lumen.

less of the cell volume while the cytoplasm is expanded with the products of secretion readily visible towards the luminal surface. The scanning electron microscopic image of a glandular stump of a day1 specimen recently exposed by functional epithelial loss in Figure 4 confirms the 'terminal differentiated' features of such epithelium with luminal microvilli and cilia producing an irregular luminal surface. All these cyclical-related changes in the endometrium are already well-documented and understood.

Not so well-recognised are the changes that happen to the retained basal glands during menstruation itself. Figure 5 is a remarkable image that summarises various aspects of the process in a single moment. The epithelium stained with cKit antibody shows evidence of some of the epithelium being shed into the cavity, leaving some very small cells exposed. Cells on either side of these show a progressive increase in the size of the cells and their nuclei until, at the opposite side of the gland, the epithelial morphology is typical of the early proliferative phase.

We primarily studied the effects of the menstrual cycle on the morphology of endometriotic lesions. Figure 6 shows the structure of an early proliferative endometriotic lesion stained with CD34. Just as in the eutopic endometrium, the gland at this stage of the cycle is lined with small cuboidal epithelial cells with the nuclei filling much of the cell volume. Importantly, just as in eutopic endometrium, glands within proliferative-phase endometriosis show considerable mitotic cell division, as shown by the expression of Ki67 (Fig. 7). Figure 8 illustrates an endometriotic lesion in the mid-secretory phase that shows columnar-shaped cells with secretions and the gland removed from deep inside a rectal nodule that is surrounded by cells expressing CD34. Figure 9 is a sample counterstained with a CD34 antigen that was removed during

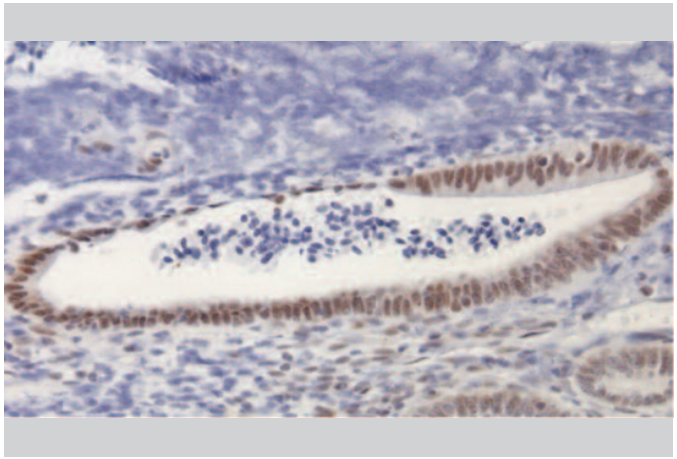

Figure 5. An endometrial gland remaining in the basalis after shedding of the functionalis during menstruation. This gland is stained with c-Kit which stains the nuclei of the glandular epithelium brown. This specimen shows shed cells lying within the lumen of the gland associated with an area devoid of glandular epithelial but exposing a line of very small, undifferentiated cells. On either side of this 'shed' area are cells of progressively increasing size to become cells of typical proliferative morphology with the c-Kit-stained nuclei filling most of the cell volume.

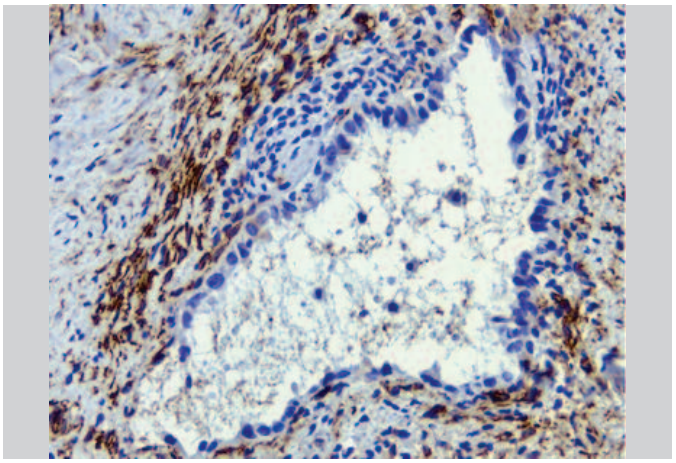

Figure 6. A gland from an early proliferative phase specimen of endometriosis of the uterosacral ligament also counterstained with CD34 antigen. The specimen shows simple small cuboidal cells without secretions or microvilli.

