

Endometrial regeneration using autologous adult stem cells followed by conception by *in vitro* fertilization in a patient of severe Asherman's syndrome

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ABSTRACT

In a woman with severe Asherman's syndrome, curettage followed by placement of intrauterine contraceptive device (IUCD) (IUCD with cyclical hormonal therapy) was tried for 6 months, for development of the endometrium. When this failed, autologous stem cells were tried as an alternative therapy. From adult autologous stem cells isolated from patient's own bone marrow, endometrial angiogenic stem cells were separated using immunomagnetic isolation. These cells were placed in the endometrial cavity under ultrasound guidance after curettage. Patient was then given cyclical hormonal therapy. Endometrium was assessed intermittently on ultrasound. On development of endometrium with a thickness of 8 mm and good vascularity, *in vitro* fertilization and embryo transfer was done. This resulted in positive biochemical pregnancy followed by confirmation of gestational sac, yolk sac, and embryonic pole with cardiac activity on ultrasound. Endometrial angiogenic stem cells isolated from autologous adult stem cells could regenerate injured endometrium not responding to conventional treatment for Asherman's syndrome.

KEY WORDS: Adult stem cells, Asherman's syndrome, endometrial regeneration, endometrial thickness, hormone replacement therapy

INTRODUCTION

In this case report, we describe a case of Asherman's syndrome treated with adult autologous stem cells for endometrial regeneration that resulted in conception after *in vitro* fertilization-embryo transfer (IVF-ET).

The basis of this case is that endometrium is a dynamic, cyclically regenerating tissue, a unique model of physiological angiogenesis in adults. Angiogenesis results either from sprouting of new vessels through recruitment of local endothelial cells from neighbouring blood vessels and/or by endothelial progenitor cells circulating in the peripheral blood after release from the bone marrow.^[1-4] Bone marrow stem cells also contribute to regeneration of the endometrium.^[5] On the basis of these facts, adult autologous bone marrow stem cells were used for regeneration of damaged endometrium.

CASE REPORT

A 33-year-old woman visited our clinic in November 2006. She was married for 8 years and had primary infertility. Her history, menstrual history and family history, was not significant. She had normal hormonal profile and husband's semen analysis was also normal. Her past treatment included a dilatation and curettage (D and C) in February 2005. This was followed by three cycles of superovulation with intrauterine insemination without success. She underwent IVF in June 2006, but did not conceive.

She presented to us with infertility and scanty menstruation since her D and C. Her first transvaginal ultrasound scan on day 3 of the menstrual cycle revealed normal size retroverted uterus with homogenous myometrium and thin single line endometrium, but intact endometriomyometrial junction. The left

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ovary measured 2.52 × 2.51 × 3.04 cm, had two antral follicles and a hemorrhagic cyst, and was adherent to uterus posteriorly. Right ovary measured 2.55 × 1.22 × 1.84 cm and had only one antral follicle. Doppler studies showed very poorly vascularised ovaries, even with minimum wall filter, pulse repetition frequency of 0.3, and gains -0.8.

A repeat scan on day 14 revealed that hemorrhagic cyst in left ovary had regressed partially. Right ovary showed a follicle of 20 mm which on color Doppler showed vascularity covering more than three-fourth of the follicular circumference with resistance index (RI) of 0.47 and peak systolic velocity (PSV) of 11.23 cm/s, but the endometrium was only 3.2 mm with branches of spiral vessels seen only up to endometrio-myometrial junction. The right uterine artery pulsatility index was 2.76.

Follow-up scan after three days still showed the same endometrial picture though follicle had ruptured. Midluteal ultrasound scan (ninth day post ovulation) showed that endometrium had failed to grow even during secretory phase, despite corpus luteum with vascular ring covering more than half of the corpus luteal circumference with RI of 0.43 and PSV of 10.43 cm/s on right side. Hysteroscopy was done in the next cycle to exclude endometrial adhesions. At hysteroscopy, severe endometrial adhesions were seen, which were cut [Figures 1 and 2].

IUCD-Cu T was placed to maintain surgically established patency of the endometrial cavity. Laparoscopy done showed bilateral cornual tubal block.

She was treated with cyclical estrogen and progesterones with ethinyloestradiol 0.05 mg from fifth to 25th day of the cycle and with medroxy progesterone acetate 10 mg from 20th to 25th day for 6 months to obtain a functional endometrium. During this period, she had withdrawal bleeding, which was scanty. After 6 months, the IUCD was removed.

Ultrasound assessment of the endometrium in the following cycle showed no growth of the endometrium in the periovulatory and secretory phase of the menstrual cycle despite normal follicular development, rupture, and corpus luteum formation. The endometrium was perpetually 3.2 mm in thickness and echogenic [Figure 3].

