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Autologous Bone Marrow Derived Cell Concentrate Therapy for Thin Endometrium

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Abstract

Objective: To observe that can cell therapy with Autologous Bone Marrow Derived Cell concentrate, post uterine artery injection through interventional radiology offer a therapeutic approach in patients with Thin Endmoetrium.

Design: Pilot Study

Setting: Referral Centre

Patient(s): This was a prospective pilot study of 12 participants ageing from 25 to 45 years old with Thin Endometrium.

Intervention: Uterine artery injection of autologous Bone Marrow Derived Cell concentrate through interventional radiology

Main outcome measures: Endometrial lining improvement, Emdoemtial Thickness and Increase in Menstrual Blood Flow.

Result: All patients exhibited an improvement in Endometrial Linning anf Endometrial Thickness. Also, increase in Menstrual Blood flow was observed.

Conclusion: Failure to construct or regenerate a functional endometrium with its correct morphology and thickness in patients with inadequate thin endometrium is believed to be one of the major clinical issues in fertility treatments. With the advancement in regenerative science, clinician can utilize cells (BMDCs) which have high regenerative & repair potential. This is the first of its kind study analyses that cell therapy with Autologous Bone Marrow derived Cell Concentrate has benefitted patients.

Introduction

Since last many years, numerous clinical trails have begun targeting endometrial infertility, in particular Thin Endometrium that is often found in woman with Asherman's Syndrome. The healthy endometrium is composed of two layers: the functionalis and the basalis. The functionalis is a superficial layer, and its growth, deterioration and shedding are hormonally regulated during the menstrual cycle [1]. The major cause of uterine infertility is when the basal layer is destroyed and the functional layer fails to respond to hormonal stimulation.

Clinically, various strategies or add – ons have been imcorporated for endometrial regeneration including extended estrogen administration, low – dose aspirin, pentoxiphyllin, tocopherol, vaginal sildenafil & intrauterine perfusion with GCSF, PRP [2].

Offlate, Regenerative Medicine is a new field of Biomedicine and is currently gaining a lot of momentum as it provides massive promise for cell therapy, tissue engineering and an alternative approach to invasive surgeries or medicinal approach for repairing damaged or chronically inflamed tissue [3].

The cellular components used for Regenerative Medicine are undifferentiated cells that are present in the Embryonic, fetal and adult stages of life and give rise to differentiated cells that are building blocks to tissues and organs.

In this regard, Bone Marrow Cell Concentrate (BMC) or Bone Marrow derived stem cell concentrate (BMDSC) has numerous applications as potential biological cells for use in regenerative medicine [4].

BMDC contains variety of stem cells and other progenitor cells, including hematopoietic and mesenchymal stem cells (MSCs).

Recently, Therapeutics based in BMC / BMDC is an expanding field seeking to alleviate number of diseases involving chronic inflammation, fibrosis and wound repair [5].

For performing multiple functions for tissue regeneration various studies have explored the use of BMDC in a wide variety of disorders like neurological & bone related malfunctioning. Certain clinical studies have elaborated the role of bone marrow derived cells in restoring the endometrial function too.

The purpose of this study was to evaluate the use of Autologous, non-expanded, Bone Marrow Derived Cell concentrate (BMDC) to treat the Asherman's syndrome or Endometrial Atrophy in an attempt to achieve normal reproductive functioning of the endometrium by injecting this concentrate into the origin of uterine branches (spiral arterioles) through interventional radiology, to achieve maximum benefit of on point injection nearest to the supply of target organ in this case uterus and endometrium.

Based on this, we conducted a pilot study designed to demonstrate the efficacy of of Bone Marrow Derived Cell Concentrate in twelve patients diagnsed with Endometrial Atrophy and Asherman's Syndrome.

Materials and Methods

The study was approved by the Institutional Ethics Committee at the Sat Kaival Hospital Ethics Committee. All participants provided writen & audio – Video consents. We enrolled 14 patients, but one patient opted out and the other patient had dry tap.

Thus, 12 patients (from age 27 to 45) diagnosed with Endometrial Atrophy & Asherman's Syndrome (Thin Endometrium) were included in our study (Table 1).

According to the study design, Thin Endometrium was confirmed by Transvaginal Ultrasound done prior to the BMDCs treatment. Each patient had underwent Hormonal Replacement Therapy (HRT) cycle and acted as their own control before the cell therapy. It was

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Table 1: Endometrial Atrophy & Asherman's Syndrome (Thin Endometrium) were included in our study.

