ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Fertility rescue and ovarian follicle growth promotion by bone marrow stem cell infusion

Sonia Herraiz, Ph.D.,[a,b,c] Anna Buigues, B.Sc.,[a,c] César Díaz-García, M.D.,[b,c,d] Mónica Romeu, M.D.,[b] Susana Martínez, M.D.,[b] Inés Gómez-Seguí, M.D.,[e] Carlos Simón, M.D.,[c,f,g,h] Aaron J. Hsueh, Ph.D.,[h] and Antonio Pellicer, M.D.[a,b]

[a] IVI Foundation, Valencia, Spain; [b] Reproductive Medicine Research Group, Valencia, Spain; [c] Department of Pediatrics, Obstetrics, and Gynecology, School of Medicine, Valencia University, Valencia, Spain; [d] IVI London, London, United Kingdom; [e] Hematology Department, La Fe University Hospital, Valencia, Spain; [f] Instituto Universitario IVI/INCLIVA, Valencia, Spain; [g] Igenomix, Paterna, Spain; and [h] Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California

Objective: To assess if infusion of human bone marrow–derived stem cells (BMDSCs) could promote follicle development in patients with impaired ovarian functions.
Design: Experimental design.
Setting: University research laboratories.
Animal(s): Immunodeficient NOD/SCID female mice.
Intervention(s): Human BMDSCs were injected into mice with chemotherapy-induced ovarian damage and into immunodeficient mice xenografted with human cortex from poor-responder patients (PRs).
Main Outcome Measure(s): Follicle development, ovulation, and offspring. Apoptosis, proliferation, and vascularization were evaluated in mouse and human ovarian stroma.
Result(s): Fertility rescue and spontaneous pregnancies were achieved in mice ovaries mimicking PRs and ovarian insufficiency, induced by chemotherapy, after BMDSC infusion. Furthermore, BMDSC treatment resulted in production of higher numbers of preovulatory follicles, metaphase II oocytes, 2-cell embryos, and healthy pups. Stem cells promoted ovarian vascularization and cell proliferation, along with reduced apoptosis. In xenografted human ovarian tissues from PRs, infusion of BMDSCs and their CD133+ fraction led to their engraftment close to follicles, resulting in promotion of follicular growth, increases in E_2 secretion, and enhanced local vascularization.
Conclusion(s): Our results raised the possibility that promoting ovarian angiogenesis by BMDSC infusion could be an alternative approach to improve follicular development in women with impaired ovarian function.
Clinical Trial Registration Number: NCT02240342. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Bone marrow–derived stem cell infusion, ovarian niche regeneration, follicle rescue, primary ovarian insufficiency, poor ovarian response

Discuss: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/28948-25076

Ovarian aging is associated with a significant decline in fecundity and oocyte quality in woman after 35 years of age, leading to complete ovarian failure in the early 50s (1). As women in modern society delay their childbearing, maternal age, which influences both the quantity and the quality of oocytes, has currently become the main determinant of fertility (2). In addition, as more cancer patients are surviving after chemotherapy, they are left with, typically irreversible, deleterious effects of chemotherapy drugs on ovaries (3, 4). In all cases of ovarian impairment, there remains a need for methods to restore fertility in patients seeking reproductive success.

At birth, each human ovary contains a nonrenewable pool of 400,000

Received September 26, 2017; revised December 20, 2017; accepted January 4, 2018.
S.H. and A.B. should be considered similar in author order.
S.H. has nothing to disclose. A.B. has nothing to disclose. C.D.-G. has nothing to disclose. M.R. has nothing to disclose. S.M. has nothing to disclose. I.G.-S. has nothing to disclose. C.S. has nothing to disclose. A.J.H. has nothing to disclose. A.P. has nothing to disclose.
Supported by grants PROMETEOII/2014/045 and GV/2014/115 from the Regional Valencian Ministry of Education and FIS PI15/00312 from the Spanish Ministry of Economy and Competitiveness. A. Buigues' participation supported by grant FPU14/02999 from the Spanish Ministry of Education, Culture, and Sport.
Reprint requests: Sonia Herraiz, Ph.D., IVI Foundation, Av. Fernando Abril Martorell, 106-TorreA—Planta1, Valencia 46026, Spain (E-mail: sonia.herraiz@ivi.es).

Fertility and Sterility® Vol. ■, No. ■, ■ 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.01.004

quiescent follicles. At menarche, intraovarian mechanisms activate a small number of resting primordial follicles to initiate growth each month during reproductive life (5). Follicle depletion occurs at menopause when <1,000 follicles remain (6).

Interestingly, competent and fertilizable oocytes can be obtained from residual follicles, even in women with primary ovarian insufficiency (POI) (7, 8). Furthermore, patients with POI due to chemotherapy can achieve spontaneous pregnancy after bone marrow (BM) transplant (9–13), suggesting that residual follicles could be rescued. This rescue could be attributable to the ability of the ovarian niche to attract undifferentiated cells from other organs, specifically BM, in a process known as "homing" (14). Together, these findings suggest that follicles could be stimulated to grow when an adequate ovarian environment is restored by factors such as stem cells present in BM.

