# Generating receptive endometrium in Asherman's syndrome

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## ABSTRACT

Intrauterine administration of bone marrow stem/progenitor cells to a woman with thin endometrium refractory to estrogen stimulation regenerated her endometrium sufficiently to support a pregnancy. Or was it local endometrial damage induced by concurrent curettage that stimulated endogenous endometrial stem/progenitor cells into action? Or both?

**KEY WORDS:** Adult stem cells, Asherman's syndrome, bone marrow, endometrial stem cells, endometrium, regeneration, transplant

## GENERATING RECEPTIVE ENDOMETRIUM IN ASHERMAN'S SYNDROME

Asherman's syndrome is defined as the complete obliteration of the uterine cavity with adhesions resulting in the cardinal symptoms of amenorrhea and infertility.[1] Intrauterine adhesion (IUA) refers to the partial adherence of the endometrial surfaces with fibrotic tissue. Presenting symptoms are related to the degree and location of IUA and include menstrual abnormalities ranging from irregular bleeding to hypomenorrhea and amenorrhea, infertility and recurrent pregnancy loss.[2] The incidence of Asherman's syndrome and IUA varies between 2% and 22% in infertile women, depending on the criteria used for diagnosis and geographical location, reflecting practices that might injure the endometrium.[1,2] In the gravid uterus, Asherman's syndrome and IUA mainly results from trauma to the endometrium and underlying myometrium from postpartum curettage, spontaneous miscarriage, or termination of pregnancy, and in the nongravid uterus from endometrial ablation procedures.[1-3] In a setting of low-circulating estrogen levels in the postpartum period the endometrium fails to regenerate following damage to the basalis layer, which has been postulated to harbor endometrial stem/progenitor cells responsible for its remarkable regenerative capacity.[4] Infection and inflammation may contribute to the inability of traumatized endometrium to regenerate and are important processes involved in the deposition of fibrotic tissue.

Normally following menstruation or parturition the endometrial surface epithelium repairs without scarring[5] in the absence of estrogen.[6] In other mucosal surfaces such as the intestine, regeneration of the damaged mucosa occurs without scarring unless the injury involves the deep muscularis layers. This suggests that failure of the endometrial functional layer to regenerate in Asherman's syndrome and IUA results from deep trauma involving the underlying myometrium[2,3] with concomitant loss of basalis endometrium and its population of resident adult stem cells. Indeed, dense fibrotic areas of the uterine cavity show no endometrial lining[7] and in regions where endometrium is present, it is thin and atrophic with inactive glands and little stroma. Patients with fibrotic lesions around the cervix or with loss of functional endometrium present with menstrual abnormalities, particularly amenorrhea, while women with dense adhesions obliterating the tubal ostia are infertile. Repeated pregnancy loss is often due to partial blockages of the ostia and uterine cavity with fibrotic adhesions and a poorly vascularised and diminished endometrium.[1]

The goal of treatment for Asherman's

syndrome is to restore fertility to enable an affected woman to deliver a normal baby. This is achieved through surgical restoration of a functional uterine cavity in size and shape, prevention of further adhesions and regeneration of the endometrium. Surgical dissection or destruction of the adhesions is performed using minimally invasive hysteroscopic procedures with low energy instruments to ensure preservation of any endometrium.[1,2] Insertion of intrauterine contraceptive devices (IUCD) or Foley catheters has been used as preventive measures against adhesion recurrence. High dose estrogen is given to promote endometrial regeneration.

In this issue, a case report, "Endometrial regeneration using autologous adult stem cells followed by conception by *in vitro* fertilization in a patient with severe Asherman's syndrome" describes an innovative treatment of a 33-year-old woman with apparent Asherman's syndrome using autologous bone marrow-derived stem cell populations which regenerated the endometrium sufficiently to enable implantation of a donor oocyte IVF embryo and support the ensuing pregnancy for at least 8 weeks.[8]

