

Successful Application of Combined Autologous Bone Marrow-Derived Stem Cells and Platelet-Rich Plasma in a Case of Severe Asherman Syndrome and Subsequent *in vitro* Fertilization Conception

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ABSTRACT

The application of stem cells in infertility is still experimental. Thin endometrium is an important cause of cancelled or less successful frozen embryo transfer cycles. Clinically, numerous strategies have been adopted to promote endometrial regeneration including extended oestrogen administration, low-dose aspirin, pentoxifylline, tocopherol, vaginal sildenafil citrate and intrauterine perfusion with granulocyte colony-stimulating factor. However, even with the use of these therapies, the endometrium in some patients still remains unresponsive. Latest research shows that autologous bone marrow-derived stem cells (ABMDSCs) can be used for regeneration of damaged endometrium. We present a case report of patient with thin endometrium who was successfully treated with the combined use of ABMDSCs mixed with platelet-rich plasma, leading to successful *in vitro* fertilization conceived pregnancy. Patient consent and due ethical clearance were taken before starting the procedure.

KEYWORDS: *Asherman's syndrome, autologous bone marrow derived stem cells, infertility, platelet rich plasma, thin endometrium*

INTRODUCTION

A successful conception results from a healthy embryo, good endometrium and a healthy dialogue between the two. The human endometrium is a dynamic remodelling tissue undergoing more than 400 cycles of regeneration, differentiation and shedding during woman's reproductive years. Although there is no officially accepted definition of a thin endometrium, the commonly accepted cutoff is <7 mm on the day of luteinizing hormone (LH) surge or human chorionic gonadotropin (HCG) administration. It is seen in around 1%–2.5% of infertile women undergoing assisted reproductive treatments.^[1]

Asherman's syndrome is a gynaecological disorder caused by the destruction of the endometrium due to endometritis or repeated or aggressive curettages, resulting in loss of basal endometrium in many areas. The uterine cavity is obliterated by intrauterine adhesions

and fibrosis, leading to amenorrhoea, oligomenorrhoea, infertility and recurrent pregnancy loss.

A normal uterine cavity and endometrial lining are necessary in order to conceive and maintain a pregnancy. Clinically, numerous strategies have been adopted to promote endometrial regeneration including extended oestrogen administration, low-dose aspirin, pentoxifylline, tocopherol, vaginal sildenafil citrate and intrauterine perfusions with granulocyte colony-stimulating factor.^[2] However, even with the use of these therapies, the endometrium of some patients still remains unresponsive, resulting in cancellation of frozen embryo transfer (FET) cycles and repeated implantation failure. Effective treatment for thin

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endometrium is still a major challenge that has not been solved, and new therapeutic approaches for increasing endometrial thickness are being attempted. Sometimes, the only therapeutic modality available for such women is surrogacy.

However, latest research shows that mobilising autologous bone marrow (BM) stem cells can lead to regeneration of basal endometrium. Woman with severe Asherman's syndrome can be treated with autologous BM-derived stem cells (ABMDSCs). They exert their influence by the secretion of massive amounts of growth factors and cytokines to result in a therapeutic outcome called 'trophic' activity.^[3] It is known that stem cells secrete bioactive molecules that inhibit apoptosis and scarring at the site of injury and stimulate angiogenesis and mitosis of tissue-specific and tissue-intrinsic progenitors.

Consent and ethical clearance

Ethical clearance was obtained from Director General of Health Services and institutional ethical committee (ECR/291/Indt/MH/2018) for application of stem cells in infertility. A written informed consent was obtained from the patient. A detailed explanation that the therapy is still experimental and the risk of failure were given to the couple.

CASE REPORT

A 31-year-old woman visited our clinic in October 2019. She was married for 7 years and was a case of secondary infertility. She had previously conceived twice with intrauterine insemination but had missed abortions at 8 weeks. Both the times she underwent dilatation and curettage. She reported scanty menses for the past one year. Her past history and family history were not significant. She had normal hormonal profile, and husband's semen analysis was also normal.

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 with antral follicular count of 12–14. The left ovary had 6–7 follicles and right ovary had 5–6 follicles.

Ultrasound assessment of the endometrium in the 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 4 mm in thickness and echogenic.

She was given oral estradiol valerate tablets in increasing doses from 4 mg daily to 12 mg daily for a total of 25 days along with aspirin 150 mg daily

for endometrial preparation. Ultrasound scans were done regularly to assess the endometrium, but it never reached a thickness more than 4 mm, and vascularity was always sparse. This hormone replacement therapy cycle was repeated for 3 months without improvement of the endometrium.

Based on reports of adult ABMDSC applications for regeneration of various organs, it was considered as an option for the regeneration of endometrium, especially because endometrium naturally has a regenerating capacity. If the basal layer of the endometrium is repaired and further stimulated, it may show improved thickness and vascularity as it has capacity of neoangiogenesis as well.

The procedure was explained to the patient. Possibilities of failure and risks of the procedure were also explained.

