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Regenerative Effects of Adipose-Derived Mesenchymal Stem Cells on Endometrial Hypotrophy: Preliminary Study

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Abstract

Cell therapy is a promising strategy for the treatment of endometrial hypotrophy, but the origin of these cells and injection site influence the therapeutic effect of cell therapy. This preliminary study aimed to investigate the efficacy of autologous adipose mesenchymal stem cells (ASCs) transplantation in regenerating endometrium in patients with endometrial hypotrophy. This cell therapy may become a promising treatment for infertile women with endometrial hypotrophy. A descriptive prospective study including 05 patients with endometrial hypotrophy was carried out at Mohammed VI University Hospital Center in Marrakesh over a period of two years (September 2020-September 2022). The patients between 18 and 45 with an infertility with endometrial hypotrophy < 7 mm, due to intra uterine adhesions (IUA), were included in this study. Autologous ADMSCs were isolated from patient's adipose tissue obtained by liposuction and then transplanted into uterus by transcervical instillation using an embryo transfer catheter followed by estrogen hormone therapy.

Subject Areas

Gynecology & Obstetrics, Women's Health

Keywords

Endometrial Hypotrophy, Intra Uterine Adhesion (IUA), Stem Cells,

Adipose-Derived Mesenchymal Stem Cell, Endometrial Regeneration, Infertility

1. Introduction

Endometrial thickness is an important parameter to evaluate endometrial receptivity. An appropriate endometrial thickness is necessary for both embryo implantation and ongoing pregnancy. Women with thin endometrium are one of the critical challenges in the clinic, but current therapeutic strategies for thin endometrium remain ineffective.

In normal conditions, the endometrium is regularly renewed under hormone stimulation. The basal layers repair and regenerate the endometrial cells. Intra uterine adhesions (IUA) due to infections or mechanical traumas, may lead to partial or complete destruction of both superficial and basal endometrial layers. Various treatments have been proposed for prevention or treatment of IUA; mainly, estrogeno-therapy and hyaluronic acid gel were proposed with disappointing results [1].

In the last twenty years, several studies have identified a small number of endometrial stem/progenitor cells in the basal and functional layers of the endometrium [2]. Researchers have identified a small number of endometrial stem cells with, self-renewal, and differentiation potential, such as endometrial epithelial progenitor cells (EEPCs), mesenchymal stem cells (eMSCs) (as shown in Figure 1 & Figure 2).

