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Regenerative medicine options in treating premature ovarian failure

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ABSTRACT

Infertility is generally defined as the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. premature ovarian failure (POF) is a cause of female infertility, being estimated to affect 1% of the general population. This condition represents a loss of hormonal and germinative activity of the ovaries due to a lack in the number of active follicles in women under 40 years old. Regenerative medicine represents a complex therapeutic option and it consists of technologies such as the use of stem cells, tissue engineering, and gene therapy alone or in different combinations. Although the boundaries of regenerative medicine are not clearly outlined at present, this paper is aiming to review its possibility of treatment in the future in what concerns POF, focusing more on the use of stem cell therapy. Stem cell therapy could be a feasible therapeutic approach for POF as the cells can be easily obtained. However, additional clinical studies are needed because until now the majority of the literature focuses on animal models for the evaluation of the role of stem cells on treating POF. Additionally, the platelet-rich plasma (PRP) approach for POF should be kept in mind as it was shown to be useful in the regeneration of multiple types of tissue.

Keywords: ovarian failure, regenerative medicine, treatment, infertility, platelet-rich-plasma

INTRODUCTION

Infertility is generally defined as the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. Not only it is a medical condition, but it is also an issue that can cause significant psychological, emotional and relational damage in one's life. For instance, people who were involved in an infertile couple had a higher rate of depression and anxiety compared to fertile couples [1].

The contribution of the female factor in a couple's fertility issues is not known specifically. It has been demonstrated that there is a novel distribution of male and female infertility prevalence and causes throughout the world [2]. However, the same study used the assumption that 50% of all cases of infertility are due to female factors alone, 20-30% are due to male factors alone, and the remaining 20-30% are due to a combination of male and female factors. The World Health Organization estimates that 9% of couples worldwide struggle with fertility issues and that male factor contributes to 50% of the issues [3]. Therefore, we cannot assess how much of a contribution a woman makes when it comes to the inability of a couple to conceive. However, it is well known that the burden of this issue most likely falls on women [4].

There is an extensive list of causes for female infertility which includes lifestyle factors, anatomical abnormalities, hormonal imbalances, infectious outcomes, etc. Among these, premature ovarian failure (POF) is a cause of female infertility, being estimated to affect 1% of the general population [5]. This condition represents a loss of hormonal and germinative activity of the ovaries due to a lack in

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the number of active follicles in women under 40 years old. Not only is it a concerning diagnosis, but it often occurs spontaneously or idiopathically [5]. However, the causes of POF include genetic factors like Turner Syndrome but also oncologic treatments [6], which is more and more often used in treating cancer in young women.

Regenerative medicine represents a complex therapeutic option and it consists of technologies such as the use of stem cells, tissue engineering, and gene therapy alone or in different combinations [7]. Although the boundaries of regenerative medicine are not clearly outlined at present, this paper is aiming to review its possibility of treatment in the future in what concerns POF, focusing more on the use of stem cell therapy.

MATERIALS AND METHODS

A thorough search through Pubmed, Scopus and Google Scholar using the following search formula: (regenerative medicine OR regenerative therapy OR stem cell therapy) AND (*fertil*) AND (women OR female) was performed. Only full text articles published in the last 10 years were chosen. In the end, we managed to include a total of 34 articles in our search.

RESULTS

Definition and types of stem cells

Stem cells are primitive cells that have the capacity of differentiating into different kinds of cells. These cells are defined by two important features. First, they are self-renewing cells, which means that they are able to stand multiple cell cycle divisions. At the same time, potency is a characteristic that gives stem cells a smaller or wider range of differentiation into mature cells [8,9]. It is also important to mention that according to their origin, stem cells classify into embryonic and adult stem cells. Further in this article, we will get to evaluate some specific subtypes of stem cells in what concerns their involvement in treating POF.