Patient	Etiology	History	Age	Preoperative Menstrual History	Maximum Preoperative Endometrium Thickness	Hysteroscopy	Right Size Artery - Intervention Radiology Findings	Left Size Artery - Intervention Radiology Findings	Post Operative Menstrual history	Maximum Post Operative Endometrial Thickness	Pregnancy
1	Endometrial Atrophy	Thin Endometrium Due to TB: EA	27	Scanty Spotting (MVV)	< 5 mm	Regular with HRT, Full Bleeding for 3 days	Big	Medium	3 - 4 pads	7 mm	Full term delivered Singleton
2	Asherman's due to TB (Asherman's stage 4)	Abdominal Koch TB - Refractory Endometrium	36	Spotting	5.5 mm	Improved thickness and Blood Flow	Big	Medium Stenotic	2 pads	8 mm	Delivered Twins
3	EA due to TB (Asherman's Stage 3)	Genital TB - Missed Pregnancy DNE	38	Spotting	4.8 mm	Improved thickness and Blood Flow	Medium	Small	2 -3 pads	7 mm	NEGATIVE
4	EA	Thin Endometrium - EA, @ missed abortions 1. 10 weeks & 2. 6 weeks, DNE Retained POC	38	Scanty Spotting	3.46 mm	Improved thickness and Blood Flow	Medium Mild Stenotic		2 - 3 pads	7 mm	ET waiting
5	Asherman's due to Myomectomy	DNE Refractory Endometrium	32	Spotting	4.46 mm	Improved thickness and Blood Flow	Medium Stenotic	Medium	Regular	9 mm	Delivered Twins
6	Asherman's Grade 4	Post delivery Infection, DNE done Intrauterine Death -- DNC -- EA	43	Scanty Spotting	4.9 mm		Small	Medium	Very good Flow		ET waiting
7	Asherman's Grade 4	Previous CS	39	Irregular Scanty period	4.1 mm		Stenotic Medium Vessel	Stenotic Thin Vessel		6.4 mm	Opted for Surrogacy
8	Severe Fibrosis adhesions	TB gold positive , History of one DNE	37	Regular, spotting	4.8 mm	Internal OS normal endometrial pale ,Uterine cavity normal, cord cell endometrium reuvination done	Small	Medium	Ongoing Pregnancy	7 mm	Ongoing Pregnancy
9	Dense adhesions in whole cavity , Menopuasal 5 years	Post Menopuasal + Asherman's syndrome	47	Menopuasal 5 years	5.4 mm	Dense adhesions present , Adhesions cut from the level of isthmus, uterine cavity treated till fundus	Equal Size Medium	Equal Size Medium	Spotting		ET waiting
10	Asherman's syndrome	Tuberculosis	36	irregular	4.5 mm		Medium	Small	Very good Flow		Trying Naturally
11	Asherman's syndrome	Due to Fibriods	47	Spotting	3.5 mm	Fibriods removed	Medium	Small	Spotting		Negative
12	Asherman's syndrome	Tuberculosis		Scanty Spotting	5.0 mm		Small	Medium	Very good Flow		ET waiting

noted that all patients experienced no or very scanty bleeding during their natural cycles or after HRT and none of them had ever achieved pregnanacy after the diagnosis.

For all the Patients Pre – Procedure Work – up was done and the patient was admitted for the procedure early morning.

Injection Ceftriaxone 1gm IV stat started, O_2 @ 2-4L/min for 30 mins,

Injection Vitamin C150 mg (diluted in 100 ml NS)

Injection Glutathione 600 mg (diluted in 100 ml NS)

Collection of Bone Marrow Aspirate (BMA)

Bone marrow aspirate (BMA, 60 to 80 ml) was collected over acid citrate dextrose-anticoagulant (5 ml) from the patient's Anterior Superior spin of Iliac Crest. The procedure was performed with IV sedation consisting of midazolam (Versed) and fentanyl. Positioning of the Jamshidi needle was confirmed by loss of resistance after piercing the compact bone. BMA was collected in a 60 ml syringe in a series of discrete pulls on the plunger (targeting a collection of 5–10 ml per pull), with repositioning of the needle tip between pulls based on the reported enrichment of progenitor cells.

Preparation of Bone Marrow Derived Cell Concentrate (BMDC)

Bone Marrow Aspirate (BMA) is processed using density gradient centrifugation as per the manufacturer's instructions. Aseptically transfer 3.0 ml of HiSep™ LSM 1077 to a 15.0 ml clean centrifuge tube and overlay with 10.0 ml Bone Marrow Aspirate (Diluted). DO NOT MIX. Centrifuge at 400 × (2000 RPM) with brake off, at room temperature (15-25°C) for 30 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and obtain a band of mononuclear cells (Bone Marrow Derived Concentrate) above HiSep™ LSM 1077 as show in Figure 1. Discard by aspirating most of the plasma and platelet containing supernatant above the interface band (granulocytes and erythrocytes will be in the red pellet). Using Pipette carefully aspirate Bone Marrow Concentrate and transfer it to a clean centrifuge tube. Add 10 ml of isotonic phosphate buffered saline to BMDC in the centrifuge tube and mix by gentle aspiration. Centrifuge at 160- 260 × g with brake off, at room temperature (15-25°C) for 10 minutes. This washing with isotonic phosphate buffered saline removes HiSep™ LSM and reduces the number of platelets. Wash the cells again with isotonic phosphate buffered saline and resuspend in an appropriate medium. Cell viability was then measured using trypan blue assay and manual cell count was performed using hemocytometer.