Due to poor endometrial development, she was advised surrogacy with IVF. Her FSH on Day 3 of cycle was 19.70 IU/ml with an antral follicle count of 2. In view of poor ovarian reserve, she was advised surrogacy with oocyte donation. The patient was ready for oocyte donation, but not for surrogacy. She was given oral oestradiol valerate tablets in

increasing doses from 4 mg daily for 3 days, followed by 6 mg daily for another 3 days, and then 8 mg daily for a total of 25 days along with aspirin 75 mg daily for endometrial preparation. Ultrasound scans were done intermittently to assess the endometrium, but it never reached a thickness more than 3.6 mm. This hormone replacement therapy cycle was repeated for 6 months without improvement of the endometrium.

Based on reports of adult autologous stem cells applications for regeneration of injured cartilage and cardiomyocytes in cardiac infarction, it was thought that use of stem cells for regeneration of endometrium was worth trying, especially because endometrium naturally has a regenerating capacity. If the basal layer of the endometrium is repaired and further stimulated, it should increase in thickness. This was the basis of this experimental therapy.

The procedure was explained to the patient and her husband in March 2009. Possibilities of failure and risks of the procedure were also explained.

On June 15, 2009, her bone marrow aspiration was done from the iliac crest under local anesthesia maintaining strict asepsis. Aspiration was done using bone marrow biopsy needle and 10 ml syringe prewashed with heparin. Total 45 ml of bone marrow was aspirated. Collection was done in CPDA (Citrate-phosphate dextrose anticoagulant) medium using 1 ml of medium for 7 ml of bone marrow. It was sent to a dedicated stem cell laboratory working as per clinical GMP/GTP standard and having a working area with laminar airflow to class 100.

Bone marrow was centrifuged using histopaque density gradient at 1000 rpm for 10 mins and 102 million mononuclear cells were separated. These cells were further treated by column separation technique and customized cocktail of CD9, CD90, and CD133 antibodies was used for immunomagnetic isolation of endometrial angiogenic stem cells. Markers used for endometrial angiogenic stem cells were CD9, CD44, and CD90. Gene expression study for CD9, CD44, and CD90 using RT-PCR technique was done for differentiated cells. Total 39 million marker-positive endometrial angiogenic cells were supplied in 0.7 ml of PBS (phosphate buffer saline) with 2% autologous (patient's own) heat-inactivated serum on the next day for transplant.^[6-16]

Patient was called with partially filled bladder on June 16, 2009, second day of her menstrual cycle. Curettage was done under anesthesia. With patient in lithotomy position, Sim's speculum in place, anterior retractor was used to retract the anterior vaginal wall, and volsellum was used to hold the anterior lip of cervix. ET cannula

