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It is Possible to Obtain Stem Cells from Menstrual Blood?

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ABSTRACT

Introduction: *Stem cells, by definition, have characteristics of self-renewal and cellular specialization in different tissues. In medicine, it is used with great importance mainly in the area of tissue regeneration. Since its discovery, in the bone marrow in 1960, other sources have also been determined, such as adipose tissue and muscle tissue. More recently, endometrial stem cells have been found from menstrual blood, an easily obtainable and non-invasive source.*

Objectives: *To perform a bibliographic review in order to analyze the use of menstrual blood as a source of stem cells, evaluating ways to obtain and preserve them, and their regenerative capacity.*

Methods: *Articles about menstrual stem cells were searched in the databases: MEDLINE, LI-LACS, Cochrane, JAMA, CAPES, PubMed and SciELO.*

Results and Conclusions: *It was possible to conclude that menstrual blood is a viable and non-invasive source of stem cells, but it's still not so much used, requiring further research to consolidate protocols that define parameters for obtaining and culturing it, so that later they can be used in regenerative medicine.*

Keywords
Stem cells, Menstruation, Regeneration.

Introduction

Definition of stem cells

The stem cells are of great importance in medicine because they are cells with a high potential for differentiation into other tissues in the body [1], which justifies their extensive use in research for tissue regeneration and in the human reproduction area [2].

For cells to fit in the definition of "stem cells", they must exhibit two essential characteristics: 1) have the capacity for unlimited self-renewal to produce daughter cells exactly the same as the original cell, but with control of the cell division and, 2) the ability to specialize in another cell type in different tissues [1,3].

Stem cells can be of two types:

- Pluripotent: they are found in the embryo until the gastrulation phase. These can differentiate into any type of cell in the human organism. Upon reaching the gastrula, the pluripotent cells become multipotent. Pluripotent cells, being found only in embryos, face ethical and religious dilemmas for their use in medical research and human reproduction [1].
- Multipotent - they have the ability to differentiate into new stem cells or more specialized cells than the previous one. This characteristic is called plasticity [4,5]. They are the most used clinically.

The use of stem cells in medicine began in the 1960's, with their discovery in bone marrow [6], and they having been used with therapeutic potential in bone marrow transplants since then [7]. In 1967, the presence of these cells in embryonic tissue was discovered [8]. In addition to these, many other sources of stem cells have been discovered, such as adipose tissue [9,10], muscle [10], endometrium and menstrual blood [11,12].

The stem cells are called mesenchymal (MSCs) when they are multipotent, non-hematopoietic, with self-renewal properties and

differentiation capacity in mesenchymal and non-mesenchymal tissues according to the culture medium's stimulus. They can be differentiated into: osteoblasts, chondroblasts, hepatocytes, neurons, epithelial, renal and cardiac cells [14,29]. Phenotypically, they are characterized as CD105, CD73 and CD90 positive [25].

Use of stem cells

Stem cells (SC) are of great interest in the field of regenerative medicine and cancer therapy due to the possibility of applying them to tissue repair [13]. The mechanisms of tissue repair are still unclear and involve a direct action of the SC through accommodation and differentiation in the cells of the damaged tissue, and also involves a paracrine action, through the secretion of growth factors and cytokines [14]. STs have been used, for example, in bone repair for therapy of osteogenesis imperfecta [15-17]. Likewise, the ability to repair cardiac tissue may not be related to an ability to differentiate into cardiomyocytes, but to the release of trophic factors together with suppression of inflammation [18,19].

The therapeutic potential of ST is also extended to neurodegenerative diseases such as Epilepsy and Parkinson [20,21], in which the repair potential may also depend on the ability of SC to secrete locally large amounts of the brain-derived neurotrophic factor, the neural growth factor and vascular endothelial growth factor [14,22]. The importance of the paracrine action of SC was also observed in regenerative studies of acute kidney injury in rats, being the release of trophic, pro-angiogenic and mitogenic immunomodulatory factors, the most accepted mechanism of action in renal repair [23,24]. Secretomema studies have indicated that the profile of proteins secreted by SC, obtained from different tissue sources, is very similar [25].

The use of stem cells has also been gaining space as a therapeutic alternative in Human Reproduction. It is long-term effect on ovarian damage has been demonstrated for stem cells from various sources such as bone marrow, amniotic fluid, adipose tissue [26,27].

Menstrual blood stem cells

An alternative and accessible source of SC that has been explored is the tissue obtained from menstrual blood. These MSCs, here called menstrual stem cells, are characterized by rapid proliferation, high clonogenic potential and long-term survival, in addition to being more easily obtained, having extended availability throughout the woman's life and being obtained from a tissue to be discarded, which avoids ethical problems [15,28] and invasive procedures (such as those required for bone marrow SC collection).

Immunophenotypically, they are characterized as CD9+, CD59+, CD41a+, hTERT+, OCT4+, CD29+, CD44+, CD73+, CD90+, CD105+, CD146+ STRO1-, CD31-, CD34, CD45, HLA-DR, CD14-, CD38-, CD133 -, SSEA4-, NANOG- [25]. They also have surface markers Oct-4, SSEA-4, Nanog and c-kit (CD117), related to their high proliferative and differentiation capacity in various cell types [30]. Nanog encodes a transcription factor that maintains the undifferentiated and self-renewing state of SC [31-35].