Mesenchymal stem cells

Mesenchymal stem cells (MSC) are multipotent stem cells that can be found in a variety of human and animal tissues. They were first discovered in bone marrow [10]. The Tissue Stem Cell Committee of the International Society for Cellular Therapy proposes minimal criteria to define human MSC. To begin with, it is required that the cells have plastic-adherent properties when placed in standard culture conditions. Secondly, they must be able to differentiate to osteoblasts, adipocytes and chondroblasts in vitro. The third condition comes down to the cell surface markers as it follows: MSC must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules [11]. As mentioned, mesenchymal cells can now be isolated from a diversity of structures. On this scientific base, we will further present some types of MSC and their ability to interact with the ovarian function.

Amniotic Fluid Stem Cells and Amniotic Membrane Stem Cells

Amniotic Fluid Stem Cells (AFSC) come with a number of advantages, besides the ethical ones. They can be easily extracted from amniotic fluid during a procedure called amniocentesis [12]. At the same time, according to certain studies, AFSC are at low risk of being immunologically rejected and, unlike pluripotent stem cells, they don't develop into teratomas in vitro [13]. Furthermore, it seems that AFSC can be turned into pluripotent stem cells [14], which we will discuss about.

The potential of AFSC in treating POF has been evaluated in mice, six weeks after induced POF by chemotherapy [13]. The results showed that transplantation of AFSC into the ovaries can help maintain the remaining follicles at an active state, most likely by inhibiting atresia. It has also been demonstrated that certain human AFSC can survive for a long time and multiply in the ovaries after inoculation [15].

Amniotic membrane stem cells (AMSC) have similar properties to AFSC. AMSC can be collected during caesarean sections [12]. There have been a few studies on animal models which suggested a paracrine mechanism of action of AMSC in supporting fertility after chemotherapy induced POF [16–18].

Human Endometrial Mesenchymal Stem Cells

Another source of stem cells, endometrial mesenchymal cells show similar properties to bone marrow stem cells [19]. Unlike bone marrow stem cells, these are easier to collect. Similar to previous studies, these cells have been inoculated in mice ovaries after being treated with busulfan and cyclophosphamide [20]. Following stem cells transplantation, an increase in body weight and a resumption of the estrous cyclicity was observed.

Bone Marrow Mesenchymal Stem Cells (BMMSC)

Although harder to collect, bone marrow stem cells can be easily isolated and cultivated and have low immunogenicity [21]. BMMSC cells have been thoroughly studied throughout the years. On animal models they have been demonstrated to increase

fertility after chemotherapy induced POF [22,23]. Interestingly, they have also been studied on human models [24]. Two caucasian women with POF were treated to BMMSC, harvested from their own bone marrow. Several improvements were reported following the engraftment of the cells in the ovaries: an increase in estrogen levels, an increase in the size of the ovaries, an improvement in menopausal symptoms. Both women had an episode of menstruation and tolerated the procedure without any incidents. It is important to mention that the safety of BMMSC has been proven on animal models [25].

Umbilical Cord Mesenchymal Stem Cells (UCMSC)

Another well studied source of multipotent stem cells, UCMSC, were inoculated in the ovaries of animal models in several studies [26–29]. All these studies showed how UCSMSC can restore ovarian function in female rats after being treated with chemotherapy. A recent study aimed to clarify the mechanism that might be supporting this effect of UCMSC, by inoculating them in mice ovaries with CTX-induced POF [30]. The therapeutical effect of UCMSC showed a regulation of SIRT7, which inhibited apoptosis in the ovaries and, therefore, improved fertility.

Fetal Liver Stem Cells

The last category of multipotent stem cells that we will review is fetal liver stem cells. There haven't been extended studies on whether they can play a role in preventing fertility loss. Huang et al. explored their potential in restoring ovarian function by creating an in vivo model of chemotherapy damaged ovary [31]. The results showed an increase in sex hormone levels and, among other, upregulated melatonin membrane receptor 1, which seems to play a role in targeting fertility loss caused by POF.

Pluripotent Stem Cells

This category of stem cells is composed of two main types of cells: embryonic stem cells (ESC) and induced pluripotent stem cells (IPSC) [32]. They have a wider spectrum of differentiation in comparison to multipotent stem cells, but they also have their limitations.

ESC are obtained from the inner cell mass of the blastocyst. The majority of these are obtained through *in vivo* fertilization [33]. Nevertheless, this process still encounters ethical issues.

