Editorial

Stem cells—The new agents in infertility treatment: The light at the end of the tunnel?

The Ministry of Health and Family Welfare, in a proposal to amend the Drugs and Cosmetics Act 1940, is pushing to bring stem cells and cell-based products under legal regulations: a move scientists have termed as long overdue.

The ministry defined the category of stem cells and their derivatives that would be termed a drug and would thereby have to follow the protocols mandated for any drug development. The notification, dated 4th April 2018, said stem cells and products that are substantially altered, amounting to a change in biological characteristics, will be treated as a drug and will, hence, have to seek the regulator's approval before coming to the market. The role of the Central Drugs Standard Control Organisation (CDSCO), under the health ministry, will be central in this case. It is the national regulatory body for Indian pharmaceuticals and medical devices and serves as a parallel function to the European Medicines Agency of the European Union, the PMDA of Japan, the Food and Drug Administration of the United States and the Medicines and Healthcare products Regulatory Agency of the United Kingdom. In other words, CDSCO is responsible for regulating and licensing pharmaceuticals or new biological drug entities. The notification is deemed to be a part of a review exercise by the CDSCO, which was in the process for a comprehensive review of Drugs and Cosmetics Act, 1940, on the directions of Parliament.

The Indian Council of Medical Research (ICMR) will be reviewing and deliberating the draft notification in consultation with the experts in the field. The National Guidelines For Stem Cell Research (2017) made by the ICMR is looking at suspending the commercial banking of stem cells derived from biological materials such as cord tissue, placenta, tooth extract and menstrual blood. In the recommendations made by ICMR, it has been stated that there is no scientific evidence to substantiate the clinical benefits of these stem cells. Accordingly, the ICMR has issued guidelines stating that the commercial banking of all biological materials, other than umbilical cord blood, is not permitted until further notification. At the same time, ICMR has approved the stem cell treatment for 30 odd categories of diseases, mostly cancer.

The apex medical research agency listed 20 types of indications (diseases) for adults and another 13 categories of indications for children below 18 years wherein stem cell treatment is permitted. Besides cancers, some complicated congenital diseases are on that list. The guidelines also mention that every other therapeutic use of stem cells shall be treated as investigational and conducted only in the form of a clinical trial after obtaining necessary regulatory approvals.

The 2017 guidelines reiterate that any stem cell use in patients, other than that for hematopoietic stem cell reconstitution for approved indications, is investigational at present. Accordingly, any stem cell use in patients must only be done within the purview of an approved and monitored clinical trial with the intent to advance science and medicine, and not offering it as therapy. In accordance with this stringent definition, every use of stem cells in patients outside an approved clinical trial is unethical and shall be considered as malpractice. The document provides important definitions for and elaborates upon the levels of manipulations, category of research (permissible, restrictive or prohibited), manufacturing process and release criteria, among others.

Government regulations in this sector are currently virtually absent, a fact taken advantage of by many who have already added a commercial angle to it without robust evidence. The notification, however, does not touch upon the use of autologous stem cells, where the individual's own stem cells are collected and minimally treated before infusing it back into the patient.

Thus, the time is ripe for us reproductive specialists to explore the applications of stem cell therapy in the field of fertility treatment. Not surprisingly, there are many centers within our country offering a myriad of techniques and protocols to the infertile couples with tall claims of success especially in infertile males. While not all that is blogged could be brushed aside as

unfounded, there is an urgent need to understand the indications, the sources of stem cell, the mode of administration and the results of treatment for both the male and female partners. The economics of the procedure of course would be an important concern for a country such as India.

BACKGROUND

Stem cells are undifferentiated cells that are present in the embryonic, fetal and adult stages of life and give rise to differentiated cells that make up the building blocks of the tissue and organs. Due to their unlimited source and high differentiation potential, stem cells are considered as potentially new therapeutic agents for the treatment of infertility. Stem cells could be stimulated *in vitro* to develop various numbers of specialized cells including male and female gametes suggesting their potential use in reproductive medicine. During the past few years, a considerable progress in the derivation of male germ cells from pluripotent stem cells has been made. In addition, stem cell-based strategies for ovarian regeneration and oocyte production have been proposed as future clinical therapies for treating infertility in women.[1]

The major characteristics of stem cells are (a) self-renewal (the ability to extensively proliferate), (b) clonality (usually arising from a single cell) and (c) potency (the ability to differentiate into different cell types). Totipotent or omnipotent cells are the most undifferentiated cells and are found in early development. A fertilized oocyte and the cells of the first two divisions are totipotent cells, as they differentiate into both embryonic and extraembryonic tissues, thereby forming the embryo and the placenta. Pluripotent stem cells are able to differentiate into cells that arise from the three germ layers—ectoderm, endoderm and mesoderm—from which all the tissues and organs develop. Commonly, stem cells are derived from the following two main sources: early embryos [embryonic stem cells (ESCs)] and adult tissue (adult stem cells).[2]

ESCs are pluripotent stem cells derived from the inner cell mass of the blastocyst. The essential characteristics of ESCs include derivation from the preimplantation embryo, prolonged proliferation in their pluripotent state and stable developmental potential to form the derivatives of all three embryonic germ layers.