Indeed, BM-derived stem cells (BMDSCs) also confer such regenerative properties, specifically BMDSCs selected for CD133 expression, which are considered to be the most immature of the hematopoietic progenitor cells (15). Transfer of these CD133+ cells promotes cell proliferation and neoangiogenesis in endometrium (16). Follicular growth is also dependent on cyclic microvascular remodeling, which occurs throughout the menstrual/estrous cycle (17), suggesting that BMDSCs could be a feasible source to induce ovarian vasculature development. We therefore hypothesized that infusion of human BMDSCs could exert positive revitalizing effects on the impaired ovaries in mice. Based on this, we used immune-deficient mouse models with decreases in ovarian reserve to test whether BMDSC infusion could rescue quiescent residual follicles to produce competent and fertilizable oocytes. The immune-deficient mice were also xenotransplanted with human ovarian tissues from poor ovarian response (PR) patients to monitor effects on human follicle growth and local vasculature. These studies serve as a proof of concept for translation into clinical settings in which BMDSC infusion could enable recovery of ovarian function in women with ovarian impairment.

MATERIALS AND METHODS

Study Approval

This study was approved by the Institutional Review Board of Hospital Universitario y Politécnico La Fe, Valencia, Spain (2014/0147) and was conducted according to the principles expressed in the Declaration of Helsinki. All animal experiments were conducted in accordance with the Institutional Review Board and the Ethics Committee (A1484581669445 and A1429108087406) of the University of Valencia, Valencia, Spain.

Study Design

BMDSCs were mobilized to the general circulation in ten PR patients (38 ± 2 years of age) after injections of granulocyte colony–stimulating factor (G-CSF) for 5 days and then recovered by apheresis following standard procedures. G-CSF treatment increased the BMDSC population from a baseline of 2 cells/mL to ≥10,000 CD34+ cells/mL (18).

To assess their potential regenerative effect on ovarian tissues, the present study was performed in two phases. In the first set of experiments, two chemotherapy (ChT) regimens (1 ChT standard and 0.1 ChT reduced dose) were used to induce ovarian damage in immunodeficient SCID mice. Then human BMDSCs were injected to promote follicle development in ovaries of treated mice (Supplemental Fig. 1, available online at www.fertstert.org). In a second experimental group, animals were infused with standard peripheral blood mononuclear cells (PBMNCs) obtained from non–G-CSF–pretreated PR patients (without BMDSC mobilization) to assess if observed regenerative properties are due to stem cells mobilized from BM and not to circulating blood cells. Animals in the control group were injected with phosphate-buffered saline solution (PBS). Once the regenerative properties of BMDSCs in mice ovaries were established, we further tested the effects of infusing human BMDSCs on human ovarian follicle growth. Ovarian cortical fragments from PR patients were xenografted into ovariectomized immunodeficient SCID mice (Supplemental Fig. 2, available online at www.fertstert.org). In addition to BMDSCs, we also infused the CD133+ subset of the BMDSCs, because these cells have been shown to have regenerative properties in other human reproductive organs (19). The selected number of CD133+ cells for injection was established according to the percentage of their population within the BMDSCs and previous reports where the dose was able to induce endometrial regeneration in mouse models (16). Human ovarian xenografts were retrieved at several time points (days 1, 7, and 14 after cell infusion).

All the BMDSCs, CD133+ cells, and PBMNCs used in both experimental designs were labeled with Molday Ion Rhodamine B (MIRB) to allow cell tracking.

BMDSC Regenerative Effects in a Chemotherapy-Induced Ovarian Damage Mouse Model

Forty-eight 8-week-old female NOD-SCID mice (Charles River Laboratories, France) were randomly allocated to the following experimental groups: 0.1 ChT Control, 0.1 ChT PBMNC, 0.1 ChT BMDSC, 1 ChT Control, 1 ChT PBMNC, and 1 ChT BMDSC. The reduced 0.1 ChT regimen consisted of a single dose of 1.2 mg/kg busulfan (Bu) and 12 mg/kg cyclophosphamide (Cy; Sigma, St. Louis Missouri); the standard 1 ChT regimen was a single dose of 12 mg/kg and 120 mg/kg, respectively. Chemotherapy drug was intraperitoneally injected. One week later, on experimental day 0, animals allocated to the control groups received an injection of 100 μL PBS in the tail vein with the use of a 25-gauge needle, mice in the PBMNC groups were injected with 1×10^6 labeled PBMNCs resuspended in 100 μL of plasma, and mice in the BMDSC groups were injected with 1×10^6 labeled BMDSCs. On experimental day 14, mice underwent ovarian hyperstimulation (COS) with 10 IU pregnant mare serum gonadotropin (PMSG; Sigma), and 48 hours later with 10 IU human chorionic gonadotropin (hCG).

Six mice per group were mated with fertile males 16 hours after hCG injection, and four of them were killed later to recover ovulated oocytes and 2-cell embryos from the oviduct; the