IPSC are somatic differentiated stem cells that are reprogrammed to reverse into a certain state from where they can become pluripotent. Reprogramming focuses on the expression of oncogenes such as Myc and Klf4 [33]. As sources of somatic stem cells used are fibroblasts, peripheral blood cells, keratinocytes and renal epithelial cells found in urine. However, the results of the procedure of converting somatic cells to pluripotent cells are quite unsatisfactory [32]. We must emphasize that IPSC, unlike ESC, have the advantages of avoiding ethical issues, as well as immunological issues, since they are collected from the patient.

We identified a study which explored, based on previous findings, if ESC have a potential effect on ovarian damage in POF [34]. Cyclophosphamide and busulfan were used to induce infertility in mice before small vesicles of ESC were transplanted. The results showed an increase to normal of sex hormones, as well as an increase in the number of follicles, while apoptotic cells decreased in number. The mechanism behind this effect seems to be a regulation of the PI3K/AKT pathway.

Just as ESC, IPSC in human infertility are not sustained by significant data. In a short article, a study by Yashimor et al. is showing that female and male IPSC were differentiated into human primordial germ-like cells, which could slowly be turned into more advanced gonocyte stages, including oogonia and pro-spermatogonia [35]. Although this study seems promising for future research, it is important to mention that the authors mentioned a rate of less than 10% of the cells surviving the process of differentiation.

On animal models, we could identify a study which differentiated IPSC into hormone-sensitive ovarian epithelial-like cells using a microRNA [36]. The mice used for the study were treated with cyclophosphamide in order to establish POF, before injection of the sensitive ovarian epithelial cells. A number of proteins in the experimental group underwent changes of expression, including fibronectin, vimentin, cytokeratin 7. Moreover, the ovarian weight increased in the same group, these results suggesting a possible treatment of POF using IPSC.

Other treatments

We focused on stem cells therapy as it is probably the most used and studied field of regenerative medicine. However, we came across an interesting study, which demonstrated that platelet-rich plasma (PRP), a frequently used blood product for improving tissue regeneration [37], is a potentially effective therapeutic strategy for restoring fertility in POF [38]. Platelets derived from medullary megakaryocytes have a healing and tissue repair role due to the α-granules they contain which consist of over 800 proteins that via the paracrine effect on the neighboring cells promote rapid tissue regeneration.

POF was induced in Wistar albino rats by administering intraperitoneal 4-vinylcyclohexene dioxide (VCD) for 15 consecutive days. Histopathological

studies showed detachment of granulosa cells from oocytes and follicular atresia. After POF induction, PRP obtained from centrifuged rat blood samples was injected into the rats' ovaries. Histopathological studies highlighted improvement in follicular quality and number in rats treated with PRP. Additionally, a reduction of the inflammation caused by POF and of the atretic follicles was observed.

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CONCLUSION

Stem cell therapy could be a feasible therapeutic approach for POF as the cells can be easily obtained. However, additional clinical studies are needed because until now the majority of the literature focuses on animal models for the evaluation of the role of stem cells on treating POF. Additionally, the PRP approach for POF should be kept in mind as it was shown to be useful in the regeneration of multiple types of tissue.

