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Research Article

The Role of Menstrual Stem Cells in Premature Ovarian Failure and Asherman's Syndrome: a systematic review

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Abstract. A new population of stem cells have recently been discovered within the menstrual fluid. These cells exhibit fibroblast-like morphology and meet the minimum criteria for stem cell classification as stipulated by the International Society for Cellular Therapy. Menstrual-derived stem cells (MenSC) exhibit mesenchymal stem cell characteristics, high proliferation and multilineage differentiation potential. MenSC are derived from endometrial cell populations which, together with a large part of the endometrium, are sloughed from the endometrium during the menstrual phase of the uterine cycle. MenSC are cyclically available in large numbers and can be obtained non-invasively and cheaply. Furthermore, MenSC are not limited by ethical dilemmas since they are obtained from menstrual blood which is considered a clinical waste. These attributes make MenSC an attractive alternative to other conventionally used adult stem cells and consequently have attracted substantial interest in the field of gynaecology and regenerative medicine. This systematic review will focus on the potential role of MenSC particularly in premature ovarian failure and Asherman's syndrome.

Keywords: Mesenchymal Stem Cells, Menstrual Stem Cells, Gynaecology, Premature Ovarian Failure, Asherman's syndrome

Abbreviations

AMH anti-mullerian hormone
BMSCs Bone marrow-derived mesenchymal stem cells
CM Culture media
c-MYC Master regulator of cell cycle entry and proliferative metabolism
COL6A5 Collagen Type 6 Alpha 5 Chain
COL9A2 Collagen Type 9 Alpha Chain 2
CTGF connective tissue growth factor
COX-2 cyclooxygenase 2
CXCR41 chemokine receptor type 41
CY cyclophosphamide
DMEM Dulbecco's Modified Eagle Medium
ECM extracellular matrix
EE endometrial epithelial cells
ES Endometrial stromal cells
EVs extracellular vesicles
FSHR follicle stimulating hormone receptor
FST follistatin
HLA-A/B/C Human leukocytic antigen
HRT hormone replacement therapy
HUVECs Human Umbilical Vein Endothelial Cells
IDO Indoleamine-pyrrole 2,3-dioxygenase
IL-4 interleukin 4
IFN-γ interferon gamma
IPSC induced pluripotent cell
IUA intrauterine adhesions
KLF-4 Kruppel-like factor 4
MAC-1 Macrophage 1
MenSC menstrual-derived stem cells
MLR mixed lymphocyte reaction
MPO Myeloperoxidase
MHC-II Major histocompatibility complex class 2
MSCs Mesenchymal stem cells
NK natural killer
PC3 Prostate Cancer 3
PD-L1 Programmed death ligand 1
PBMC peripheral blood mononuclear cell
ROS reactive oxygen species
SOX-2 Sex determining region Y-box 2
SSEA-4 Stage specific embryo antigen 4
STRO1 Mesenchymal stem cell marker 1
TAZ Hippo/Transcriptional coactivator with PDZ-binding motif

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Th2 T-helper 2 cells
T-regs Regulatory T cells
TNF-α tumour necrosis factor alpha
OCT-4 Octamer-binding transcription factor 4
POF/POI Premature Ovarian Failure/Insufficiency
qRT-PCR Quantitative real-time polymerase chain reaction
VEGF Vascular endothelial growth factor

1 Introduction

Since their discovery, menstrual-derived stem cells (MenSC) have been at the centre of research in the field of regenerative medicine. Having the necessary qualities to be classified as stem cells, these cells have been met with great enthusiasm by researchers due to the advantages of being freely available, obtained non-invasively and without any ethical dilemmas. Characteristic evaluation of MenSC shows that these cells employ a dose dependent pro/anti-inflammatory response and are able to resolve inflammation in mice models with different pathologies (H. Wang et al., 2012). MenSC are also able to induce angiogenesis and are superior to fibroblasts in terms of induced pluripotent stem cell (IPSC) production. Additionally, when compared to bone marrow-derived mesenchymal stem cells (BMSCs), MenSC have superior proliferative, migratory, healing and angiogenic potential yielding better results. Finally, the use of MenSC derived exosomes gave impressive results and were shown to be superior to MenSC in wound healing. Several studies have investigated the role of MenSC in the treatment of gynaecological disorders. These include premature ovarian failure and Asherman's syndrome, which to date have been manageable but incurable disorders, which are both associated with infertility. (Feng et al., 2019; Lai et al., 2015; T. Liu et al., 2014; Y. Liu et al., 2018; Ma et al., 2020; Manshadi et al., 2019; Noory et al., 2019; Tan et al., 2016; S. Zhang et al., 2019; S.-X. Zheng et al., 2018; Zhu et al., 2019). Asherman's syndrome occurs secondary to damage to the endometrium (Yu et al., 2008). In this review, the *in vitro* and *in vivo* modulatory effects of MenSC are outlined followed by a description of their angiogenic and induced pluripotential effect, as compared to BMSCs, with a special focus on their role in the management of premature ovarian failure and Asherman's syndrome.

2 Methods

This systematic review aims to evaluate the potential use of MenSC, with a focus on the gynaecological applications of MenSC. The non-gynaecological applications were already reported (Galea et al., n.d.). As previously described, the electronic databases MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials were searched from 2007 up to August 2020, week 4, using the keywords and MeSH/EMTREE terms reported in the Appendix. Articles published in the English language assessing the clinical applications of MenSC were retrieved. We identified 1295 potentially relevant papers. Among these, 349 papers were excluded because they were duplicate, and 792 were excluded after evaluating titles and abstracts. Thus, 154 articles were retrieved as full text. Another 110 papers were excluded after evaluating the full texts. Finally, 40 studies were included in this systematic review.