Preparation of autologous bone marrow-derived stem cells

BM aspiration was done from the posterior superior iliac spine under local anaesthesia using the Jamshidi needle (13G) and 20 ml syringe prewashed with heparin maintaining strict asepsis. Around 150 ml of BM was aspirated. Sixteen milliliter ABMDSCs were separated using the fully automated cell separator, which uses optical sensor technology and simultaneous application of centrifugation and sedimentation. Processing of BM sample was carried out in a completely closed circuit centrifugation unit. Stem cell count (using flow cytometer and hemocytometer) varies from 05 million to 13 million cells/ml of the final stem cell concentrate. In our patient, it was 11 million cells/ml.

Preparation of platelet-rich plasma

Around 20 ml of peripheral blood in the heparinized syringe was taken and 2 ml of platelet-rich plasma (PRP) was prepared after double centrifugation. This was mixed with 16 ml of ABMDSCs.

Hysteroscopic instillation of autologous bone marrow-derived stem cells mixed with platelet-rich plasma

After written informed consent, the patient underwent hysteroscopy. Adhesiolysis was performed, and subendometrial instillation of ABMDSCs and PRP was done [Figure 1].

Subendometrial instillation was done as it induces local trauma and improves inflammatory response and may lead to better regeneration of basal endometrium.

The patient was given antibiotics in the perioperative period but nonsteroidal anti-inflammatory drugs were avoided so that adequate inflammatory response can

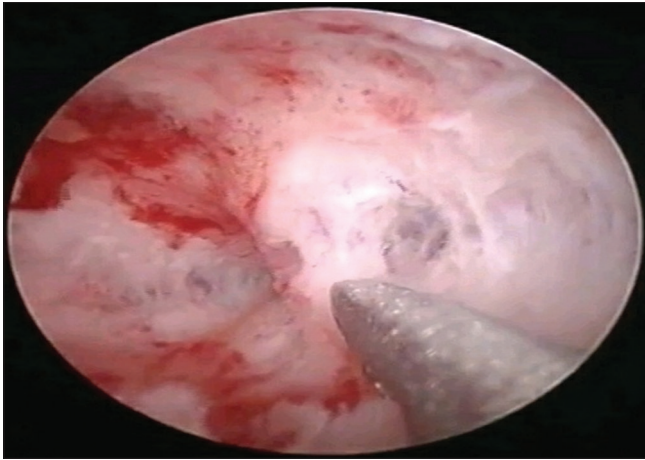


Figure 1: Hysteroscopic adhesiolysis followed by stem cell with platelet-rich plasma instillation being done

be generated. She was given 6 mg estradiol valerate with 150 mg aspirin for 3 weeks. A three-dimensional transvaginal ultrasound was performed 3 weeks later which showed a markedly improved endometrial cavity with neoangiogenesis. The endometrial thickness improved to 6 mm with excellent zone 3 vascularity. Progesterone was added for the last 7 days to achieve withdrawal bleeding.

After assurance of good cavity, she was offered IUI but as the patient was extremely anxious, she refused IUI and wished to proceed with *in vitro* fertilization (IVF). She underwent progesterone primed ovarian stimulation. Her ovarian stimulation was done with successful formation of 8 good-quality day 3 embryos which were vitrified, and FET was planned; the patient was started on hormone replacement therapy to build up the endometrium for FET. Estradiol valerate 12 mg/day and aspirin 150 mg/day were given to the patient. Serial ultrasound assessment showed an endometrial thickness of 7.2 mm with zone 4 vascularity. Progesterone supplementation was started with dydrogesterone 10 mg thrice a day (TDS) and vaginal micronised progesterone 400 mg BD. A day 3 embryo transfer was done with 2-day three early compacting embryos in the same cycle after 3 days of progesterone supplementation, resulting in successful intrauterine pregnancy with a beta HCG value of 1240 mIU/ml on day 16. A follow-up ultrasonography showed single live intrauterine pregnancy.

DISCUSSION

Stem cell-based therapies hold promise for future use in activating non-functional endometrium and reconstructing the endometrium *in vivo*. A few studies so far have evaluated the role of intrauterine instillation of ABMDSCs and PRP in suboptimal endometrium.

The first published case report of role of stem cells in endometrial rejuvenation dates back to 2004 when Taylor^[4] demonstrated that endometrial cells can originate from donor-derived BM cells and suggest that non-uterine stem cells contribute to the regeneration of endometrial tissue.

In 2011, case study by Nagori *et al.*^[5] showed endometrial regeneration using autologous adult stem cells followed by conception by IVF in a patient of severe Asherman's syndrome.

In 2014, Singh *et al.*^[6] published a case series of six cases of autologous stem cell implantation in cases of Asherman syndrome. Successful endometrial regeneration was reflected by restoration of menstruation in five out of six cases.

In 2016, Santamaria *et al.*^[7] did a pilot study, in which 16 cases with refractory Asherman syndrome underwent autologous BM stem cell treatment. They followed up the study subjects at 3 and 6 months to assess menstrual history, ET, adhesion score and neoangiogenesis. Fifteen out of 16 patients resumed menses though duration and intensity of menstruation decreased progressively 6-month post-stem cell treatment. Hysteroscopic visualisation of the uterine cavity, ET and neoangiogenesis observed through immunofluorescence markedly improved. Two term live births and two ongoing pregnancies were achieved.