menstruation and shows an endometriosis lesion in which the epithelial cells are clearly being shed into the gland cavity leaving exposed stroma.

The composite image in Figure 10 shows the similar morphological changes observed in both eutopic endometrium and endometriosis during each of he phases of the menstrual cycle. The changes begin with small cuboidal epithelium in the proliferative phase, evolving into tall columnar epithelium with microvilli on the luminal surfaces in the secretory phase, followed by evidence of extensive shedding of the glandular epithelial cells during the early menstrual phase.

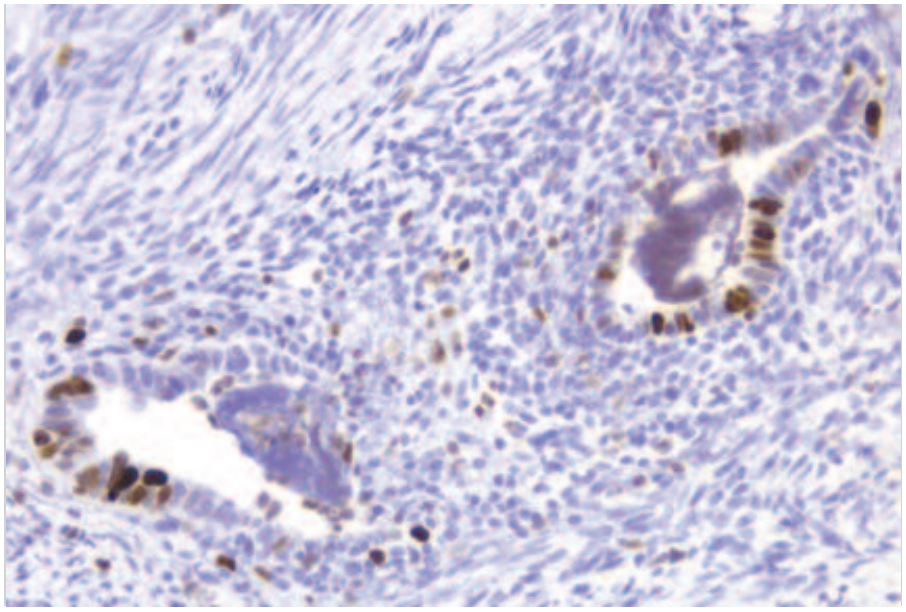

Figure 7. A proliferative phase sample of endometriosis stained with Ki-67 with two glands showing a number of cells expressing the proliferation marker indicating at this early stage in the menstrual cycle that the epithelial cells making up the glands are actively dividing just as they are in eutopic glands.

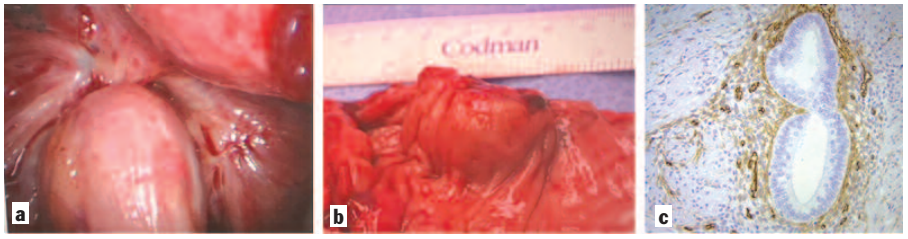

Figure 8. A composite image of a mid-secretory phase rectal endometriosis lesion. The laparoscopic image shows the lesion in situ with the rectal lesion pulled over to the left-hand side of the pelvis and surrounded by fibrotic material (a). The gross histology of the excised rectum shows the nature and size of the lesion (b). The CD34 immunohistochemistry of the same lesion shows a single gland with mid-secretory phase columnar epithelial cells with nuclei occupying less than half the cellular volume and with an occasional secretory vacuoles and luminal microvilli surrounded by CD34+ cells (c).