Depending on the hormonal stimulus, which varies according to the menstrual cycle, the endometrium undergoes changes [36]. In the proliferative phase, which begins shortly after menstruation, the estrogen produced by the ovary binds to receptors in the endometrium (ER alpha), leading to the activation of endometrial SC. Therefore, the menstrual blood is made up of blood cells predominantly of arterial origin, prostaglandins, fibrinolysin and endometrial cells, which have among them the SC, in a lesser extent [37,38]. It is the presence of endometrial SC that justifies the continuous renewal of the uterine endometrium even after menstruation, pregnancy or trauma [39, 40]. It was previously believed that SC was present only in the basal layer of the endometrium. With the discovery of SC in menstrual blood, we now have an accessible and non-invasive source for conducting cell cultures, research and treatments [41].

Although studies with menstrual stem cells are relatively recent, there is evidence that, when compared to other sources of mesenchymal stem cells, the doubling time is shorter and the clonogenic potential in culture is better [41,42]. The menstrual stem cells also appear to be more resistant to senescence [42]. Cellular senescence is defined as the decreased ability to differentiate. After the seventh passage in culture, the cell derived from the bone marrow begins to enter senescence, whereas in the menstrual SC this occurs after the 26th pass. Such potential ensures high bioavailability of these mesenchymal cells [42].

Research with cultured menstrual SC showed differentiation in neurogenic, cartilaginous, muscle, bone and beta insulin-secreting cells. Likewise, myocardial tissue repair was performed using this cells after ischemic injury in rats and of the endometrium in animal models of Asherman's syndrome [43], proving the potential and the need for further studies on this new source of stem cells.

Objective

Conduct a bibliographic review in order to analyze the use of menstrual blood as a source of stem cells, evaluating ways to obtain and preserve these cells, and their potential use in the regeneration of injured tissues.

Materials and Methods

This is a bibliographic review article, carried out through the virtual page of the Regional Library of Medicine, in the databases: MEDLINE, LI-LACS, Cochrane, JAMA, CAPES, PubMed and SciELO. The main studies already published on the subject were analyzed, taking into account, in Portuguese and English, the following terms: células-tronco x fontes de obtenção; células-tronco x sangue menstrual; regeneração tecidual x células-tronco; células-tronco menstruais x proliferação celular; menstrual blood x stem cells; stem cells x ways of obtaining; células-tronco menstruais x obtenção x preservação; células-tronco x usos medicinais; stem cells x tecidual regeneration.

Results

51 articles were selected and studied, published between 2001 and 2019. We used as inclusion criteria articles that approached stem cells, their discovery, their sources of obtaining and their use in Regenerative Medicine. Among these, only thirteen brought menstrual blood as a source of such cells.

The discovery of stem cells in menstrual blood occurred in 2008 [41], emerging as a promising option as a source of stem cells, as it does not need invasive procedures for its collection, it does not face ethical and moral problems because it's a tissue to be discarded [44], and it has the possibility of autologous use by the donor patient itself, avoiding problems of incompatibility and risk of transmission of diseases [45].

Published studies have shown that the collection of menstrual blood can be done through the use of menstrual collectors on days of greater menstrual flow, in a simple way, with low levels of contamination and little discomfort to the patient [44]. In addition, the use of hormonal contraceptives doesn't alter the availability of stem cells in the menstruation blood. After collection, the material can be sent for processing within 24 hours [44,46], to perform cellular immunophenotyping with specific multipotent embryological and cell stroma surface markers (such as oct-4, SSEA, CD90, CD105) [44], and subsequent cell culture and cryopreservation.

Menstrual stem cells have a high power of cell differentiation and, with appropriate stimuli, can differentiate into osteocytes, adipocytes, chondrocytes, hepatocytes and cardiomyocytes [30,41,44,47,48,49]. It has also been shown to be effective in regenerating nerve cells after stroke in vitro [50]. In addition, in mice with ovarian failure, menstrual stem cells could differentiate into granulosa cells, resulting in increased hormone secretion and weight gain in these ovaries [51].

This data is of significant importance for future treatments of women with premature ovarian failure, caused by autoimmune diseases, cancer treatments (irradiation, chemotherapy) or ovarian surgery.

Discussion

According to the articles read, we conclude that the use of menstrual blood as a source of stem cells is still small and limited, despite being an option discovered more than a decade ago. There are few articles in the literature addressing the use of menstrual stem cells even with all the advantages demonstrated in relation to them: easy to obtain, absence of moral and ethical dilemmas for their use, low cost of obtaining, viable source of stem cells, possibility autologous use, and differentiation capacity in another tissues.

At the University of Ribeirão Preto (UNAERP), we made several attempts of obtaining these stem cells in the laboratory (in unpublished data), but we find difficulties due to the high rate of contamination of menstrual blood during collection, which differs from some published articles. Such contamination made the processing, isolation and cultivation of menstrual stem cells unfeasible. The contamination of the material may be one of the reasons for the small amount of scientific literature on the subject. However, the menstrual blood is an important source of stem cells with a promising future in several areas of Regenerative Medicine, among them in Gynecology and Obstetrics, especially in the ovarian regeneration of patients with premature ovarian failure.

Conclusion

The analyzed studies demonstrated the existence of stem cells in menstrual blood, and the main benefits of it as a source of these cells:

- It is a tissue to be discarded.
- The way of obtaining it is simple and non-invasive, without bringing risks to the donor patient.
- Possibility of autologous use, avoiding the need for immunosuppression and risk of tissue rejection.
- High clonogenic potential and long survival.

Analyzes were also made regarding the possibility of cultivation, proliferation and preservation of these cells, all demonstrating the viability of this tissue, with possible use in reparative medicine. However, despite all the positive points, there are still few studies and research in this area which can establish definitive protocols for the collection, obtaining and proliferation of menstrual stem cells, and which allow its use in tissue regeneration.

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