2.1 *In Vitro* Immunomodulatory effect

The immunomodulatory properties of MenSC were investigated in a mixed lymphocyte reaction (MLR) and it was shown that they inhibited the secretion of interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) whereas interleukin 4 (IL-4) secretion was upregulated. MenSC also successfully inhibited peripheral blood mononuclear cell (PBMC) proliferation and altered T-cell responses towards T-helper 2 cells (Th2) (Murphy et al., 2008). Furthermore, the inhibitory effect brought about by MenSC on allogenic MLR was shown to depend on the ratio between MenSC and PBMCs (Nikoo et al., 2012). At high MenSC to PBMC ratios of 1:1 and 1:2, the MenSC were able to suppress PBMCs, but on dilution to a ratio of 1:32 or lower, MenSC were remarkably able to stimulate allogenic PBMC proliferation. Thus, MenSC have a dose dependent anti-inflammatory /pro-inflammatory effect (Nikoo et al., 2012). MenSC co-cultured with PBMCs at a relatively high ratio of 1:10, exhibited similar T-cell suppression as that caused BMSCs. However, following dilution to 1:100 MenSC exhibited a lower suppressive effect on PBMCs than BMSCs. This is possibly a result of lower expression of cyclooxygenase 2 (COX-2) and IL-6 and lower secretion of Activin A and Indoleamine-pyrrole 2,3-dioxygenase (IDO), an immunosuppressive agent which can limit T cell function in comparison to BMSCs. Consequently, at low suppressor: effector ratios MenSC induced less anti-inflammatory IL-4, IL-10, and CD4 lymphocytes. As a result, pro-inflammatory CD8 T cells and CD4 Th1 cells were less inhibited (Luz-Crawford et al., 2016). MenSC exposed to pro-inflammatory cytokines such as IFN-γ and IL-1β secreted more IL-6 and TGF-β, which in turn downregulated NK cell cytotoxicity and proliferation. Uterine natural killer (NK) cells constitute 50–70% of all lymphocytes in the early pregnant uterus. Effective downregulation of these cells is important to preserve the semi-allograft foetus to term (Moffett-King et al., 2002). This suggests that endometrial stem cells may play a potential

role in NK modulation and subsequent gestational success (Shokri et al., 2019).

2.2 *In vivo* Immunomodulatory effect

MenSC administration has been shown to significantly ameliorate colitis in various mouse models and improve prognosis (Lv et al., 2014; Shi et al., 2019; Xu et al., 2018). The resolution of colitis was mediated though the immunomodulatory effect of MenSC on the inflammatory milieu. Following MenSC treatment, the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 were significantly less concentrated in serum, whereas the anti-inflammatory cytokines IL-10 and IL-4 were significantly more concentrated in comparison to the untreated control (Xu et al., 2018). Furthermore, MenSC treatment decreased the levels of Macrophage 1 (MAC-1) positive cells and Myeloperoxidase (MPO) positive neutrophils. Additionally, MenSC treatment also significantly decreased the number of splenic dendritic cells and significantly decreased the Major histocompatibility complex class 2 (MHC-II) expressed on these cells. This limits the antigen-presenting capacities of these cells and therefore limits effector cell activation and subsequent inflammation (Lv et al., 2014). MenSC treatment also significantly alters immune cell populations in colitis. Regulatory T cells (T-regs) have been shown to be significantly elevated whereas CD3+CD25+, CD3+CD8+, CD3+CD4+,CD3+ T cells were decreased (Lv et al., 2014; Shi et al., 2019; Xu et al., 2018). These immunomodulatory effects are mediated by Programmed death ligand 1 (PD-L1); in fact if this is blocked, MenSC fail to attenuate colitis (Shi et al., 2019).

MenSC treatment has been shown to double the survival of heart transplants in treated mice over untreated controls through the attenuation of acute vascular rejection. At MenSC:B-cell ratio of 1:10 or higher, MenSC inhibited B cell proliferation, repressed the expression of surface molecules CD80, CD83 and CD86; and consequently resulted in decreased IgM and IgG levels (Xu et al., 2017). MenSCalso exhibit antimicrobial activity and secrete higher levels of hepcidin (Alcayaga-Miranda et al., 2015). *In vivo* MenSC administration to a caecal ligation puncture model of sepsis mediated the uncontrolled inflammatory response through the reduction of various cytokines including TNF-α, MCP, IL-6 and IL-10, which resulted in a 43% survival rate in comparison to 6% in the untreated model. Moreover, with the combined treatment of antibiotic and MenSC, the survival rate stood at 95% which was even higher than the 73% survival rate obtained with antibiotic treatment alone (Alcayaga-Miranda et al., 2015).