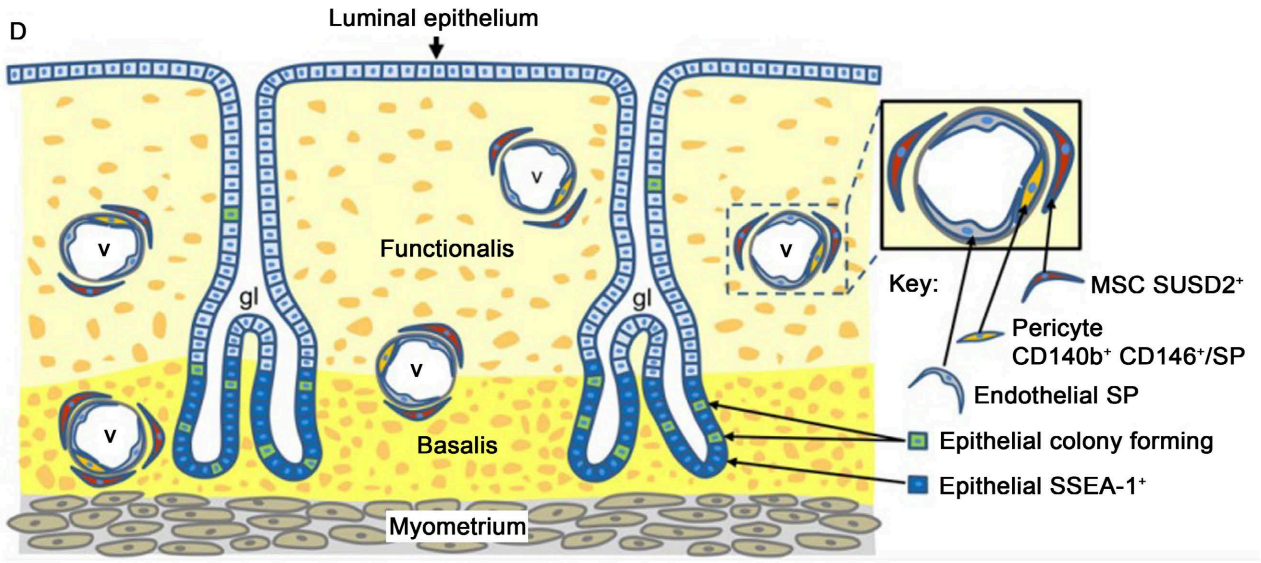

Figure 1. Stem/progenitor cells identified in the human endometrium. Endometrium is composed of endometrial epithelium, functionalis and basalis. Epithelial progenitor cells are postulated to be located in the base of the glands in the basalis; Perivascular SUSD2+ (W5C5 antibody) cells with *in vitro* and *in vivo* mesenchymal stem/stromal cells (MSCs) properties are found in basalis and functionalis; PDGFR-*β*/CD140b+CD146+ endometrial MSCs (eMSCs) are pericytes. ESP cells consist of CD31+ endothelial cells and CD140b+ CD146+ pericytes (Adapted from Gurung *et al.* [3]).

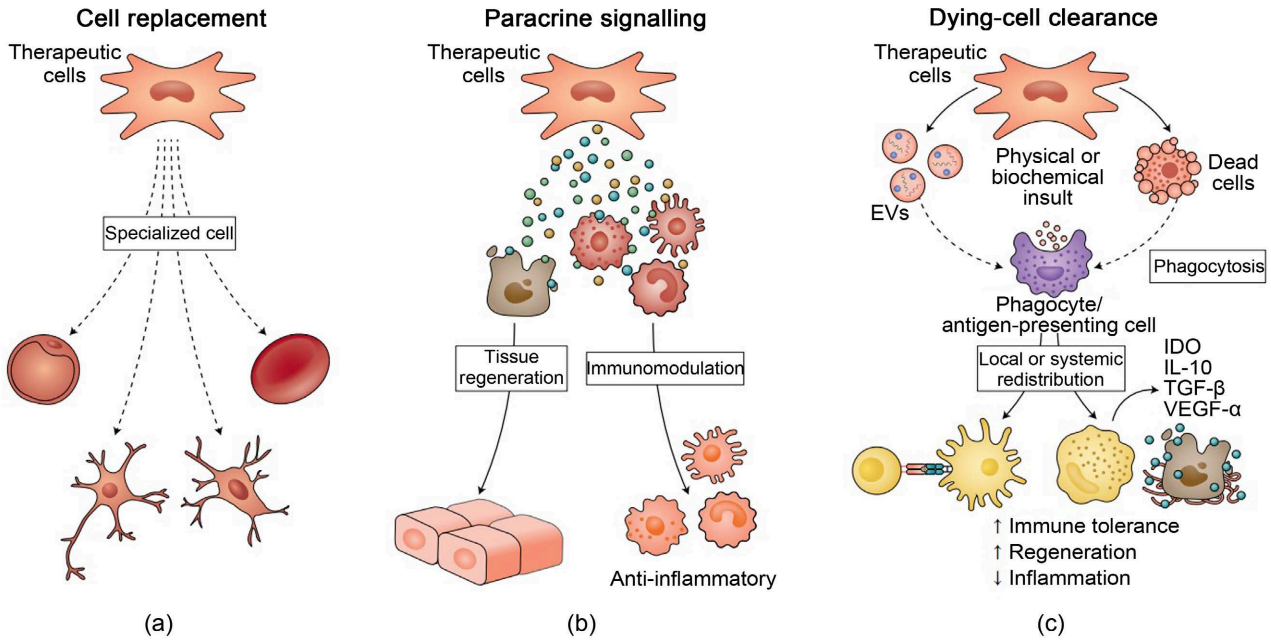

Figure 2. Potential mechanisms of stem cell-based therapy. (a) Cell replacement by stem cells multi-lineage differentiation; (b) Cell communication, though paracrine signaling; (c) Dying cell clearance through phagocytosis. (Adapted from Wagoner and Zhao [4]).