Intra Arterial Injection

After proper consent and all aseptic precautions, bilateral femoral artery access was taken with 6Fr sheath via seldinger technique under local anasthesia. 6Fr diagnostic JR cathether was used to reach contralateral internal iliac artery over 0.035 inch terumo guide wire. Then, it was exchanged to 6Fr JR 3.5 guiding catheter over 0.035 inch stiff wire.

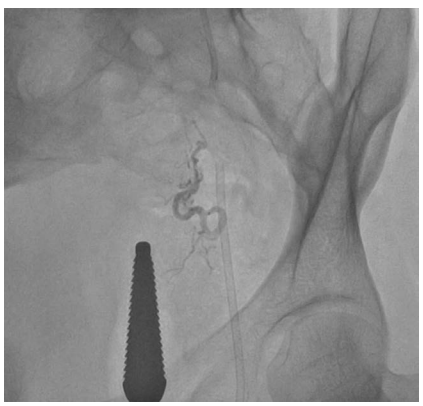

Figure 1: Intra Arterial Injection.

Uterine artery was identified by contrast dye injection in internal iliac artery. Super selective cannulation of uterine artery and subsequently spiral arterioles was done with microcatheter over 0.014 inch PTCA work horse wire. Bone marrow derived concentrate was injected into the vessel. Patency of artery post injection was confirmed by contrast dye injection from guide catheter through internal iliac artery. Post procedure femoral sheaths were removed in cath lab with manual compression and lower limb immobilization was done in supine position for 6 hours recovery room. Slowly patient was mobilized under observation and discharged next day Figure 1.

Post procedure

All the Patients were put on HRT for 3 months sequential Estrogen and Progesterone therapy and endometrium was monitored every month till the month of FET.

The patient showed increase in the volume of menstrual blood flow, which was scanty before and endometrium showed blood flow in color Doppler reaching upto zone 3, distinct 3-4 vessels were lucidly appreciated.

Results

From the enrolled 12 patients, 3 patients achieved Biological Pregnancy, 1 patient has ongoing pregnancy, 2 patients have Negative outcome and 6 are yet to do their embryo transfer. No major complications were noted in any of the 12 patients. Menstrual History of patients revealed that 10 patients had scanty blood flow and 2 had amenorrhea with previous HRT treatment. Despite the number of treatment the patients had undergone previously, none of the patients had shown any improvement. Before undertaking the cell therapy, the endometrial thickness of each patient was recorded and was less than 5 mm in each patient.

After the Cell Therapy

After the instillation of Bone Marow derived Stem Cell Concentrate, the Menstrual Cycles of all patients resumed cycles with normal to heavy bleeding upto 3 days.

Hysteroscopic observations performed before and after in all patients before and after the cell therapy releaved improvements in the endometrium lining, thickness and uterine cavity (Figure 2 & 3).

Discussion

Endometrium is a very important aspect of treating infertility. The morphology, thickness & vascularity are its important components, any defect in any of these aspects leads to compromised outcomes in ART. Asherman's Syndrome & Endometrial Atrophy is two grave conditions which have a huge impact on the condition of Endometrium & ART outcomes.

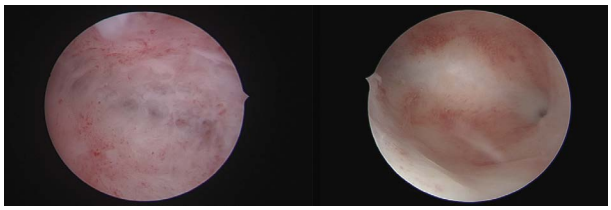

Figure 2: Cell therapy releaved improvements in the endometrium lining, thickness and uterine cavity.

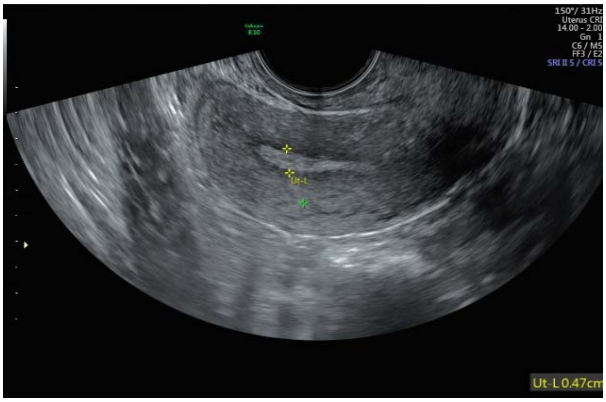

Figure 3: Hysteroscopic observations.