Mesenchymal stem cells (MSCs) are one of the most common adult, multipotent stem cells. They can be derived from a variety of tissues including the bone marrow, adipose tissue, bone, Wharton's jelly, umbilical cord blood and peripheral blood. MSCs are adherent to cell culture dishes and are characterized by specific surface cell markers. Recently, MSCs were differentiated into neuronal tissue, which is derived from the ectoderm. This is an example of transdifferentiation, that is, when a cell from one germ layer (mesoderm) differentiates into neuronal tissue (ectoderm).[3]

Stem cells can also be derived from the extraembryonic tissues (amnion, chorion, placenta and umbilical cord). The amnion and chorion contain stromal cells that display characteristics and differentiation potential similar to bone marrow-derived MSCs and are able to differentiate into adipocytes, endothelial cells, hepatocytes, osteocytes, myocytes and neurons. Placental-derived stem cells have the capacity to differentiate into ectodermal, mesodermal and endodermal cell types, while umbilical cord matrix stem cells, after transplantation, enhanced muscle regeneration in a mouse model of severe muscle damage and promoted blood vessel formation and neurological function in the animal models of ischemic brain disease. The main advantage of stem cells derived from extraembryonic tissues is the efficient isolation from tissues normally discarded at birth avoiding ethical concerns that plague the isolation of human ESCs (hESCs).[2]

STEM CELLS AND MALE INFERTILITY

Male infertility accounts for approximately half of all cases of infertility, and nonobstructive azoospermia is the most severe form. Besides genetic factors, azoospermia also occurs due to injuries, exposure to toxicants, immunosuppression and anticancer treatment. However, a large proportion of infertile males are diagnosed as idiopathic with unknown causes, reflecting a poor understanding of the mechanisms regulating spermatogenesis and sperm function in humans.[4]

Significant progress has been made in ART for the treatment of infertility. However, current method of ART has been unable to help the infertile couples who lack functional gametes, unless donor gametes were used. In fact, most couples wish to have their own genetically related child. With the rapid development of stem cell technology, the possibility to derive artificial gametes from human pluripotent stem cells may provide new therapeutic strategies for infertile couples.

ESCs can differentiate into male germ-like cells *in vitro*, but they are genetically unrelated to the patients, and the sources of hESCs are limited and accompanied by ethical issues regarding the destruction of embryos.[5]

The ectopic expression of transcription factors leads to the reprogramming of somatic cells to induced pluripotent stem cells (iPSCs), which resemble ESCs in morphology, pluripotency marker expression and differentiation ability. To some extent, human iPSCs (hiPSCs) are superior to hESCs for reproductive medicine application because there are few ethical issues and the sources are abundant. Furthermore, hiPSCs can be generated from patients' somatic cells and are immunocompatible for autotransplantation. Therefore, the generation of patient-specific spermatozoa from hiPSCs will provide the foundation for the future treatment of male infertility. However, hiPSCs may not faithfully recapitulate the characteristics of hESCs at both genetic and epigenetic levels. Especially, hiPSCs are reported to keep some epigenetic marks of the donor cell type from which they were reprogrammed.[6]

Hayashi *et al.* made the remarkable finding that PGCLCs could be obtained from mouse ESCs and mouse iPSCs. The PGCLCs could be differentiated into spermatozoa *in vivo* resulting in the birth of healthy offspring via ICSI.[7] In spite of the progress in mice, the differentiation of hiPSCs to male germ cells still presents a significant challenge. Unlike miPSCs in naive state, hiPSCs exhibit a primed pluripotency with less potential for the germ cell fate. Therefore, it may not be surprising that the success rate of germ cell derivation from hiPSCs is much lower than that from miPSCs.[4]

All in all, the discovery of hiPSCs may not only lead to clinical approaches addressing infertility resulting from defects in gametogenesis, but also provide an opportunity to investigate the molecular mechanism of human germ cell development. In this review, we summarize the current advances in the derivation of male germ cells from hiPSCs and raise perspectives regarding the usage of hiPSCs in medical application for the treatment of male infertility, as well as for basic research into the mechanisms of human germ cell development.

STEM CELLS TO THE RESCUE OF FEMALE INFERTILITY

Stem cell-based strategies for ovarian regeneration and oocyte production have been proposed as future clinical therapies for treating infertility in women. The pioneering work by White *et al.* has identified a rare population of mitotically active germ cells in human ovaries that can be purified and cultured *in vitro* to spontaneously form oocytes.[8]

In allogeneic stem cell transplant (SCT), the recovery of ovarian function ranges from 14 to 24%, and the interval from SCT to first spontaneous menstruation ranges from 21 to 87 months (median 49 months). Recovery rates as high as 84% have been reported among patients with favorable predictors. These patients were young, and none received total body irradiation as a part of transplant conditioning. The rates for the recovery of ovarian function after autologous SCT are expected to be higher than after allotransplantation, because autologous SCT does not require subsequent immunosuppressive therapy, and recipients do not experience graft-versus-host disease.[9]

Ability to generate new oocytes, the treatment of Asherman syndrome and recurrent implantation failures due to poor endometrial receptivity have all been addressed with the treatment of stem cells in various research centers across the globe. However, these are under various stages of research at approved highly advanced centers with strict enrolment and monitoring criteria. Young women with premature ovarian insufficiency may be able to use their own bone marrow stem cells to rejuvenate their ovaries and avoid the effects of premature menopause as new research suggests in a paper presented at the 100th Meeting of the Endocrine Society in March 2018 at Chicago.

While the treatment with stem cells for various indications in reproductive medicine seems very attractive, one has to proceed with cautious optimism and be careful not to overstate the promises of the technology in its current form. There is certainly a promising vision as to how the research could change the world of fertility and aging. However, what is surprising is the open invitation to the desperate infertile couples from various infertility centers across India claiming the success of stem cell therapy for both male and female infertility.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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Access this article online

Quick Response Code:

Website: www.fertilityscienceresearch.org

DOI: 10.4103/fsr.fsr_16_18

How to cite this article: Radhakrishnan G. Stem cells—The new agents in infertility treatment: The light at the end of the tunnel? Fertil Sci Res 2017;4:70-3.