Adult stem cells derived from other tissues have also a great potential in repairing endometrial injuries [5] [6]. eMSCs particularly participate in endometrial regeneration and remodeling and are one of the candidate adult stem cell types for stem cell therapy. They have proangiogenic, antiapoptotic, immunomodulation, and chromatin stability functions and maintenance without tumorigenicity and low immunogenicity [2] [7] [8].

However, differentiation is extremely sensitive to the microenvironment and senescence is one of the major reasons for stem cell therapy failure.

Adipose tissue-derived mesenchymal stem cells (AD-MSCs) have the advantages of large reserves *in vivo*, they are easy to obtain and have the strong proliferative capacity.

There are relatively few studies on AD-MSCs in endometrial repair. AD-MSCs and their exosomes promote regeneration of the endometrium in rats with intrauterine adhesion, improving endometrial receptivity [9] [10]. This indicates the local application of AD-MSC exosomes in the uterus may be a promising treatment for patients with intrauterine adhesion. However, limited evidence has demonstrated the efficacy and safety in patients with thin endometrium.

The aim of our preliminary study is to inject mesenchymal stem cells from homologous fatty tissue in view to treat the endometrial hypotrophy of five patients suffering from infertility.

2. Methodology

This study is a preliminary single-center, longitudinal, descriptive and prospec-

tive self-control study to investigate the preliminary efficacy and safety of autologous adiposis stem cells in improving the pregnancy outcome of infertile patients with thin endometrium.

It was carried out at Mohammed VI University Hospital Center in Marrakesh over a period of two years (September 2020-September 2022). All our patients were informed by pre-established formulary on the basics of the study and signed their consent before the study. The ethics committee of the hospital gave their consent to this study also.

3. Materials

Five patients diagnosed with thin endometrium were recruited based on the inclusion and exclusion criteria. Inclusion criteria were patients with an age between 18 and 45 years, suffering from an infertility due to an endometrial atrophy with a thickness < 7 mm in echography measures, after an estrogenic treatment well conducted. Exclusion criteria included the patients having contraindications to liposuction, pregnancy itself, or another cause of infertility. All the patients in this study benefits from hysteroscopicadhesiolysis followed by hormone therapy.

Liposuction was performed in the plastic surgery department at the abdominal level, associated with a blood sample for the preparation of PRP. In coordination with the regenerative medicine department, the sample of adiposis cells undergoes processing resulting in the obtaining of the stem cell concentrate (optimal number of cells 15 - 30 million).

MSCs were isolated from the stromal vascular fraction (SVF) of the sample, which was obtained from adipose tissue after mechanical washing, enzymatic digestion and centrifugation.

The immunophenotyping of the different cells contained in the SVF was based on flow cytometry. The cells are washed by adding physiological saline. This operation is repeated as much as necessary until a clear final solution is obtained. The enzyme used in the second part of the process is collagenase type I and II. The collagenase-saline mixture is added to the tubes. The tubes are placed on a thermal shaker, preheated to 37˚C. for approximately 20 min. The digestion process is visually inspected by product homogenization. It is stopped once the digestion process is complete.

Then, the patient benefits from intra-myometrial insemination of the collected product (stem cells + PRP), after gentle curettage, under ultrasound guidance. The needle is introduced sub-endometrially until it reaches the myometrium. The injection was performed in 7 sites: 3 on anterior/front side of the uterus, 2 lateral and 2 on the posterior side. The suspension will be transferred into the uterine cavity via an embryo transfer catheter.

Hormonal therapy with oral estradiol valerate 8 mg for 20 days.