REFERENCES

1. Fallahzadeh H, Zareei Mahmood Abadi H, Momayyezi M, Malaki Moghadam H, Keyghobadi N. The comparison of depression and anxiety between fertile and infertile couples: A meta-analysis study. *Int J Reprod Biomed.* 2019 May 5;17(3):153–62.
2. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol Endocrinol.* 2015 Apr 26;13:37.
3. Fainberg J, Kashanian JA. Recent advances in understanding and managing male infertility. F1000Res. 2019 May 16;8:F1000 Faculty Rev-670.
4. Cui W. Mother or nothing: the agony of infertility. *Bull World Health Organ.* 2010 Dec 1;88(12):881-2.
5. Rudnicka E, Kruszewska J, Klicka K, Kowalczyk J, Grymowicz M, Skórska J, et al. Premature ovarian insufficiency - aetiopathology, epidemiology, and diagnostic evaluation. *Prz Menopauzalny.* 2018 Sep; 17(3):105-8.
6. Jankowska K. Premature ovarian failure. *Prz Menopauzalny.* 2017 Jun; 16(2):51-6.
7. Ciccocioppo R, Cantore A, Chaimov D, Orlando G. Regenerative medicine: the red planet for clinicians. *Intern Emerg Med.* 2019 Sep;14(6):911-21.
8. Hosseinkhani M, Mehrabani D, Karimfar MH, Bakhtiyari S, Manafi A, Shirazi R. Tissue engineered scaffolds in regenerative medicine. *World J Plast Surg.* 2014 Jan;3(1):3-7. PMID: 25489516; PMCID: PMC4236978.
9. Sobhani A, Khanlarkhani N, Baazm M, Mohammadzadeh F, Najafi A, Mehdinejadiani S, Sargolzaei Aval F. Multipotent Stem Cell and Current Application. *Acta Med Iran.* 2017 Jan;55(1):6-23. PMID: 28188938.
10. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells - current trends and future prospective. *Biosci Rep.* 2015 Apr 28;35(2):e00191.
11. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8(4):315-7.
12. Roubelakis MG, Trohatou O, Anagnou NP. Amniotic fluid and amniotic membrane stem cells: marker discovery. *Stem Cells Int.* 2012;2012: 107836.
13. Xiao GY, Liu IH, Cheng CC, Chang CC, Lee YH, Cheng WT, Wu SC. Amniotic fluid stem cells prevent follicle atresia and rescue fertility of mice with premature ovarian failure induced by chemotherapy. *PLoS One.* 2014 Sep 8;9(9):e106538.
14. Srivastava M, Ahlawat N, Srivastava A. Amniotic Fluid Stem Cells: A New Era in Regenerative Medicine. *J Obstet Gynaecol India.* 2018 Feb;68(1):15-19.
15. Liu T, Huang Y, Guo L, Cheng W, Zou G. CD44+/CD105+ human amniotic fluid mesenchymal stem cells survive and proliferate in the ovary long-term in a mouse model of chemotherapy-induced premature ovarian failure. *Int J Med Sci.* 2012;9(7):592-602.
16. Liu R, Zhang X, Fan Z, Wang Y, Yao G, Wan X, et al. Human amniotic mesenchymal stem cells improve the follicular microenvironment to recover ovarian function in premature ovarian failure mice. *Stem Cell Res Ther.* 2019 Oct 2;10(1):299.
17. Zhang Q, Bu S, Sun J, Xu M, Yao X, He K, Lai D. Paracrine effects of human amniotic epithelial cells protect against chemotherapy-induced ovarian damage. *Stem Cell Res Ther.* 2017 Nov 28;8(1):270.
18. Ling L, Feng X, Wei T, Wang Y, Wang Y, Wang Z, et al. Human amnion-derived mesenchymal stem cell (hAD-MSC) transplantation improves ovarian function in rats with premature ovarian insufficiency (POI) at least partly through a paracrine mechanism. *Stem Cell Res Ther.* 2019 Jan 25;10(1):46.
19. Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 10 years. *Hum Reprod Update.* 2016 Mar-Apr;22(2):137-63.
20. Lai D, Wang F, Yao X, Zhang Q, Wu X, Xiang C. Human endometrial mesenchymal stem cells restore ovarian function through improving the renewal of germline stem cells in a mouse model of premature ovarian failure. *J Transl Med.* 2015 May 12;13:155.
21. He Y, Chen D, Yang L, Hou Q, Ma H, Xu X. The therapeutic potential of bone marrow mesenchymal stem cells in premature ovarian failure. *Stem Cell Res Ther.* 2018 Oct 4;9(1):263.
22. Badawy A, Sobh MA, Ahdy M, Abdelhafez MS. Bone marrow mesenchymal stem cell repair of cyclophosphamide-induced ovarian insufficiency in a mouse model. *Int J Womens Health.* 2017 Jun 15;9:441-7.
23. Bao R, Xu P, Wang Y, Wang J, Xiao L, Li G, Zhang C. Bone marrow derived mesenchymal stem cells transplantation rescues premature ovarian insufficiency induced by chemotherapy. *Gynecol Endocrinol.* 2018 Apr;34(4): 320-26.
24. Herraiz S, Pellicer N, Romeu M, Pellicer A. Treatment potential of bone marrow-derived stem cells in women with diminished ovarian reserves and premature ovarian failure. *Curr Opin Obstet Gynecol.* 2019 Jun;31(3):156-62.
25. Park HS, Chugh RM, Elsharoud A, Ulin M, Esfandyari S, Aboalsoud A, et al. Safety of Intraovarian Injection of Human Mesenchymal Stem Cells in a Premature Ovarian Insufficiency Mouse Model. *Cell Transplant.* 2021 Jan-Dec;30:963689720988502.
26. Song D, Zhong Y, Qian C, Zou Q, Ou J, Shi Y, et al. Human Umbilical Cord Mesenchymal Stem Cells Therapy in Cyclophosphamide-Induced Premature Ovarian Failure Rat Model. *Biomed Res Int.* 2016;2016:2517514.
27. Shen J, Cao D, Sun JL. Ability of human umbilical cord mesenchymal stem cells to repair chemotherapy-induced premature ovarian failure. *World J Stem Cells.* 2020 Apr 26;12(4):277-287.
28. Mohamed SA, Shalaby S, Brakta S, Elam L, Elsharoud A, Al-Hendy A. Umbilical Cord Blood Mesenchymal Stem Cells as an Infertility Treatment for Chemotherapy Induced Premature Ovarian Insufficiency. *Biomedicines.* 2019 Jan 18;7(1):7.
29. Lv X, Guan C, Li Y, Su X, Zhang L, Wang X, et al. Effects of single and multiple transplantations of human umbilical cord mesenchymal stem cells on the recovery of ovarian function in the treatment of premature ovarian failure in mice. *J Ovarian Res.* 2021 Sep 15;14(1):119.
30. Ding C, Zhu L, Shen H, Lu J, Zou Q, Huang C, et al. Exosomal miRNA-17-5p derived from human umbilical cord mesenchymal stem cells improves ovarian function in premature ovarian insufficiency by regulating SIRT7. *Stem Cells.* 2020 Sep;38(9):1137-1148.
31. Huang B, Qian C, Ding C, Meng Q, Zou Q, Li H. Fetal liver mesenchymal stem cells restore ovarian function in premature ovarian insufficiency by targeting MT1. *Stem Cell Res Ther.* 2019 Nov 29;10(1):362.