2.3 Angiogenic Potential of MenSC

Analysis of growth media following MenSC culture identified the presence of multiple angiogenic factors including Angiopoietin 2 and vascular endothelial growth factor (VEGF) (Meng et al., 2007). Hence, these cells influence angiogenesis through the production and secretion of these factors. Six hours after the introduction of MenSC into an *in vitro* angiogenesis assay, vessel formation was visualised. MenSC were grown under hypoxic and normoxic conditions, following which the culture media was used to grow human umbilical vein endothelial cells (HUVECs). HUVECs cultured on media obtained from the hypoxic culture group contained more VEGF than the culture medium obtained from the normoxic group. Consequently, the hypoxic cultured medium showed increased tube formation in comparison to HUVECs grown in the culture medium obtained from the normoxic group. This was quantified by a significant increase in length, percentage of covered area and loop number, due to more angiogenic factors being secreted (Alcayaga-Miranda et al., 2015). MenSC extracted from patients with a previous history of preeclampsia and from normal patients were cultured, following which, the conditioned medium was used for HUVEC culture. Growth of HUVECs occurred more in the cultured media obtained from healthy patients and consequently the vessels developed significantly more branches (Varas-Godoy et al., 2019). Following these observations, the levels of secreted VEGF was measured (Alcayaga-Miranda et al., 2015; Varas-Godoy et al., 2019) VEGF is a pro-angiogenic molecule which is upregulated through various pathways, such as through the hypoxia inducible factor alpha under hypoxic conditions (Akimoto et al., 2013). VEGF levels were shown to be 174 times higher in the hypoxic group than in the normoxic group and MenSC obtained from preeclampsia patients secreted significantly less VEGF (Alcayaga-Miranda et al., 2015; Varas-Godoy et al., 2019). Matrigel was mixed with MenSC and with Dulbecco's Modified Eagle Medium (DMEM) and both were subsequently injected within mice. *In vivo* transplantation of MenSC in matrigel plug assay promoted the development of vessels and haemoglobin which were significantly higher than changes induced by DMEM in Matrigel (Alcayaga-Miranda et al., 2015). These data support the hypothesis that in women with a previous pregnancy complicated by preeclampsia, their angiogenic and inflammatory properties of MenSC have reduced angiogenic capacity and are more proinflammatory than those with a previous normal pregnancy. This may be present before the development of the pathology, leading to limited vascular remodeling within the developing placenta, and can be used in the future for targeted therapeutic intervention.

2.4 Induced Pluripotent stem cells generated from MenSC

The reprogramming of a human adult fibroblast back to an induced pluripotent stem cell (IPSC) was a major breakthrough in the field of regenerative medicine. The conversion occurs through the expression of Yamanaka's factor genes; Octamer-binding transcription factor 4 (OCT-4), Kruppel-like factor 4 (KLF-4), Sex determining region Y-box 2 (SOX-2) and master regulator of cell cycle entry and proliferative metabolism (c-MYC) (Takahashi et al., 2007). Depending on the cell's genetic makeup all or some of the Yamanaka's factors may be required for IPSC production (Kim et al., 2009; Kim et al., 2008). Mesenchymal stem cells (MSCs) have been shown to reprogram at a faster rate than other cells, since they express some of the Yamanaka factors (C. Zheng et al., 2009). Despite this, difficult extraction procedures limit their potential. The discovery of MenSC, which are freely available in the menstrual fluid, addresses this limitation (Meng et al., 2007). Moreover, MenSC express some pluripotent genes, including OCT-4, KLF-4, c-MYC, SOX-2 and NANOG which make them even more attractive for remodelling into IPSCs (de Carvalho Rodrigues et al., 2012; Li et al., 2013). de Carvalho Rodrigues et al. (2012) managed to reprogram MenSC into IPSCs through lentiviral mediated transduction of KLF-4, SOX-2 and OCT-4. Subsequently, Li et al. (2013) successfully reprogrammed MenSC with OCT-4 and SOX-2, thus avoiding the need for the oncogenic KLF-4 factor. IPSCs produced from MenSC develop at a faster rate (15–17 days vs. 20 days) and with improved efficiency (2–5% vs. 0.01–0.1%) from fibroblast derived IPSCs (de Carvalho Rodrigues et al., 2012; Li et al., 2013). Moreover MenSC-IPSCs are safer than fibroblast derived IPSCs since c-MYC and KLF-4 factors are not needed for their reprogramming (Li et al., 2013). MenSC derived IPSCs express the pluripotent genes OCT-4, SOX-2, TRA-1-60, TRA-1-80 and subsequently develop embryoid bodies containing cells from all three germ lines (de Carvalho Rodrigues et al., 2012; Li et al., 2013). Furthermore *in vivo* transplantation of MenSC-IPSCs develop into a trilaminar teratoma (Li et al., 2013).