Then, comparisons between pretreatment and post-treatment were analyzed, and the outcomes, including endometrial thickness, menstrual volume and du-

ration, frequency and severity of adverse events and early pregnancy outcomes, will be measured within a 3 - 6 and 9 month/follow-up.

4. Results

A total of five patients were included in this study. Mean age was 39 years old with a mean duration of infertility of 06 years. No antecedent of invasive genital surgery was reported. Genital tuberculosis was reported in 02 cases. Amenorrhea was found within one patient, hypomenorrhea within 02 patients and normal regular cycles within 02 others. All our patients benefit from a diagnostic and therapeutic hysteroscopy for adhesiolysis. The AS staging was severe within 03 patients, and moderate within 02 others patients (Table 1).

Our first patient (M.F) benefit from intra-cervical insemination into the uterine cavity, whereas the four other patients benefit from intra-cervical intra-myometrial insemination via an embryo transfer catheter. We organized a follow-up on first month, 3 and 6 months later. The outcomes were listed in Table 2. No side effects nor complications attributable to our intervention was reported.

Table 1. Demographic and clinical characteristics of the patients.

	Age	Infertility Etiology	AS staging	BMI	Ovarian dysfunction	Menstrual pattern
M.F	38	Genital Tuberculosis	severe	25	Poor Ovarian reserve	Hypomenorrhea
N.A	38	-	moderate	25	Normal	Normal
H.B	38	-	Severe	30	Poor Ovarian reserve	Hypomenorrhea
F.Y	39	Genital Tuberculosis	Severe	25	Normal	Amenorrhea
S.L	37	-	Moderate	30	Normal	Normal

Table 2. Evolution of endometrial thickness after injection of stem cells.

	Before injection	M1	M3	M6	Menstrual pattern
M.F	1 mm	-	-	-	-
N.A	2 mm	2 mm	-	-	Normal
H.B	4 mm	7 mm	6 mm	5.8 mm	Normal
F.Y	1 mm	5 mm	4 mm	2 mm	Amenorrhea
S.L	4 mm	7 mm	6 mm	6 mm	Normal

5. Discussion

5.1. Endometrium

The endometrium is a regenerative tissue that undergoes periods of growth and turn over during the menstrual cycle in female reproductive years. Endometrial regeneration also occurs after parturition and endometrial resection, and in postmenopausal women taking estrogen replacement therapy [7].

It has been suggested that adult stem or progenitor cells are responsible for the cyclic regeneration of both functional and basalis layers [11]. Many reasons can be responsible of this thickness anomaly including [12] postpartum curettage, spontaneous miscarriage, pregnancy termination, endometrial ablation, infection and inflammation, or genetic vulnerability. This lack of functional endometrium leads to both quantity and functionality anomalies for embryo implantation.

Asherman's syndrome (AS) is defined histopathologically [13] as a loss of functional endometrium, which is replaced with fibromuscular tissue, resulting in obliteration of the uterine cavity by intrauterine adhesions. AS is not associated with any clinical symptom if there is no fertility concern, and clinical manifestations include amenorrhea, hypomenorrhea, recurrent pregnancy loss, infertility, and a history of abnormal placentation.

It is important to underline that there is plenty of cases reported in literature where the presence of intrauterine adhesion (IUA) is not associated with any symptoms. Under these circumstances, some authors believe that the term of AS should be avoided [13].

The standard treatment of AS is hysteroscopicadhesiolysis, and an intrauterine device, uterine balloon stent, Foley's catheter, or an anti-adhesive barrier may be used to prevent recurrence. Even after hysteroscopicadhesiolysis, the endometrium may not be fully recovered. For restoring normal endometrium, various therapeutic methods have been used, including supplemental administration of estradiol, low-dose aspirin, vaginal sildenafil citrate, gonadotropin-releasing hormone agonists, human chorionic gonadotropin (hCG), and intrauterine granulocyte colony-stimulating factor instillation [13].