In 2017, a pilot study by Tandulwadkar *et al.*^[8] evaluated the role of PRP in thin endometrium. Their study suggested that the use of autologous PRP holds promise in the treatment of women with suboptimal ET and vascularity for embryo transfer. It helps to reduce the incidence of cycle cancellations and thus even help reduce the financial and psychological burden of repeated cancelled cycles.

In 2017, Zadehmodarres *et al.*^[11] conducted a similar pilot study for treatment of thin endometrium with autologous PRP which showed successful results.

In 2018, Eftekhar *et al.*^[9] again proved that PRP may be effective in improving the endometrial growth and possibly pregnancy outcomes in women with a thin endometrium.

In 2020, a pilot study by Tandulwadkar and Karthick^[10] assessed the synergistic effect of ABMDSCs with PRP for ovarian rejuvenation. They observed that autologous PRP attracts stem cells to the site of injury and boosts their response. BMDSCs secrete chemokines, growth factors and hormones to influence adjacent cells (the paracrine effect). Paracrine signalling is important in anti-inflammation, immunoregulation, antiapoptosis,

antifibrosis and controlling oxidative stress, thus improving the microenvironment to promote the recovery of damaged tissues.^[11] ABMDSCs express genes relative to vascular endothelial growth factor, fibroblast growth factor 2 and interleukin-6 and promote angiogenesis *in vivo* and *in vitro*.^[12] PRP treatment assists in tissue regeneration, enhancement of anabolic signalling pathways, cell differentiation and proliferation and angiogenesis.^[13]

Currently, commercial surrogacy is banned in many countries and may not be socially acceptable and economically viable option to many. Hence, endometrial rejuvenation remains a ray of hope for such patients. However, legal and ethical issues need to be addressed before stem cell therapy becomes a standard of care for such patients.

The possible limitations of stem cell therapy include that it is invasive, expensive and still experimental. The possible contraindications include acute infections of endometrial cavity, haematometra, pyometra, chronic medical illnesses and immunocompromised states.

Our case report shows successful pregnancy following ABMDSCs mixed with PRP application in patients with thin endometrium. Although this therapy is currently experimental, more such case reports and larger studies in future may provide evidence for application of this therapy in larger population.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understand that name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest

Sunita Tandulwadkar is a member of the National Advisory Board of the Journal of Human Reproductive

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REFERENCES

1. Zadehmodarres S, Salehpour S, Saharkhiz N, Nazari L. Treatment of thin endometrium with autologous platelet-rich plasma: A pilot study. *JBRA Assist Reprod* 2017;21:54-6.
2. Singh N, Shekhar B, Mohanty S, Kumar S, Seth T, Girish B. Autologous bone marrow-derived stem cell therapy for Asherman's syndrome and endometrial atrophy: A 5-year follow-up study. *J Hum Reprod Sci* 2020;13:31-7.
3. Benor A, Gay S, DeCherney A. An update on stem cell therapy for Asherman syndrome. *J Assist Reprod Genet* 2020;37:1511-29.
4. Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *JAMA* 2004;292:81-5.
5. 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.
6. Singh N, Mohanty S, Seth T, Shankar M, Bhaskaran S, Dharmendra S. Autologous stem cell transplantation in refractory Asherman's syndrome: A novel cell based therapy. *J Hum Reprod Sci* 2014;7:93-8.
7. Santamaria X, Cabanillas S, Cervelló I, Arbona C, Raga F, Ferro J, et al. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: A pilot cohort study. *Hum Reprod* 2016;31:1087-96.
8. Tandulwadkar SR, Naralkar MV, Surana AD, Selvakarthick M, Kharat AH. Autologous intrauterine platelet-rich plasma instillation for suboptimal endometrium in frozen embryo transfer cycles: A pilot study. *J Hum Reprod Sci* 2017;10:208-12.
9. Eftekhari M, Neghab N, Naghshineh E, Khani P. Can autologous platelet rich plasma expand endometrial thickness and improve pregnancy rate during frozen-thawed embryo transfer cycle? A randomized clinical trial. *Taiwan J Obstet Gynecol* 2018;57:810-3.
10. Tandulwadkar S, Karthick MS. Combined use of autologous bone marrow-derived stem cells and platelet-rich plasma for ovarian rejuvenation in poor responders. *J Hum Reprod Sci* 2020;13:184-90.
11. Tilly JL, Johnson AL. Modulation of hen granulosa cell steroidogenesis and plasminogen activator activity by transforming growth factor alpha. *Growth Factors* 1990;3:247-55.
12. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote *in vitro* and *in vivo* arteriogenesis through paracrine mechanisms. *Circ Res* 2004;94:678-85.
13. Alsousou J, Ali A, Willett K, Harrison P. The role of platelet-rich plasma in tissue regeneration. *Platelets* 2013;24:173-82.