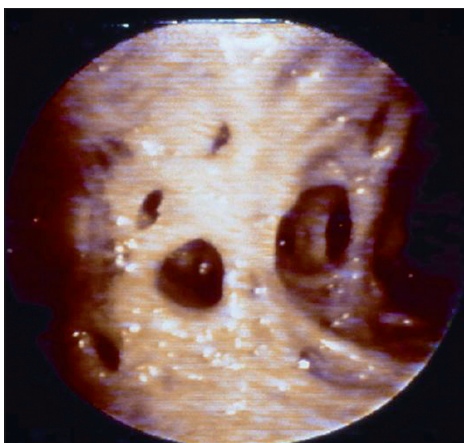


Figure 1: Hysteroscopic picture - Endometrial adhesions

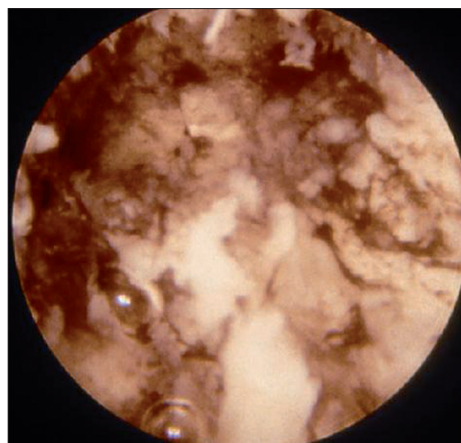


Figure 2: Postadhesiolysis hysteroscopic picture

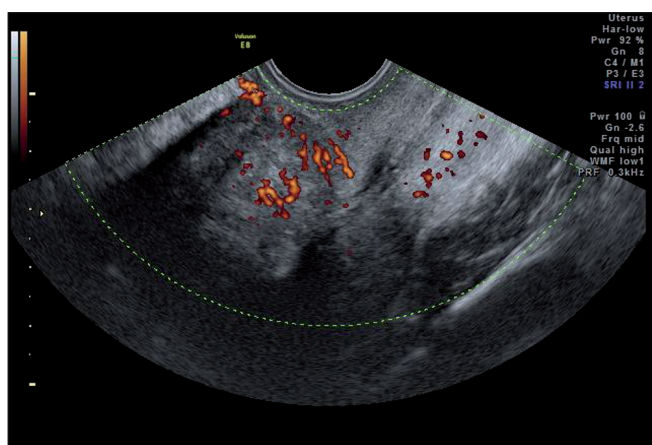


Figure 3: Thin endometrium after removal of IUCD in preovulatory period

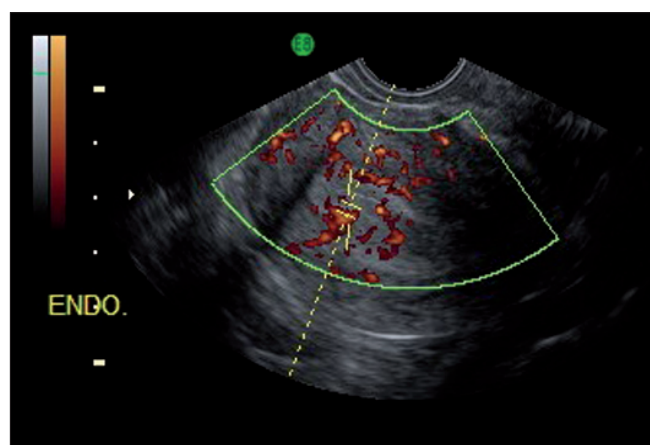


Figure 4: Well-developed endometrium with low-resistance vascularity reaching zone 4

(Frydman classic catheter 4.5, Laboratoire C.C.D., France) attached with 1-ml syringe, filled with 0.7-ml stem cell suspension, was advanced through cervix upto the fundal end of the endometrium under transabdominal ultrasound guidance. When the tip of the catheter was 0.5 cm below the fundus, piston was slowly advanced to allow slow steady flow of cell suspension in the uterine cavity. After instilling 0.3 ml of stem cell suspension at the fundus, injection was continued when cannula was gradually withdrawn out, till the tip reached mid cavity of the uterus. It was further very gently and slowly withdrawn out of the internal os and then external os, maintaining continuous pressure on the piston to prevent any back flow. Speculum and volsellum were removed, and patient was shifted when she recovered from anesthesia. She was discharged after 2 hours.

She was given oestradiol valerate 6 mg daily, starting on the same day for 25 days. Aspirin 75 mg was started from the same day and was continued.

Ultrasound was done on 14th day and 19th day of the cycle, which showed endometrial thickness of 5.0 and 5.2 mm,

respectively, and color Doppler showed spiral vessels reaching subendometrial zone. Medroxy progesterone acetate 10 mg was further given from 20th to 25th day. After withdrawal bleeding, cyclical estrogen and progesterone therapy was repeated for four cycles. Ultrasound scans done at midcycle showed improvement in endometrial thickness, morphology, and vascularity [Figure 4].

On November 2, 2009, endometrial thickness at ultrasound was 6.9 mm, with vascularity reaching intraendometrial region. There were dominant follicles in either ovary, which excludes the possibility of any endogenous luteinizing hormone surge. On November 6, 2009, three 4-6 cell Grade I donor oocyte IVF embryos were transferred. At this time, her endometrium was multilayered, with thickness of 7.1 mm and intraendometrial vascularity. She was given progesterone vaginal gel (Crinone 8%, manufactured by Fleet Laboratories, UK, marketed by Merck Serono) twice a day after ET, as luteal support and ethinyl oestradiol was continued in a dose of 6 mg daily along with Aspirin 75 mg daily.

Her serum β -hCG on November 21, 2009 was 8 297.1 mIU/

ml. Once β -hCG was positive, the dose of oestradiol valerate was increased to 8 mg daily. Progesterone vaginal gel 8% was continued twice a day along with aspirin 75 mg daily. A single gestational sac of 12 mm, yolk sac of 2.9 mm, embryonic pole of 2.4 mm, with M-mode showing embryonic heart rate of 112/mt were seen at ultrasound on November 30, 2009. Follow-up scan was done at 8 weeks of pregnancy which showed a healthy fetus [Figures 5–7].

DISCUSSION

This was a case of infertility with severe Asherman's syndrome and bilateral cornual tubal block with low ovarian reserve. Anti-mullerian hormone, which is now considered to be the most reliable marker for ovarian reserve, was not routinely used at that time (2007) and was not easily available locally, and therefore was not done. Low ovarian reserve with blocked fallopian tubes had left her with an option of oocyte donation. This had to be delayed due to her poor endometrial thickness. Placement of IUCD after surgery and cyclical hormonal therapy in association with low-dose aspirin or nitroglycerine is an established protocol for development of functional endometrium.^[17,18] Placing IUCD after curettage, followed by cyclical oestrogen progesterone therapy with aspirin, could not improve her endometrium.