5.2. Stem Cells

Stem cells are known as undifferentiated cells that have the potential to be multiplied as a stem cell in undifferentiated form (self-renewal) and to mature and differentiated cells. They owe their regenerative capability to telomerase activity.

There are two types of stem cells: embryonic, originating from blastocysts, and adult stem cells, located in regenerating tissues. Embryonic stem cells are highly proliferative but also tumorigenic. Adult stem cells have a low malignant potential and thus are the type used for cell therapy.

Adult stem cells (ASCs), also referred to as somatic stem cells, are a genre of multipotent stem cells located in specific differentiated organs and can differentiate into a limited type of mature cell to maintain tissue homeostasis [14]. The

necessary conditions are provided by the specific anatomical location surrounding the ASCs. This microenvironment, called the stem cell niche, gives rise to autocrine, paracrine, and systemic signals that enable stem cell maintenance and differentiation into specific cell types that participate in tissue repair or regeneration.

Considering the self-renewal and the capacity of multi-lineage differentiation, stem cells therapy can be useful in the treatment of many degenerative diseases and situation that therapeutic choices are limited or do not exist.

Most of the described ASCs reside in the bone marrow, but they are also detected in several organs as adiposis tissue, umbilical, endometrium, placenta and amnios. Traditionally, stem cells can be classified according to their location and differentiation potency. the multipotent stem cells can differentiate into a confined number of cell types limited to the same lineage, such as mesenchymal stem cell (MSC).

Mesenchymal stromal cells (MSCs) owing to their easy separation from various tissues, plentiful proliferation capacity in vitro without any change in their biological features, injured tissues tropism, weakly immunogenic, secretion of anti-inflammatory molecules, and lowest hurt to normal cells/tissues, have been the best option for cell/gene therapy and regenerative medicine in various tissues such as uterus [15].

Stem cells have the potential of substituting damaged cells in the endometrium. Cell replacement strategies have been suggested and examined in some animal models of endometrial pathology and also ovarian pathology. Since multiples sources and ways to stem cells insemination exists, many researches are conducted in this way to change the prognosis of these incurable pathologies and infertility issues.

5.3. Endometrium Thickness and Implantation Rate

In most cases, a thin endometrium is defined as ≤7 mm by ultrasound examination. However, the definition of "thin" varies between ≤ 6 - 8 mm [16]. Its incidence increases with age and reaches 25% in women older than 40 years.

Sufficient growth of the endometrium is one of the essential factors for prosperous implantation. According to some reports [17], low rates of implantation are associated with "thin" endometrium. A prospective observational cohort study also suggested that the probability of pregnancy and live birth in patients with thin endometrium was significantly reduced (15.2% vs 29.2%) [18].

Recovering the endometrium in patients suffering from thin endometrium is time-consuming. Various regimens have been explored without giving the expected satisfaction. The use of MSCs seems to be promising. Various cell types have been explored in clinical and preclinical models for endometrium regeneration-based cell therapy.

Several angiogenic factors associated to MSC have been target to be responsible for the raise of endometrial thickness. However, the heterogenicity in studied

populations and the lack of large data does not allow the identification of a precise effective fraction of MSCs.

5.4. Thin Endometrium and Genital Female Tuberculosis

Female genital tuberculosis (FGTB) is considered the 4th most common site of tuberculosis after pulmonary, pleural and abdominal sites. The diagnosis remains difficult because of the absence or lack of symptoms. Also, this form of tuberculosis is underestimated by clinicians due to non-specific symptoms and rarity nowadays.

The evaluation of the prevalence of genital tuberculosis [19] has always been challenging and no study, to our knowledge, has found exact results.

Several studies [20] tend to do a screening of all at-risk infertile patients for latent tuberculosis because of the unknown prevalence, the latency of symptoms and the multi-factorial etiologies of infertility. They found a large proportion of patients with positive QuantiFERON Gold test in the infertile groups. However, it has been reported that uncontrolled diabetes mellitus may alter the results of QuantiFERON Gold and this parameter has not been reported in these studies, leading to ask the question: can we lean on this dosage of QUANTIFERON or QuantiFERON gold to make the diagnosis of FGTB?