32. Romito A, Cobellis G. Pluripotent Stem Cells: Current Understanding and Future Directions. *Stem Cells Int.* 2016;2016:9451492.
33. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem Cell Res Ther.* 2019 Feb 26;10(1):68.
34. Liu M, Qiu Y, Xue Z, Wu R, Li J, Niu X, et al. Small extracellular vesicles derived from embryonic stem cells restore ovarian function of premature ovarian failure through PI3K/AKT signaling pathway. *Stem Cell Res Ther.* 2020 Jan 3;11(1):3.
35. Gell JJ, Clark AT. Restoring Fertility with Human Induced Pluripotent Stem Cells: Are We There Yet? *Cell Stem Cell.* 2018 Dec 6;23(6):777-779.
36. Liu T, Qin W, Huang Y, Zhao Y, Wang J. Induction of estrogen-sensitive epithelial cells derived from human-induced pluripotent stem cells to repair ovarian function in a chemotherapy-induced mouse model of premature ovarian failure. *DNA Cell Biol.* 2013 Dec;32(12):685-98.
37. Amable PR, Carias RB, Teixeira MV, da Cruz Pacheco I, Corrêa do Amaral RJ, Granjeiro JM, Borojevic R. Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors. *Stem Cell Res Ther.* 2013 Jun 7;4(3):67.
38. Ahmadian S, Sheshpari S, Pazhang M, Bedate AM, Beheshti R, Abbasi MM, et al. Intra-ovarian injection of platelet-rich plasma into ovarian tissue promoted rejuvenation in the rat model of premature ovarian insufficiency and restored ovulation rate via angiogenesis modulation. *Reprod Biol Endocrinol.* 2020 Aug 5;18(1):78.