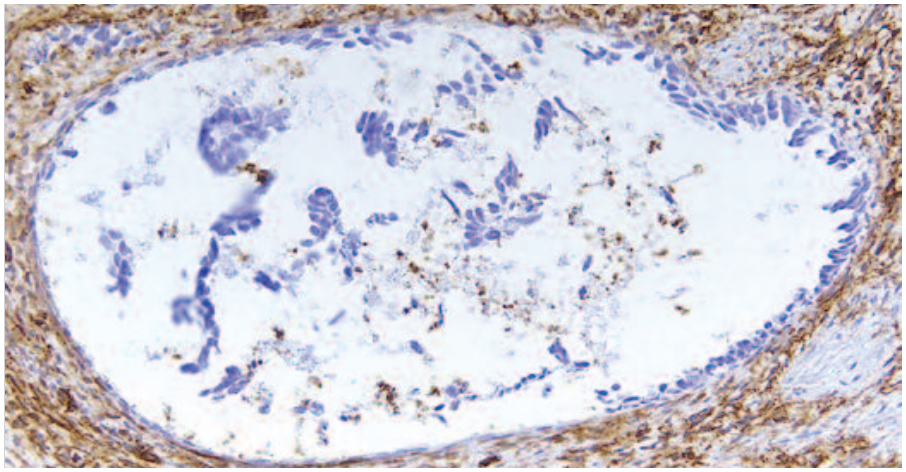

Figure 9. An endometriosis lesion removed on the second day of bleeding. There are numerous CD34+ cells in the stroma surrounding the gland but the most important feature is of the obvious loss of most of the glandular epithelial cells into the cavity leaving many small undifferentiated cells exposed.

Figure 11 shows CD34-stained images from the solitary case of extensive adenomyosis obtained during the early menstrual phase. The three views of different lesions from the same case each show evidence of complete shedding of epithelial glands into the glandular cavities. Although the cause of such epithelial shedding is not clear, all but one of the cases of shedding were seen during the menstrual phase of the cycle. Endometriotic glandular epithelium is shed during the menstrual phase of the cycle.

CD34 antigen is expressed in the stromal cells in a linear zone on the upper surface of the basalis (Fig. 12) as well as in cells surrounding glands within the eutopic basalis (Figs. 1 and 3) and endometriosis lesions.[6,8,9] C-Kit (CD117) is also known as a stem cell factor receptor and is shown expressed within the nuclei in Figure 5 and the eutopic menstrual image in the composite Figure 10. Figure 13 shows the intimate relationship between CD34+ cells and c-Kit cells. The surface of the cells surrounding an isolated lesion of endometriosis stains with CD34 while the nuclei within the same cells stain with c-Kit.

DISCUSSION

Understanding any disease begins with a sound knowledge of anatomy. Unlike most structures, the endometrium is not static, but each of its components is in continuous, non-synchronous and dynamic change. There is no image that can represent a 'normal' endometrium. This fluid structure makes study of the anatomy of the endometrium, particularly during menstruation, unusually difficult. The presence of unshed, shedding, and regenerated endometrium in the same sample further complicates interpretation as we previously described in 2009–10. The speed of change is more rapid than usually appreciated. Markee, who is the only person who has observed endometrium continuously throughout multiple cycles, showed that a new luminal epithelium regenerates within two hours of menstrual shedding.[6] Our observations are compatible with this rapid rate of change.