2.5 Comparison between MenSC and BMSCs

Both MenSC and BMSCs exhibit a fibroblast-like morphology (Alcayaga-Miranda et al., 2015). Phenotypic characterisation revealed a largely similar profile between the two cells (Darzi et al., 2012; Fathi-Kazerooni et al., 2019; Meng et al., 2007). Mesenchymalmarkers, such as CD44, CD29, CD105, CD73 and CD146, are strongly expressed in both cell types; however, MenSC do not express Mesenchymal stem cell marker 1 (STRO1) and Stage specific embryo antigen 4 (SSEA-4). Instead, they show marked expression of the OCT-4 embryonic stem cell marker in comparison to BMSCs (Darzi et al., 2012; Fathi-Kazerooni et al., 2019). Additionally, MenSC express higher levels of CD49a and Human leukocytic antigen (HLA-A/B/C) (Alcayaga-Miranda et al., 2015). Mitochondrial dehydrogenase analysis showed that MenSC proliferate at a significantly faster rate than BMSCs at all intervals assessed. The proliferative potential of MenSCincreased from 2.84-fold at day 3 to 4.97-fold at day 9 over that of BMSCs (Alcayaga-Miranda et al., 2015), while the MenSC doubling time was approximately halved in comparison to BMSCs (Darzi et al., 2012). Following induction, both cell types successfully differentiated into adipocytes, chondrocytes and osteoblasts. BMSCs showed a higher capacity to differentiate into adipocytes but no significant variation in chondrocytic and osteoblastic differentiation was noted (Alcayaga-Miranda et al., 2015). Contrary to this result, MenSC showed decreased osteoblast differentiation potential than BMSCs. This was attributed to lower levels of alkaline phosphatase (ALP) expression by MenSC which subsequently resulted in decreased mineralization (Darzi et al., 2012). BMSCs showed a higher potential to differentiate to glial-like cells. Induction with neurogenic factors induced morphological changes which were more pronounced in BMSCs (Azedi et al., 2014). Scratch wound assay showed complete resolution following 24 hours of MenSC treatment, while BMSCs only partially resolved the wound within the same timeframe (Alcayaga-Miranda et al., 2015).24 hours after addition to a migration assey MenSC were shown to migrate more than BMSCsat. The enhanced migratory capacity is correlated to a larger number of MenSC expressing chemokine receptor type 41 (CXCR41) over BMSCs (Luz-Crawford et al., 2016). As mentioned before, HUVEC culturing on conditioned medium derived from MenSC under hypoxic conditions have a greater angiogenic effect on HUVECs than MenSC cultured medium at normoxic conditions (Alcayaga-Miranda et al., 2015). However, BMSC derived culture medium in hypoxia or normoxic conditions show no significant different effect on HUVECs. Therefore, MenSC have an ability to induce angiogenesis based on the microenvironment which BMSCs lack. Moreover, under normoxic conditions MenSC had greater angiogenic effect on HUVECs than BMSCs Finally, in a rat model of acute liver failure, MenSC administration suppressed miR-122 more than BMSCs. Furthermore, MenSC significantly decreased the collagen content in the liver and reduced the levels of AST and ALT. Therefore, MenSC show more improvement in acute liver failure (Fathi-Kazerooni et al., 2019).

2.6 MenSC derived exosome

MenSC produce three types of extracellular vesicles (EVs) of which exosomes are the smallest subtype ($< 150nm$). Exosomes are generated through the endosomal pathway and are released following fusion of multivesicular bodies to the plasma membrane. These EVs carry bioactive cargo content from the mother cell to recipient cells where they exert their effect (Hessvik et al., 2018). EVs derived from MenSC are positive for CD81, CD63 and TSG101 exosomal markers and negative for calnexin. Furthermore, these spherical vesicles have a size of 30–170 nm and thus they exhibit the minimum criteria for exosome classification as stipulated by the International Society for extracellular vesicles (Dalirfardouei et al., 2018).

Wound healing has been shown to significantly improve in the exosome treated patients' group (Dalirfardouei et al., 2019). Inflammation at the site of injury was significantly attenuated as a result of a shift towards the tissue healing M2 macrophage phenotype. VEGF upregulation with subsequent increase in CD34 positive vessels indicated improved neoangiogenesis. The wound had faster re-epithelialization and better resolution as a result of increased Collagen I: Collagen III ratio with subsequent reduced scar formation. Furthermore, MenSC-derived exosome treatment resulted in better would healing than the MenSC treated group (Dalirfardouei et al., 2019). In a diabetic mouse model, administered exosomes successfully homed into the diabetic pancreas after 48 hours. Exosome treatment improved the number of islets within the pancreas, enhanced B-cell mass and insulin production capacity. Improved serum insulin level was observed following treatment although levels remained sub-optimal and showed no effect on the non-fasting glucose (Mahdipour et al., 2019). In the Prostate Cancer 3 (PC3) cells, MenSC-derived exosomes downregulated the NF-kB transcription factor activity and reactive oxygen species (ROS) production, which subsequently resulted in decreased VEGF production. HUVECs cultured on conditioned medium derived from exosome treated PC3 cells showed no tube formation and therefore VEGF inhibition by ROS was confirmed. Following encouraging *in vitro* results, *in vivo* effects of exosomes on PC3 tumours was investigated. Following MenSC-derived exosome treatment, extra- and intra- tumour angiogenesis was significantly inhibited as visualised by decreased vessel formation (Alcayaga-Miranda et al., 2016).

Exosomes may therefore serve as a potential treatment instead of the direct transplantation of MenSC since exosomes are more stable, not limited by the possibility of rejection and have no genetic risks. However, being so small they are efficiently cleared by the body and may therefore require a very high dose and repeated treatment (Bozorgmehr et al., 2014).

2.7 The Possible Applications of MenSC treatment for Gynaecological Disorders

The treatment of gynaecological disorders by various types of MSCs obtained from various adult tissues, i.e., bone marrow, adipose and amniotic tissue, have shown encouraging results (Rungsiwiwut et al., 2021). Despite this, their clinical application is restricted due to limited sources, invasive extraction procedures and the risk of infection. The discovery of MenSC in the menstrual fluid represents as an attractive alternative which is cyclically available and freely shed (Meng et al., 2007). These cells meet all the requirements for stem cell classification by the International Society for Cellular Therapy (Hu et al., 2019; Y. Liu et al., 2018). To date, studies on their application for the treatment of gynaecological disorders are still limited. However, from the results obtained so far, there is potential for future clinical use, since MenSC may offer an exciting alternative to the conventionally used adult stem cells.