Bone marrow like many other organs harbors small adult stem populations. Adult stem cells are rare undifferentiated cells that have been identified by their functional properties in most adult tissues and organs in the body. Their role is to maintain tissue homeostasis, providing replacement cells lost through cellular turnover and following tissue damage. Recently rare populations of epithelial progenitor cells and mesenchymal stem/stromal cells (MSC) were identified in human endometrium (reviewed in[4,9]) and are likely responsible for regenerating the functional layer of the endometrium following menstruation and parturition. Defining markers of human endometrial MSC (eMSC) indicate that they are a subpopulation of pericytes located perivascularly in both basalis and functionalis layers.[10] Besides these endogenous endometrial stem/progenitor cells which may have originated from residual fetal stem cells, evidence also suggests that bone marrow stem cells, which circulate in very low numbers, may populate human endometrium and other organs.[9] HLA- and gender-mismatched transplant studies in human and mouse suggest that bone-marrow-derived cells may incorporate into the endometrium[11,12] in a setting of ongoing tissue damage and inflammation. The actual bone marrow cell type that transdifferentiates into endometrial cells has not yet been elucidated and could be myeloid cells, hemopoietic stem cells, MSC, or endothelial progenitor cells.

The case described in this issue presented with a diagnosis of primary infertility. It would appear that a previous dilatation and curettage caused her hypomenorrhea, although the procedure was not done in the postpartum period. Endometritis was not reported and the patient has premature ovarian failure, indicating that she was not a classical case of Asherman's syndrome or IUA. It was demonstrated that the patient's thin endometrium failed to respond during a normal ovulatory cycle monitored by sonography and Doppler, although plasma sex steroid hormone levels were not examined. Subsequent hysteroscopy revealed severe endometrial adhesions which were surgically dissected. To prevent the development of further adhesions an IUCD-Cu-T was inserted, and to regenerate the endometrium the patient was artificially cycled with estrogen and progesterone for 6 months. However, in the following natural cycle, the endometrium failed to respond and remained at 3.2 mm. A schedule of increasingly high doses of estradiol valerate was then administered in cycles for another 6 months, but again the endometrium failed to grow beyond 3.6 mm. Given the patient's refractory endometrium, her ovarian failure and previous reports that bone marrow cells incorporate into the endometrium,[11,12] it was decided to use autologous bone marrow-derived adult stem cell therapy to generate a receptive endometrium. The patient's bone marrow was collected and prepared for transplantation in a GMP facility. Bone marrow cells were selected with three different markers, CD9, CD90, and CD133 in attempt to isolate angiogenesis-promoting cells. While CD133 specifies a very rare subset of hemopoietic stem cells with endothelial progenitor cell activity, CD90 and CD9 select both MSC and fibroblasts.[13,14] This mix of fibroblasts, MSC and endothelial progenitor cells was instilled into the uterine cavity immediately following curettage on the second day of the patient's menstrual cycle, ensuring that some of the cells gained access to endometrial stem cell niches through the damaged surface. To promote endometrial regeneration, high doses of estradiol valerate were also given. Within 14 days the endometrium had thickened to 5.0 mm with improved vascularity, as observed by color Doppler. Four further artificial cycles with withdrawal bleeds resulted in growth of the endometrium to 6.9 mm. Three grade 1 donor oocyte embryos were then transferred into the uterine cavity with further progesterone and estradiol support. Fifteen days later, β-hCG levels indicated pregnancy and at 8 weeks a healthy fetus was visualized by ultrasound.

This success story generates some interesting questions. Firstly, while the patient was informed of the risks and possible failures of the procedure, was human ethics approval obtained? Did the pregnancy go to full term and was a healthy baby born? Was the baby genotyped? And was the offspring really from the donor oocyte or was it from the patient? Secondly, what bone marrow cell type was involved in regenerating the thin endometrium of this patient that had been refractory to estrogen administration? The release criteria (CD9, CD44, CD90 expression) for

the cell product suggest that fibroblasts and MSCs[13] rather than endothelial progenitor cells ($CD34^+$, $CD133^+$, $VEGFR2^+$) were the main cell types delivered into the uterine cavity. Questions also arise on the mechanism involved in regenerating the endometrium. Did the bone marrow cells incorporate into endometrium and transdifferentiate into endometrial epithelium, stroma, and/or vascular cells? Or did the bone marrow cells provide trophic factors that promoted angiogenesis, prevented apoptosis of remaining endometrial cells, or provided growth factors that induced resident endometrial stem/progenitor cells to proliferate to replace the cells lost via the curettage?

In studies to date, the main mechanism of action attributed to transplanted bone marrow-derived-mononuclear cells, $-CD34^+$ or $-CD133^+$ cells, involves cell–cell contact and secretion of bioactive molecules that promote angiogenesis and tissue repair, inhibit scarring, modulate inflammatory and immune reactions, and activate tissue specific progenitor cells, rather than by engraftment.[15,16] For example, these mechanisms operate in other better characterized systems where bone marrow-derived cells have been shown to limit damage to the myocardium in experimental models of myocardial ischemia and in several clinical trials (reviewed in[14,15]). It is possible that similar mechanisms may operate in the endometrium when bone marrow-derived cells are transplanted into the uterine cavity. Studies addressing these issues using animal models are required to reveal the mechanisms involved.