These problems, may explain why there has, as yet, been no attempt to document the anatomical variations of endometriosis throughout the menstrual cycle.

This paper is based on a retrospective review on the detailed morphology of 17 cases of endometriosis studied at various stages of the menstrual cycle. The findings are compared to those observed in eutopic endometrium. For the first time, we have shown that the epithelial cells lining endometriotic glands follow the same well-defined growth and shedding patterns observed in eutopic endometrial glands. During the proliferative phase, there is mitotic driven cellular growth. During the secretory phase, the cells progressively enlarge while accumulating secretions and luminal microvilli. During the menstrual phase, residual glands within the basalis are not retained unaltered as previously suggested[7] but are shed and replaced. Here we show that in endometriosis, the epithelial cells lining the glands are similarly shed and rapidly replaced by small new undifferentiated cells.

The implications of the concept that endometriosis undergoes cyclical morphological changes similar to those seen within eutopic endometrium could have profound implications in understanding both the aetiology and management of this most enigmatic of conditions. In particular, the novel concept that endometriosis lesions undergo cyclical shedding of terminally differentiated secretory cells during menstruation, along with their almost simultaneous replacement by small new cells that then undergo mitotic cell division, provides a new disease model.

This paper is not the result of a clinical trial or experiment. It is simply observations of clinical anatomy observed at timed intervals during the menstrual cycle. The study is of a very small size (17 cases) and the selection of cases are chosen in a highly selective manner and obviously require repeating by others. Nonetheless, the observations were seen repeatedly and fitted a rational pattern. If endometriotic glandular epithelial cells are derived from eutopic mother cells, directly or indirectly, they would be expected to respond in a comparable manner to their parent cells, to the same complex hormonal and cytological stimuli. The sole case of adenomyosis observed during the bleeding phase also showed the total shedding of glandular epithelia supporting the concept that all epithelia, wherever the glandular epithelial cells are located, are shed during the menstrual phase of the cycle. By definition, it is also unlikely that 'terminally differentiated' (i.e., epithelia that become columnar and develop specialised apical microvilli and lose their ability to further differentiate) cells can give rise to the new small epithelial cells that are seen in the cycle. It is more probable that these 'old' columnar cells are shed and replaced by new undifferentiated cells as suggested by these images.

These observations do not provide an explanation for the mechanisms for these changes, but it is interesting to note that in Figures 1 and 3, the glands in eutopic endometrium are surrounded by cells expressing CD34. Figures 6 and 8 show glands within endometriosi that are also surrounded by CD34+ cells. This antibody is sometimes a marker of stem cell potential. Such stomal-resident CD34

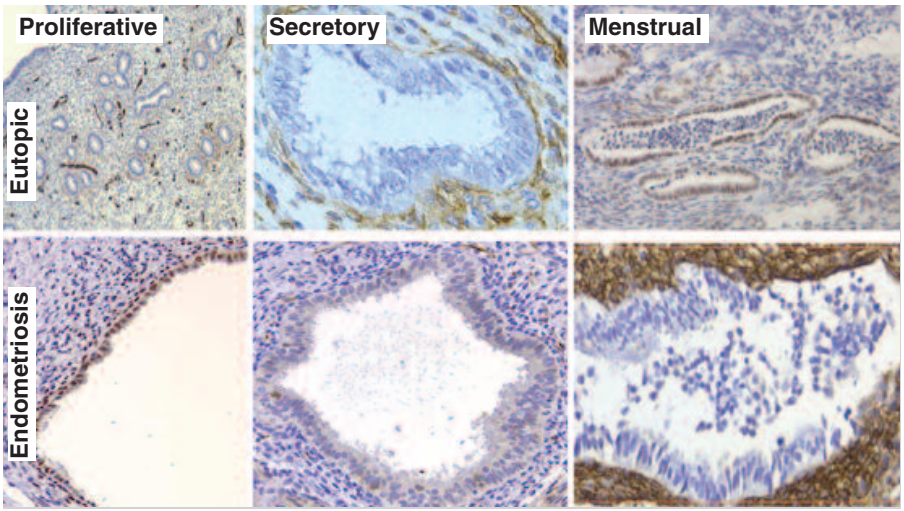

Figure 10. This is a composite slide to show the mirroring of the morphological appearances of the glandular structures in eutopic endometrium and endometriosis lesions. The epithelial cells begin as small cuboidal cells in the proliferative phase, become larger and distended with secretions and with luminal surfaces distorted with microvilli in the secretory phase, and then during menstruation are shed into the gland cavity.