2.8 MenSC and premature ovarian failure

Premature Ovarian Failure/Insufficiency (POF/POI) is a secondary infertility disorder effecting woman of reproductive age (Anasti, 1998). POF is diagnosed in women under the age of 40 and it is estimated to affect 1 percent of women below this age. POF is characterised by amenorrhea lasting more than 4 months and by hypergonadotropic hypoestrogenism (Jiao et al., 2017; Tucker et al., 2016). POF does not only impact fertility but can lead to osteoporosis, cardiovascular and neurological disorders and also emotional manifestations (Z. Wang et al., 2017). Chemotherapy is an essential component of cancer treatment however the use of alkylating drugs such as cyclophosphamide (CY) and busulfan lead to the development of POF (Sakurada et al., 2009). CY was shown to stimulate the development of primordial follicles through PI3K/Akt/mTOR, Rictor/ mTORC2/Akt/Foxo 3a signalling thus depleting the finite ovarian reserve (Kalich-Philosoph et al., 2013; B.-F. Zhang et al., 2018; Zhou et al., 2017). Busulfan leads to ovarian cytotoxicity (Kalich-Philosoph et al., 2013). Hormone replacement therapy (HRT) is used to alleviate symptoms caused by POF however it fails to restore ovarian function. Therefore HRT is only a symptomatic treatment, which carries increased risks of thrombosis, stroke, oestrogen sensitive tumours, ovary and breast cancers. Thus, there is an urgent need for alternative treatments (Rantanen et al., 2013).

The regenerative and restorative potential of MenSC has been assessed favourably in many studies involving multiple different cell types (Allickson et al., 2011; Rodrigues et al., 2012) and therefore the possible regenerat-

ing effect of these cells on POF has been investigated in chemotherapy-induced POF murine models. After POF induction, MenSC have been transplanted by intravenous or direct intra-ovarian injection to the murine models and their effect on ovarian function was evaluated (Feng et al., 2019; Lai et al., 2015; T. Liu et al., 2014; Manshadi et al., 2019; Noory et al., 2019). Chemotherapy resulted in weight loss and a decrease in the size and weight of the ovaries (Feng et al., 2019; Manshadi et al., 2019). Z. Wang et al. (2017) reported that from the 9th day after MenSC transplantation, the body weight of the transplanted mice was found to be significantly higher compared to untreated POF mice. In addition, after 21 days, the ovaries showed a statistically significant higher weight than in the untreated POF model. T. Liu et al. (2014) reported that there was no statistical significance between the weight of ovaries from the MenSC treated group and the positive control group which were not treated with chemotherapy. Pathological analysis showed that a large number of follicles in all stages of development were present in the ovarian stroma of healthy mice (T. Liu et al., 2014). Following chemotherapy, the quantity and quality of the follicles were shown to decrease (Feng et al., 2019). Furthermore, the ovarian stroma was mainly composed of interstitial cells and collapsed oocytes largely in primary and secondary follicles (T. Liu et al., 2014). MenSC treatment was shown to increase the number of healthy follicles in all stages of development and improve ovary microstructure (Feng et al., 2019; Manshadi et al., 2019; Z. Wang et al., 2017). Inflammatory molecules which develop as a result of chemotherapy act as signals for mesenchymal stem cells to migrate to the injured site where they can either regenerate tissue by engraftment or induce tissue repair through the secretion of bioactive molecules. Granulosa cell specific genes for anti-mullerian hormone (AMH), follistatin (FST) and follicle stimulating hormone receptor (FSHR) were downregulated following chemotherapy indicating apoptosis of the granulosa layer (Manshadi et al., 2019). Granulosa cell apoptosis was confirmed by tunnel assay (Feng et al., 2019; Noory et al., 2019). One month after DIL-labeled MenSC transplantation, the levels of granulosa cell specific genes were shown to be restored (Manshadi et al., 2019). MenSC were able to migrate towards the apoptotic granulosa layer after which they were able to engraft and differentiate into granulosa cells (Lai et al., 2015; Manshadi et al., 2019). Z. Wang et al. (2017) reported decreased apoptosis of the granulosa cells following treatment with MenSC conditioned media. The presence of cytokines and growth factors in the conditioned media was analysed and an extremely high level of Fibroblast Growth Factor 2 was identified. Conditioned media obtained from MenSC previously treated to inhibit FGF2 production had no effect on the rate of GC apoptosis (Z. Wang et al., 2017). In another experiment the levels of B-cell lymphoma-2-associated X protein (BAX) and B-cell lymphoma 2 (BCL2) were evaluated following MenSC treatment. BAX and BCL2 are two important genes which are involved in the regulation of apoptosis. BAX is proapoptotic while BCL2 is antiapoptotic. Quantitative real-time polymerase chain reaction (qRT-PCR) showed a significant variation in the levels of expression of BAX and BCL2 in the POF model in comparison with the normal untreated group. BAX levels were seen to increase while BCL2 levels decreased in the POF mice model. This shift towards BAX resulted in increased atrophied follicles. Following MenSC treatment, the level of BAX showed a statistically significant decrease in comparison to the POF mice model. The decrease in the level of BAX is attributed to the regulatory functions of MenSC which through paracrine factors can influence genetic expression and in this case lead to the cessation of apoptosis (Noory et al., 2019). Pregnancy rates were seen to be restored after MenSC treatment of chemotherapy injured mice. After mating with normal healthy male mice, the MenSC treated group and untreated control group had three pregnancies, while chemotherapy treated mice had only one pregnancy (Lai et al., 2015). Successful pregnancies in both normal untreated controls and MenSC treated groups were significantly higher than in the POF mice model (Feng et al., 2019; Lai et al., 2015). Chemotherapy treatment resulted in high levels of follicle stimulating hormone (FSH) and low levels of oestrogen and progesterone thus giving the classical hypergonadotropic hypoestrogenism seen in POF (Feng et al., 2019; Guo et al., 2019; Y. Liu et al., 2018; Manshadi et al., 2019; Z. Wang et al., 2017). Z. Wang et al. (2017) reported that at 7 and 21 days following MenSC transplantation the level of FSH was lower while the level of E2 was higher than that in the untreated POF model. Meanwhile Liu et al found no statistical significance between the serum hormone levels of positive control group and treated group thus MenSC reversed the hypergonadotropic hypoestrogenism (T. Liu et al., 2014; Z. Wang et al., 2017). The ovarian stroma is composed of extracellular matrix (ECM) which serves an important role in follicular support (Rodgers et al., 2003). Chemotherapy induces changes to the ECM microfibrillar system which results in the disruption of homeostasis (Alipour et al., 2015). Two important proteins in the micro fibrillar system are Collagen Type 6 Alpha 5 Chain (COL6A5) and Collagen Type 9 Alpha Chain 2 (COL9A2) (Pan et al., 2014). The mRNA expression of these two molecules was decreased with chemotherapy but, after the introduction of MenSC, there was an increase in the