Stem cells derived from tissues such as bone marrow, cord blood, adipose tissue, or the amniotic fluid have demonstrated regenerative potential in a variety of diseases and degenerative disorders.^[19]

Therefore, we tried to regenerate the endometrium with adult autologous stem cells after curettage, which was further supported with cyclical estrogen, progesterone, and aspirin for improving vascularization.

Bone marrow aspiration is fairly safe procedure with the risk of bleeding or infection, which are extremely rare when done meticulously in the hands of the expert.

Curettage was done to evoke injury-induced inflammatory reaction and hyperemia as homing induction, which in turn would enhance the response of endometrium to cyclical hormones.

We would like to quote a study that has shown regeneration of injured endometrium using adult bone marrow cells.

'Donor-derived bone marrow cells have been identified in human uterine endometrium.' Recent evidence has implicated bone marrow-derived cells as possible endometrial progenitors. It is unknown whether these cells



Figure 5: Gestational sac, yolk sac, and embryonic pole after embryo transfer and positive β -hCG test

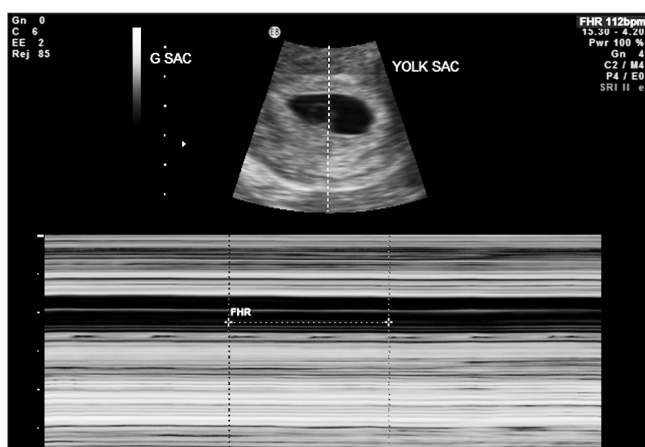


Figure 6: M-mode of cardiac activity of embryo



Figure 7: 3D picture of 8 weeks scan

originate from bone marrow mesenchymal stem cells or, alternatively, are circulating endometrial cells originally derived from the endometrium and harbored in bone marrow. These cells, regardless of their origin, may serve as a source of reparative cells for the reproductive tract. Both stromal and epithelial cells were derived from bone marrow origin. These data show the potential for stem cells

to have a role in the regeneration or repair of this tissue after injury.^[20] Significant engraftment of endometrium by bone marrow is likely to occur after endometrial injury or inflammatory insult.

Additionally, the proliferation and development of endometrium are entirely regulated by hormonal stimuli. Ovarian estrogen and progesterone drive endometrial growth and apoptosis.^[21,22]

In the case described in this study, cyclical hormonal therapy resulted in development of endometrium with good vascularity after four cycles.

We tried to regenerate the endometrium by endometrial angiogenic stem cells isolated from autologous adult stem cells and transplanting them in the endometrium immediately after curettage. This was based on a study that describes that mononuclear cells collected from the menstrual blood contains a subpopulation of adherent cells and retains expression of the markers CD9, CD29, CD41a, CD44, CD59, CD73, CD90, and CD105; the markers used were CD9, CD44, and CD90 to isolate the desired cells.^[19]

There is a minimal risk of developed cells presenting certain other markers during the process, but immunomagnetic isolation with specific marker antibodies reduces this possibility.

This experimental therapy may be tried on larger scale when the basal layer of endometrium is also damaged by surgical insult. The risk for malignancies after the use of adult autologous stem cells may be a concern in view of several reports, but we would like to draw attention to the fact that most of these reports discuss the cases where adult autologous stem cells were used to treat malignancies. Larger studies can completely exclude this risk.

The total cost of the therapy in an Indian set up comes to approximately Rs. 50 000 (Rupees fifty thousand), excluding the expense of IVF-ET. The cost is significantly lower than that to be spent for a surrogate mother. Moreover, it has an emotional and social advantage that the patient can bear her own child as against surrogacy.

To the best of our knowledge, no case of Asherman's syndrome conceived after endometrial regeneration with adult autologous stem cells after failure of all other conventional modes of treatment has been reported in literature. This therapy can be used as an alternative to surrogacy in females with severe Asherman's syndrome, though larger trials may be needed to establish this as proved line of treatment.

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