AS was popularized by Joseph Asherman and corresponds to replacement of the endometrial stroma by fibrous tissue where as glands are usually replaced by an inactive cubo-columnar epithelium that is generally non-responsive to hormonal stimulation. As a consequence, leads to complete disappearance of Endometrial structure [6] and alters the functioning of this organ in addition to obliterating the uterine cavity. Also Endometrial Atrophy (EA) is another rare grave condition with poor prognosis. The prevalence of this pathology is 0.5% of infertile women undergoing ARTs [7]. EA patients also have poor reproductive outcomes and although many therapies have been attempted [8] none have proven effective. With the popularity of endoscopy, new methods for diagnosis & treatment of both these conditions have been reported, but despite these advances 50% of these cases have remained without a comprehensive cure [9].

Both these conditions AS and EA results in thin endometrium, which interferes in normal implantation. Within a thin endometrium, embryo's implantation is close to the basal layer spiral arteries, which has copious blood flow and high oxygen tension, with the later resulting in the production of reactive oxygen species (ROS), that interrupts with embryonic development and implantation. With the instillation of BMDCs, there is a reduction in the oxygen tension in the functional layer, which improves and facilitates implantation.

Usually Gestational Surrogacy is the only alternative in cases of extensive Asherman's Syndrome or resistant Endometrial Atrophy. Gestational Surrogacy being banned in lot of countries around the world for legal or for religious reasons, this procedure gives hope to a lot of women suffering from these conditions.

Here we describe the first instance in 12 cases where BMDC therapy was used to treat these conditions. BMDCs were isolated from Bone marrow and then reintroduced into the uterine arterioles of the same patient using non-invasive interventional radiology so as to facilitate direct infusion in the affected endometrial milieu via uterine artery radical and arcuate artery followed by spiral arterioles.

There are various reports suggesting that stem cells populations do exist in human endometrium [10]. Reduced regenerating capacity or damage to these cells may induce pathological consequences in endometrium, which can be the underlying cause of thin or atrophic endometrium [11]. Bone marrow derived cell concentrate contains hematopoietic stem cells, mesenchymal stromal cells and endothelial progenitor cells which, in combinations, have the capacity to integrate into damaged tissues and trans-differentiate into host tissues, including endometrium [12].

BMDCs regenerate vascularization and induce endometrial proliferation to the creation of an autologous reconstruction of the endometrium BMDCs have been safely used for more than decade in clinical trails to regenerative medicine in non - hematological applications [13].

Carvello etal. 2015 in published the engraftment of BMDCs predominantly around the Endometrial blood vessels of traumatized endometrium [14], this study also demonstrated that BMDCs induce proliferation of the neighboring endometrial cells in the damaged endometrium mainly at the epithelial component.

Our theory is that hematopoietic stem cells in BMDC can stimulate the angiogenesis and secretion of tropic factors, which have immunomodulatory effects by upregulating anti inflammatory cytokines such as IL- 2, IL – 4, IL 10 and downregulating for inflammatory cytokines such as IL – 17 &TNF alpha. [15]. These Bone Marrow consists of a hematopoietic component and storma in general. The hematopoetic components includes hematopoietic progenitors and hematopoietic stem cells while the stoma of bone marrow contains cells with multipotential non hematopoietic cells capable of differentiating into various tissues of mesenchymal origin, including osteoblasts, chondrocytes, tenocytes, endothelial cells, myocytes, fibroblasts, nerves and adipocytes. Apart from tissue repair and regenration, Bone Marrow Derived mononuclear cells are known to enhance angiogenesis which further advances the process of regeneration in various tissue locations.

Till date only one article has been identified that reported autologous stem cells transportation by non-invasive radiological procedures in the endometrium milieu.

However, that study utilized CD133+ BMDCs (periphery mobilization) [16] against this study which implicates minimal manipulation by harvesting all of the cell populations in bone marrow concentrate rather targeting specific cell population like earlier CD+133 cells. This approach reduces the complexity of the procedure by limiting the number of steps involved and thereby reducing the over procedure time and risk of contaminating the sample with similar output.

Conclusion

Endometrium plays an important role in implantation. Since years Asherman's Syndrome and Endometrial Atrophy due to various causes have not hold good prognosis, with treatments available till date. Gestational Surrogacy is the only last option in such case. Stem cells have shown some promise in treatment of endometrial factor. To the best of our knowledge this is the only case series of 10 patients in the world where BMDCs were isolated and instilled in the uterine artery through interventional radiology leading to a live birth. This rare and novel form of treatment paves a way for future research in the treatment of Endometrial factor of infertility by Regenerative medicine.

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