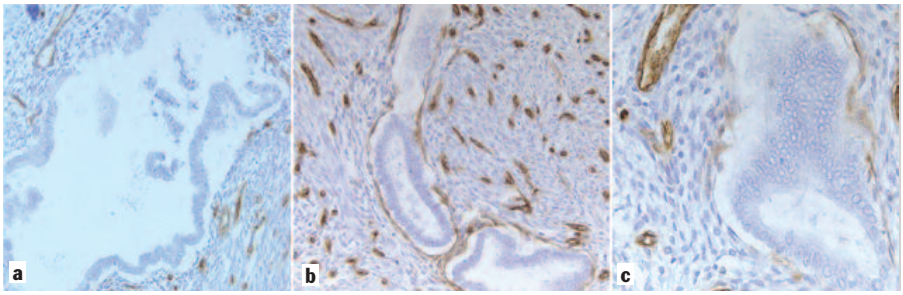

Figure 11a-c. Three samples from different areas of the same uterus with extensive adenomyosis stained with CD34 and removed during menstruation. In each of the images, the epithelial cells are being shed into the glandular cavities during the menstrual phase of the cycle.

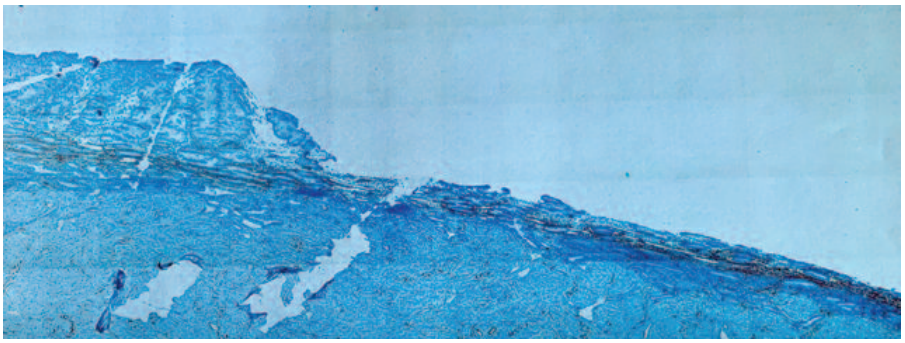

Figure 12. This is a composite image of a large sample of an endometrium sampled during menstruation. Part of the endometrium is late secretory in morphology and clearly unshed while a large portion of the same specimen taken at the same time shows almost complete shedding of the pars functionalis. This specimen is counterstained with CD34 and also shows a continuous horizontal band of brown-staining CD34+ cells in the stroma around the upper basalis in the unshed areas and on the exposed surface of the shed areas.