This case report also highlights the potential role of invoking tissue damage to promote endometrial regeneration. Women presenting with thin endometrium refractory to estrogen treatment has been an intractable problem in ART. Thin endometrium is characterized by poor growth of the glandular epithelium, high uterine blood flow impedance and poor vascular development.[17] Successful embryo implantation requires the development of a thick (7-8 mm) receptive endometrium.[17] In the present case, the combination of curette, intrauterine bone marrow cell transplant and ultrasound monitoring resulted in a successful IVF-ET pregnancy when embryo transfer was only attempted after the endometrium reached 7 mm and Doppler measurements indicated intraendometrial vascularity. This case raises the question about why some thin endometria fail to respond to estrogen stimulation? Is there diminished activity of the resident endometrial stem/progenitor cells or are they present in lower numbers? Alternatively, are the niche cells, postulated to regulate the function of endometrial stem/progenitor cells, faulty or diminished in thin endometrium associated with Asherman's syndrome? Studies in mouse endometrium suggest that ER$\alpha$-expressing niche cells respond directly to estrogen and transmit proliferative signals to neighboring endometrial stem/progenitor cells identified as label retaining cells.[4,18]

This case report highlights the importance of inducing tissue damage to activate resident adult stem cell populations into cell cycle to initiate cellular replacement. In studies examining the role of adult stem cells in regenerating hemopoietic tissue in the bone marrow or intestinal epithelium for example, ablation of the original tissue with cytoreductive agents or irradiation is first undertaken before administration of stem cell populations or examining their role during subsequent tissue regeneration. Quiescent adult stem cell populations are recruited into cell cycle to initiate cellular replacement, fulfilling their role in restoring tissue homeostasis. In general these rare adult stem cell populations overproduce replacement cells and in the case of the bone marrow, there can be spillage of small numbers of hemopoietic stem cells and their progeny into the peripheral blood during the restoration phase. Recent studies have shown that curetting or biopsying the endometrium in preceding cycles or even within the same menstrual cycle as IVF-ET doubles the pregnancy and live birth rates.[19,20] It has been suggested that local injury to the endometrium may induce decidualization of proliferative stage endometrium,[19] or that a wound healing response is initiated with release of growth factors and cytokines that produce a receptive endometrium, or that the histological advancement of the endometrium induced by IVF procedures is delayed or reversed in accordance with the "backward development hypothesis."[20] From an adult stem cell perspective we hypothesize that single or repeated biopsy of thin endometrium induces an injury response that stimulates resident endometrial stem/progenitor cells to initiate a regenerative response that restores endometrial tissue homeostasis. In keeping with other tissue systems, it appears that the stem/progenitor cell response to local injury is greater than that generated each menstrual cycle resulting in production of a much thicker receptive endometrium. Similar to the present case, another recently reported case of Asherman's syndrome initially treated with hysteroscopic adhesiolysis with restoration of the uterine cavity also had a thin endometrium not detectable by sonography that failed to respond to estrogen therapy.[21] In this case the patient was subsequently treated with multiple Pipelle biopsies in two preceding artificial cycles prior to IVF-ET producing a 7 mm thick endometrium, successful pregnancy and live birth. Other studies have shown that ER$\alpha$ expression increases in the stroma following biopsy-induced endometrial injury.[20] Together these case reports suggest that biopsy/curette-induced injury to thin endometrium activated resident endometrial stem/progenitor cells and/or their stromal niche cells to produce a much thicker, receptive endometrium. Whether the presence of activated endometrial stem/progenitor cells promoted the incorporation of rare

circulating bone marrow stem cells into the endometrium to enhance the response is not known. Neither is it known whether the trophic factors released by the bone marrow cells contributed to the activation of the resident endometrial stem/progenitor cells.