level of expression of these proteins. Thus, MenSC could ameliorate POF through the restructuring of the ovarian stroma microenvironment through the activation of the ECM FAK/AKT signalling pathway (Feng et al., 2019). The positive effects of MenSC treatment on POF are explained by both the secretory and regenerating effect of MenSC. These results merit favourable prospects for the future clinical application of MenSC in the treatment of POF tables 1a and 1b.

2.9 MenSC in Endometrial Dysfunction

Asherman's Syndrome, also known as intrauterine adhesions (IUAs) is a disease of the female reproductive tract (Asherman, 1948). The prevalence of IUA has increase and presently the disease accounts for 4.6% of female factor infertility (Baradwan et al., 2018). IUAs are caused by damage to the endometrium by direct repeated mechanical damage or inflammatory responses to infections which effect the basalis layer (Yu et al., 2008). A functioning basalis is necessary for the cyclic regeneration of the functionalis layer in preparation for implantation (Cervelló et al., 2015). Insults effecting the stratum basalis result in the loss of function of endometrial stem cells thus leading to a decline in the normal function of the endometrium. This results in endometrial functional disorders with clinical manifestations such as painful menses, hypomenorrhea or complete amenorrhea, thin endometrium and fibrotic scarring, which together make the uterine cavity hostile to implantation (Gargett et al., 2012). Conventional treatment is based on surgical adhesion resection followed by hormone replacement therapy. However, this has a poor prognosis with adhesion recurrence commonly seen, especially with severe cases of IUA (Deans et al., 2010; Guo et al., 2019). MenSC supplementation to injured endometrium has attracted some interest and although limited research had been conducted thus far, results obtained are encouraging and may represent a potential cure for IUA in the future (Ma et al., 2020; Tan et al., 2016; S. Zhang et al., 2019; S.-X. Zheng et al., 2018; Zhu et al., 2019). Endometrial stromal cells (ES) and endometrial epithelial cells (EE) are cell types which are found abundantly in healthy normal endometrium. However, following histological analysis these cells were seen to be replaced by fibrotic tissue in patients with IUAs. After culturing with specific growth factors in vitro, MenSC were found to differentiate into both vimentin-expressing ES cells and cytokeratin expressing EE cells (S. Zhang et al., 2019). At 9- and 18-days post MenSC treatment immunocytochemistry analysis showed that cytokeratin and vimentin expression was significantly higher in the MenSC treated group than in the control groups, thus confirming that MenSC differentiated in vivo to ES and EE cells (Hu et al., 2019; S. Zhang et al., 2019). Furthermore, combined treatment with platelet rich plasma (PRP) had a synergistic effect with consequential better endometrial repair (S. Zhang et al., 2019). Treatment with MenSC stimulated angiogenesis which subsequently increased micro-vessel density in the treated endometrium (Hu et al., 2019; X. Wang et al., 2020). Significantly higher levels of pro-angiogenic VEGF were secreted in the treatment groups (Hu et al., 2019). VEGF induced micro vessel formation as detected by increased expression of CD34 protein (X. Wang et al., 2020). Effect on angiogenesis is brought about by paracrine influences on the AKT/ERK pathway which is activated by phosphorylation. Activation leads to increased levels of transcription of angiogenic genes resulting in increased expression of angiogenic factors. Asherman's Syndrome showed no improvement in endometrial thickness with oestrogen therapy alone; however, following MenSC transplantation and HRT, endometrial regeneration was induced. This indicated that stem cell loss may be the main cause of Asherman's (Tan et al., 2016). Immunohistochemistry analysis of endometrium obtained from patients with IUAs showed a 20-fold decrease in OCT-4 positive cells. In vitro analyses of harvested cells showed a 9-fold decrease in their cloning efficiency when compared to cells obtained from healthy endometrium, thus supporting Tan et al's findings (S.-X. Zheng et al., 2018). MenSC transplantation in murine IUA model significantly increased the thickness of the endometrium which consequentially led to improved embryo implantation and development (Hu et al., 2019). Following assessment of refractory IUA patients, Ma et al reported an endometrial thickness of 3.9±0.9 mm which was described by Tan et al as having a rough morphology (Ma et al., 2020; Tan et al., 2016). Treatment with autologous transplantation of in vitro proliferated MenSC and hormone replacement therapy ameliorated IUA. The duration of menses in treated patients increased and the endometrium showed normal morphology and developed a triple line appearance. An increase in thickness to 7-8 mm was observed in 5 of the 7 (71%) women recruited, of whom 3 (43%) successful conceived (Tan et al., 2016). Ma et al increased the dose of transferred MenSC ten-fold from that administered Tan et al. (2016) and while noting the same improvements 83% of treated patients showed increased endometrial thickness. However, the pregnancy rate fell slightly to 41.7% (Ma et al., 2020). Endometrial stem cell expression of collagen I, alpha SMA, connective tissue growth factor (CTGF) and fibronectin were downregulated following MenSC co-culture (Zhu et al., 2019). Additionally, treatment with MenSC conditioned media had the same effect (Lin et al., 2018; Zhu et al., 2019). Reduction of CTGF and Collagen I was also ob-