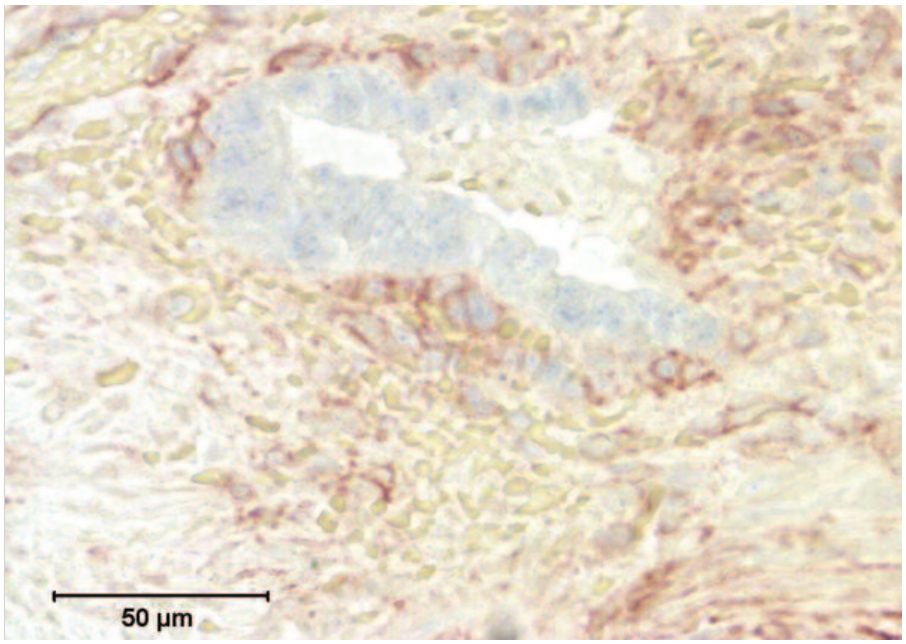

Figure 13. This a double-stained specimen of a mid-cycle endometriotic lesion. The cells expressing CD34 are stained red and those with blue coloured nuclei are stained with cKit. Showing a consistent and close anatomical relationship between these two antigens.

expressing stem cells have been reported to directly contribute to endometrial regeneration.[8] In Figure 5, the eutopic endometrial glands express c-Kit, particularly in their nuclei, while Figure 13 shows a double-stained endometriosis gland that is surrounded by CD34+ cells that also express cKit + cells within the same CD34+ cells while also staining the nuclei of all the glandular epithelium of the endometriosis. CKit is a proto-oncogene that responds to stem cell factor and appears to be a critical regulator of cell proliferation, survival, and migration involved in several physiological processes.[9] The role of these two antigens cannot be determined by these observations, but their consistent expression in areas of shedding and regeneration suggest a possible relationship.

CONCLUSION

In summary, endometriosis lesions undergo cyclical shedding and regeneration and suggest that this concept may have important theoretical and therapeutic implications. STI

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AUTHORS' DISCLOSURES

The author has no conflicts of interest to disclose.

REFERENCES

1. Sampson JA. Peritoneal endometriosis due to the menstrual. Dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927 ;14:422–69.
2. Cousins FL, McKinon BD, Mortlock S, et al. New concepts on the ethology of endometriosis. J Obstet Gynaecol Res 2023:4:1090–105.
3. Garry R, Hart R, Karthigasu KA, et al. A re-appraisal of the morphological changes within the endometrium during menstruation: a hysteroscopic, histological and scanning electron microscopic study. Hum Reprod 2009;24:1393–401.
4. Garry R, Hart R, Karthigasu KA, et al. Structural changes in endometrial basal glands during menstruation. BJOG 2010;117:1175–185.
5. McMenamin PG, Crewe J, Morrison S, et al. Immunomorphologic studies of macrophages and MHC class11-positive dendritic cells in the iris and ciliary body of the rat, mous and human eye. Invest Opthalmol Vis Sci 1994;35:3224–50.0
6. Markee JE. Menstruation in intraocular endometrial transplants in the rhesus monkey. Contrib Embryol 1940;177:228–64.
7. Novak E, Te Linde EW. The Endometrium of the menstruating uterus. JAMA 1924;53:900–6.
8. Yin M, Zhou HJ, Lin C, et al. CD34+ KLF4+ stromal stem cells contribute to endometrial regeneration and repair. Cell Reports 2019;27(9):2709–24.
9. Sheilkh E, Tran T, Vranic S, et al. Role and significance of c-Kit tyrosine kinase in cancer: A review. Bosn J Basic Med Sci 2022;22(5):683–98.