In summary, the thin nonresponsive endometrium of the Asherman's case described in this issue was successfully treated with intrauterine transplantation of bone marrow fibroblasts and MSC and concurrent curette, both of which may have independently or possibly synergistically promoted the growth of endometrial cells. The bone marrow cells by production of trophic factors promoting angiogenesis and tissue growth and the curette by stimulating dormant endometrial stem/progenitor cells into active cell cycle to regenerate endometrial tissue. Given, that this is a case of adult stem cell-based medical innovation[22] for treating a patient with a form of Asherman's syndrome and that the community has high hopes in curing many diseases with stem cells, it will be important that a small carefully designed clinical trial be conducted with human ethics approval according to the guidelines established by the International Society for Stem Cell Research for the clinical translation of stem cells[23,24] to determine whether autologous bone marrow transplantation into the uterine cavity does indeed regenerate a thick receptive endometrium capable of carrying a conceptus to term. Further studies are also required to delineate the mechanisms responsible for these successful treatments of Asherman's syndrome, a previously intractable infertility disorder.

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## REFERENCES

1. Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman's Syndrome-One century later. Fertil Steril 2008;89:759-79.
2. Panayiotides I, Weyers S, Bosteels J, Van Herendael B. Intrauterine adhesions (IUA): has there been progress in understanding and treatment over the last 20 years? Gynecol Surg 2009;6:197-211.
3. Lo ST, Ramsay P, Pierson R, Manconi F, Munro MG, Fraser IS. Endometrial thickness measured by ultrasound scan in women with uterine outlet obstruction due to intrauterine or upper cervical adhesions. Hum Reprod 2008;23:306-9.
4. Gargett CE. Uterine stem cells: what is the evidence? Hum Reprod Update 2007;13:87-101.
5. Salamonsen LA. Tissue injury and repair in the female human reproductive tract. Reproduction 2003;125:301-11.
6. Kaitu'u-Lino TJ, Morison NB, Salamonsen LA. Estrogen is not essential for full endometrial restoration after breakdown: Lessons from a mouse model. Endocrinology 2007;148:5105-11.
7. Schenker JG, Margalioth EJ. Intrauterine adhesions: an updated appraisal. Fertil Steril 1982;37:593-610.
8. Nagori CB, Panchal SY, Patel H. Endometrial regeneration using autologous adult stem cells followed by conception by *in vitro* fertilization in a patient of severe Asherman's syndrome. J Hum Reprod Sci 2011;4:43-8.
9. Gargett CE, Masuda H. Adult stem cells in the endometrium. Mol Hum Reprod 2010;16:818-34.
10. Schwab KE, Gargett CE. Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. Hum Reprod 2007;22:2903-11.
11. Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. Stem Cells 2007;25:2082-6.
12. Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. JAMA 2004;292:81-5.
13. Halfon S, Abramov N, Grinblat B, Ginis I. Markers distinguishing mesenchymal stem cells from fibroblasts are downregulated with passaging. Stem Cells Dev 2011;20:53-66.
14. Alaiti MA, Ishikawa M, Costa MA. Bone marrow and circulating stem/progenitor cells for regenerative cardiovascular therapy. Transl Res 2010;156:112-29.
15. Prockop DJ. Repair of tissues by adult stem/progenitor cells (MSCs): Controversies, myths, and changing paradigms. Mol Ther 2009;17:939-46.
16. Caplan AI. Why are MSCs therapeutic? New data: New insight. J Pathol 2009;217:318-24.
17. Senturk LM, Erel CT. Thin endometrium in assisted reproductive technology. Curr Opin Obstet Gynecol 2008;20:221-8.
18. Chan RW, Gargett CE. Identification of label-retaining cells in mouse endometrium. Stem Cells 2006;24:1529-38.
19. Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I. Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing *in vitro* fertilization. Fertil Steril 2003;79:1317-22.
20. Li R, Hao G. Local injury to the endometrium: its effect on implantation. Curr Opin Obstet Gynecol 2009;21:236-9.
21. Barash A, Granot I, Fieldust S, Or Y. Successful pregnancy and delivery of a healthy baby after endometrial biopsy treatment in an *in vitro* fertilization patient with severe Asherman syndrome. Fertil Steril 2009;91:1956.e1-3.
22. Lindvall O, Hyun I. Medical innovation versus stem cell tourism. Science 2009;324:1664-5.
23. Hyun I, Lindvall O, Ahrlund-Richter L, Cattaneo E, Cavazzana-Calvo M, Cossu G, et al. New ISSCR guidelines underscore major principles for responsible translational stem cell research. Cell Stem Cell 2008;3:607-9.
24. Available from: http://www.isscr.org/clinical_trans/pdfs/ISSCRGLClinicalTrans.pdf [Last accessed on 2011 Apr 18].

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