Considering that Morocco is a country where FGTB is still a huge public health burden; side effects on the reproductive anatomy can be devastating. Clinical presentation of FGTB can be varied, ranging from infertility, menstrual abnormalities, and chronic pelvic pain to pregnancy loss.

Infertility is seen in up to 40% - 80% of cases of FGTB. Sharma *et al.* reported [21] several mechanisms for this infertility "Distortion and obstruction of fallopian tubes, involvement of the endometrium with resulting disorders of endometrial receptivity, destruction of normal endometrium and resulting intrauterine synechiae, defective ovarian function caused by involvement of the ovaries in tubo-ovarian inflammatory phlegmon formation, and destruction of normal ovarian tissues with resulting compromise to ovarian reserve".

Many advances in assisted reproductive technology and access to treatment and in vitro fertilization have made it possible for women to conceive. Yet, this etiology is still considered one of the most challenging in infertility.

The absence of diagnosis within our patient who had FGTB in adolescence period and had a primal amenorrhea, made the cure of AS really difficult and the possibility of increasing endometrial thickness tough. Our first patient had FGTB too and experienced multi-focal TB (pulmonary and genital) with a long treatment due to relapses. The side effects on genital anatomy were not only on the endometrium but also the ovarian reserve. The diagnosis of FGTB was made on endometrial biopsy within the 2 patients.

5.5. ADMSCs and Endometrial Effect

Since our trial was based on ADMSCs (adipose-derived mesenchymal stem cells),

we will discuss the relative results of these particular stem cells in treatment of female infertility.

ADMSCs have the advantages of large reserves in vivo, they are easy to obtain by liposuction and have the strong proliferative capacity, and their applications in the tissue engineering field have gradually shown great potential, including the treatment of diabetes, osteoarthritis, and nerve injury repair.

ADMSCs have been explored in the infertility caused by ovarian dysfunction. Premature ovarian insufficiency (POI) is the main cause of female infertility. ADMSCs are ideal candidates for the treatment of POI, by restoring ovarian hormone levels and improving ovarian function through the paracrine mechanism.

There are relatively few studies on ADMSCs in endometrial repair. Many genes/proteins are responsible for the development and regeneration of endometrium, of which Wnt, c-kit (CD117), Oct-4, CD34/KLF4, and Musashi-1 are best classified [22].

Controversial results was found between Hosseinzadeh Shirzeily [23] and Cakici [24]: The first group indicated that ADMSCs had less differentiation ability than BM-MSCs following treatment with RA, whereas the Cakici *et al.* [24] group demonstrated that these MSCs could differentiate into functional sperms; the observation of birth of 9 live offspring after mating healthy females with males transplanted with ADMSCs was the direct evidence of the role of ADMSCs in the restoration of fertility ability.

Another trial from Fang [25] showed that overexpression of CD61 in canine ADMSCs promotes their differentiation into primordial germ cell (PGC)-like cells by relying on the activation of TGF-β pathway. Thus, ADMSCs possess a considerable potential in treating the infertility of rare animal species.

ADMSCs and their exosomes promote regeneration of the endometrium in rats with intrauterine adhesion, improve endometrial receptivity, and reshape endometrium fertility [9], which indicates the local application of ADMSCs exosomes in the uterus may be a promising treatment for patients with intrauterine adhesion.

Other studies suggest that ADMSCs could have an effect on oncofertility which has become an emerging field of medicine and research. Since cancer treatment brings unexpected side effects on the reproductive system, successful applications of adult stem cells to reprogram female fertility will change prognosis and the management of endometrial cancers.

It has been reported that normal endometrial stromal cells cultured in the appropriate extracellular matrix can modify the malignant phenotype of a well-differentiated endometrial cancer cell line [26]. Various reports have shown that MSCs can inhibit tumor growth and angiogenesis by secreting exosomes, paracrine factors, and by regulating the local immune environment, thereby inhibiting tumor progression. For example, omental adipose stromal cells, a multipotent population of MSCs contained in the omental tissue, can promote tumor growth and induce therapy resistance by upregulating glycolysis and reducing

oxidative stress in endometrial and ovarian cancer cells [27]. However, there remains a lack of research on the repair of the damaged endometrium after reversal of endometrial cancer. These statements need further study to explore the safety of ADMSCs in cancer uses.