	Animal Model	Root of administration of MenSC	Findings after MenSC treatment	Findings after MenSC CM treatment
Feng,2019	CTX induced POI in C57BL/6 female mice	Tail Vein injection	↑ body weight ↑ ovarian weight ↑ live births ↑ AMH ↑ E2 ↓ FSH ↑ COL6A5 ↑ COL9A2	N/A
Lai 2015	Busalfan induced POI in C57BL/6 female mice	Tail Vein injection	↑ body weight ↑ ovary size ↑ pregnancy rate ↑ Litter size ↑ oocytes in al stages of development ↓ depletion of germline cells ↑ FSHR (granulosa cell marker)	N/A
Liu,2014	Cyclophosphamide induced POI in C57BL/6 female mice	Intra-ovarian injection	↑ ovary weight ↓ atrophield follicles ↑ AMH ↑ FSHR ↑ E2 ↓ FSH	N/A

Table 1a: Preclinical animal studies showing the response of POF to MenSC treatment

10.7423/XJENZA.2021.3.08

Manshadi,2019	Busulfan induced POI in female Wistar rats	Intravenous injection	↑ AMH ↑ FST ↑ FSHR ↑ E2 ↑ P4 ↑ FGF2 ↓ FSH	N/A
Noory,2019	Busulfan induced POI in female Wistar rats	Injection	↑ ovary weight ↑ follicles in all stages of development ↑ BCL2 ↓ BAX	N/A
Wang,2017	Cisplastin induced POI in female C57BL/6	Tail Vein injection	↑ body weight ↑ ovarian weight ↑ follicles in all stages of development ↑ E2 ↓ FSH ↓ TUNEL + cells (↓ apoptosis)	↑ E2 ↑ FSH ↓ ovary fibrosis ↓ apoptosis

Table 1b: Preclinical animal studies showing the response of POF to MenSC treatment

served in vivo (S. Zhang et al., 2019). Downregulation of these molecules leads to decreased deposition of collagen within the endometrium, thus ameliorating fibrosis caused by IUA. MenSC exert part of their therapeutic effect on IUA resolution by the paracrine activation and the subsequent regulation of endometrial stem cell genes involved in fibrogenesis. This is achieved through various molecular pathways (Lin et al., 2018; S. Zhang et al., 2019; Zhu et al., 2019). MenSC activate the Hippo/Transcriptional coactivator with PDZ-binding motif (TAZ) pathway (B.-F. Zhang et al., 2018; Zhu et al., 2019). Following coculture with MenSC or MenSC culture media (CM), TAZ was phosphorylated and was subsequently sequestered in cytoplasmic granules. Sequestered P-TAZ cannot interact with TGF-β therefore downstream mRNA expression of fibrotic genes is blocked since their expression depend on TGF-β (Zhu et al., 2019). Zinc finger protein (GLi2), a pro-fibrinogenic transcription factor was found to be expressed at a statistically significant higher level in patients with mild to severe IUA. MenSC CM analysis identified extremely high levels of Granulocyte colony stimulating factor (G-CSF). ESC co-culture with CM treated with anti G-CSF had no effect on ESC fibrosis and thus it was concluded that MenSC exhibits their anti-fibrotic effect as a result of G-CSF. G-CSF acts through the inhibition of Gli2 expression and by inducing G0/G1 arrest in endometrial stem cells (Lin et al., 2018). MenSC treated groups showed no statistical difference in body and organ weight from untreated controls and further in vitro analysis showed that MenSC are negatively tumorigenic and toxigenic thus they are safe for clinical application for the treatment of Asherman's syndrome (Chang et al., 2020) (table 2).

3 Conclusion

Initial studies on these relatively new type of stem cells yield a favourable prospect for their application as a potential source of therapy for the treatment of gynaecological disorders and resulting secondary infertility. These cells have been assessed favourably in many animal studies and have been shown to reverse POF and Asherman's syndrome in murine models. Additionally, MenSC gave impressive results when tested against BMSCs, the most studied type of mesenchymal stem cells. Given their innate advantages these cells are the perfect replacement for BMSCs. The advent of their application as mainstream therapy requires more studies and clinical trials to assess their root of application, long term safety and dosing. However despite these grey areas, they seem to be a suitable versatile cellular therapy agent yielding impressive results in the field of regenerative medicine.

4 Declaration

There is no conflict of interest.

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	Animal Model	Root of administration of MenSC	Findings after MenSC treatment	Findings after MenSC and PRP treatment	Findings after G-CSF treatment
Zhang, 2019	IUAs induced by mechanical damage in female Sprague Dawley rats.	Intrauterine injection.	↑ endometrial proliferation ↑ angiogenesis ↑ endometrial thickness ↑ implantation ↓ fibrosis ↓ inflammation	↑↑ endometrial proliferation ↑ angiogenesis ↑ endometrial thickness ↑↑ implantation ↓ fibrosis ↓↓ inflammation	N/A
Hu, 2019	IUAs induced by mechanical damage in female BALB/c nude mice.	Transplanted in right uterine cavity.	↑ endometrial thickness ↑ vitamin ↑ VEGF ↑ keratin ↑ pregnancy rate	N/A	N/A
Zheng, 2018	NOD-SCID mice injected with endothelial cell programmed MenSC.	Transplanted in axillary subcutaneous tissue.	↑ cytokeratin ↑ vimentin	N/A	N/A
Lin, 2018	Female Sprague Dawley rats injected with 95% ethanol to induce IUAs.	G-CSF injected in the right uterine horn.	N/A	N/A	↓ fibrosis relate proteins (COL1, CTGF, αSMA ↓ GLi2

Table 2: Preclinical animal studies showing the response of IUAs following MenSC treatment.