However, some notable hurdles, such as stemness loss, immunogenicity, low retention and survival rate, limit their clinical application. One essential factor that we report in our study is the long-term engraftment of transplanted adult stem cells to target tissues. It has been proved that even if such cells can survive without estrogen, their stem cell niches require estrogen to activate them. Yet, even with the estrogenic environment, the immediate effect of raising the endometrial thickness does no longer exist after 3 or 6 months. Some microenvironmental factors such as hypoxia and nutritional deficiency might disturb the viability of transplanted stem cells in the injured tissue [28]. Factors such as IGFBP3, which induces oxidative stress, can drive adult stem cell senescence through inhibiting stem cell differentiation and expansion.

Some biomaterials have been tested to improve the viability and stability of stem cells: Hyaluronic-acid gel, Collagen scaffold and hydrogels. Yet, the superiority of the effects cannot be guaranteed with these biomaterials.

The perfect solution will be to find molecules that block the senescence of MSCs or introduce materials into MSCs therapeutic systems which might effectively maintain stem cell differentiation and secretion spectrum.

Finding a certain subtype among these MSCs, that can not only effectively repair immediately the damaged endometrium, but also be stable and have a long-term effect for recovering normal endometrial function.

In preclinical models, therapeutic systemic injection of stem cells seems to be more effective than direct intrauterine injection in regenerating the endometrium.

Liu *et al.* [29] experimented local and systemic administration of stem cells (which were obtained from the uterus and bone marrow) with a mild uterine injury but no adhesions.

Systemic injection of BmMSCs and USCs led to greater increase of endometrial thickness at 2- and 3-weeks post-injection by recruitment of GFP+ cells. Their group concluded that systemic injection is superior to local injection in homing stem cells to the uterus.

Another study by Zhao *et al.* [10] demonstrated encouraging effects of BmMSCs transplantation not only on endometrial regeneration but also on its receptivity. Rats were administered ethanol in the uterine cavity to induce thin endometrium. Bone marrow mesenchymal stem cells were transplanted directly into the uterus. The endometrial thickness was significantly thicker, pro-inflammatory cytokines (IL-1β, TNF-α) were attenuated, anti-inflammatory cytokines (IL-6, bFGF) were upregulated, and markers of endometrial regeneration (cytokeratin, vimentin) were significantly greater in the treatment group compared to the control.

It is hypothesized that blood provides the stem cells with various trophic factors, which may enhance their survival when injected systemically as compared to local injection into the uterus.

Homing is the key aspect to stem cell efficacy since their effect is substantiated only if appropriately located. Hence, systemic injection of MSCs may led to greater results.

Sanchis *et al.* [30] evaluated the capacity of ADMSCs to induce endometrial proliferation and angiogenesis in mice with Asherman syndrome. The findings assess that combining ADMSCs with estrogen has a synergistic effect on the regrowth of the endometrium. The same procedure was used in our study, but since we didn't have a control group with only estrogen nor ADMSCs, we can only argue that combining ADMSCs and estrogen is synergic.

6. Conclusion

Cell therapy is a promising strategy for the treatment of endometrial hypotrophy, either within ADMSCs or BmMSCs. This study demonstrates clinical efficacy of ADMSCs for increasing endometrial thickness and menstrual flow in patients with severe AS. Application of autologous mesenchymal stem cells may increase endometrial receptivity and the chance for pregnancy using assisted reproductive technologies. However, more investigations are needed to confirm the improvement in pregnancy outcomes.

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Authors' Contributions

All authors contributed to conception and design of the study. The main idea was from YAB.

YAB and MN wrote the first draft of the manuscript. All authors listed contributed to revision and approved the submitted version.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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