	Recruited for study	Findings after autologous MenSC transplantation
Tan, 2016	7 patients with sever IUAs.	↑ endometrial thickness in 5 patients 1 spontaneous pregnancy 2/4 patients conceived following frozen embryo transplant.
Ma, 2020	12 patients with refractory IUAs.	↑ endometrial thickness ↑ duration of menstruation ↑ pregnancy rate

Table 3: Clinical studies showing the response of IUAs to MenSC treatment.

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	Search	Results
1	Menstrual blood-derived stem cell*.mp.	37
2	Menstrual blood-derived stromal stem cell*.mp.	3
3	Menstrual blood-derived mesenchymal stem cell*.mp.	15
4	Menstrual blood-derived endometrial stem cell*.mp.	4
5	Menstrual blood-derived cell*.mp.	3
6	Menstrual blood-derived stromal cell*.mp.	4
7	Menstrual blood-derived progenitor cell*.mp.	0
8	Menstrual blood-derived regenerative cell*.mp.	0
9	Menstrual stem cell*.mp.	5
10	Menstrual blood stem cell*.mp.	23
11	Menstrual blood stromal stem cell*.mp.	3
12	Menstrual blood progenitor cell*.mp.	1
13	Menstrual-derived stem cell*.mp.	2
14	Endometrial stem cell*.mp.	139
15	Endometrial stromal stem cell*.mp.	7
16	Endometrial mesenchymal stem cell*.mp.	59
17	Endometrial progenitor cell*.mp.	8
18	Endometrial regenerative cell*.mp.	17
19	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18	286
20	exp Menstruation/	15,751
21	exp Endometrium/	32,210
22	20 or 21	46,533
23	exp Stem Cells/	216,632
24	exp Mesenchymal Stem Cells/	37,597
25	23 or 24	216,632
26	22 and 25	571
27	19 or 26	660
28	limit 27 to yr="2007–2020"	612
29	limit 28 to english language	584

Table 4: MEDLINE (Ovid) (from 2007 to August 2020, Week 4) (Galea et al., n.d.).

	Search	Results
#1	'menstrual blood-derived stem cell*'	68
#2	'menstrual blood-derived stromal stem cell*'	4
#3	'menstrual blood-derived mesenchymal stem cell*'	34
#4	'menstrual blood-derived endometrial stem cell*'	7
#5	'menstrual blood-derived cell*'	5
#6	'menstrual blood-derived stromal cell*'	8
#7	'menstrual blood-derived progenitor cell*'	0
#8	'menstrual blood-derived regenerative cell*'	0
#9	'menstrual stem cell*'	15
#10	'menstrual blood stem cell*'	59
#11	'menstrual blood stromal stem cell*'	10
#12	'menstrual blood progenitor cell*'	1
#13	'menstrual-derived stem cell*'	8
#14	'endometrial stem cell*'	320
#15	'endometrial stromal stem cell*'	15
#16	'endometrial mesenchymal stem cell*'	144
#17	'endometrial progenitor cell*'	12
#18	'endometrial regenerative cell*'	32
#19	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18	632
#20	'menstruation'/exp	23,693
#21	'endometrium'/exp	34,082
#22	#20 OR #21	56,053
#23	'stem cell'/exp	378,513
#24	'mesenchymal stem cell'/exp	60,648
#25	#23 OR #24	378,513
#26	#22 AND #25	808
#27	#19 OR #26	1,212
#28	#27 AND ([conference abstract]/lim OR [conference paper]/lim OR [conference review]/lim)	434
#29	#27 NOT #28	778
#30	#29 AND [english]/lim	728
#31	#30 AND [2007-2020]/py	690

Table 5: EMBASE (from 2007 to August 2020, week 4) (Galea et al., n.d.).

	Search	Results
S1	Menstrual blood-derived stem cell*	4
S2	Menstrual blood-derived stromal stem cell*	1
S3	Menstrual blood-derived mesenchymal stem cell*	1
S4	Menstrual blood-derived endometrial stem cell*	0
S5	Menstrual blood-derived cell*	4
S6	Menstrual blood-derived stromal cell*	1
S7	Menstrual blood-derived progenitor cell*	0
S8	Menstrual blood-derived regenerative cell*	0
S9	Menstrual stem cell*	8
S10	Menstrual blood stem cell*	5
S11	Menstrual blood stromal stem cell*	2
S12	Menstrual blood progenitor cell*	0
S13	Menstrual-derived stem cell*	0
S14	Endometrial stem cell*	12
S15	Endometrial stromal stem cell*	0
S16	Endometrial mesenchymal stem cell*	7
S17	Endometrial progenitor cell*	5
S18	Endometrial regenerative cell*	0
S19	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18	21
S20	(MW menstrual) OR (MW menstruation)	1,489
S21	(MW endometrial) or (MW endometrium)	1,673
S22	S20 or S21	3,047
S23	MW stem cells	2,493
S24	MW mesenchymal stem cells	213
S25	S23 OR S24	2,493
S26	S22 AND S25	1
S27	S19 OR S26	22
S28	S27	21
	Limiters—Published Date: 20070101-20201231	

Table 6: Cochrane Central Register of Controlled Trials (EBSCO) (from 2007 to August 2020, week 4) (Galea et al., n.d.).