Autologous stem cell ovarian transplantation to increase reproductive potential in patients who are poor responders

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

[a] Fundación IVI, [b] IVI-RMA Valencia, [c] Reproductive Medicine Research Group, IIS La Fe, [d] Women's Health Area, [g] Hematology Department, and [h] Radiology Department, La Fe University Hospital; [e] Department of Pediatrics, Obstetrics and Gynecology, University of Valencia, Valencia, Spain; [f] IVI-RMA London, London, United Kingdom; and [i] IVI-RMA Rome, Rome, Italy

Objective: To evaluate effects of autologous stem cell ovarian transplant (ASCOT) on ovarian reserve and IVF outcomes of women who are poor responders with very poor prognosis.
Design: Prospective observational pilot study.
Setting: University hospital.
Patient(s): Seventeen women who are poor responders.
Intervention(s): Ovarian infusion of bone marrow-derived stem cells.
Main Outcome Measure(s): Serum antimüllerian hormone levels and antral follicular count (AFC), punctured follicles, and oocytes retrieved after stimulation (controlled ovarian stimulation) were measred. Apheresis was analyzed for growth factor concentrations.
Result(s): The ASCOT resulted in a significant improvement in AFC 2 weeks after treatment. With an increase in AFC of three or more follicles and/or two consecutive increases in antimüllerian hormone levels as success criteria, ovarian function improved in 81.3% of women. These positive effects were associated with the presence of fibroblast growth factor-2 and thrombospondin. During controlled ovarian stimulation, ASCOT increased the number of stimulable antral follicles and oocytes, but the embryo euploidy rate was low (16.1%). Five pregnancies were achieved: two after ET, three by natural conception.
Conclusion(s): Our results suggest that ASCOT optimized the mobilization and growth of existing follicles, possibly related to fibroblast growth factor-2 and thrombospondin-1 within apheresis. The ASCOT improved follicle and oocyte quantity enabling pregnancy in women who are poor responders previously limited to oocyte donation.
Clinical Trial Registration Number: NCT02240342. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Poor responder, bone marrow-derived stem cell transplant, ovarian reserve, AMH, antral follicular count

Up to 15% of couples trying to conceive are considered infertile and require medical assistance to achieve pregnancy (1). The deleterious effects of aging on ovarian reserve and oocyte quality are well known (2). Aged women with low ovarian reserve are typically referred to as poor responders and make up 9%–24% of patients seeking therapy at assisted reproductive technology (ART) clinics (3). Oocyte donation is the practical therapeutic option (4), but it involves significant psychological burdens (5) and encounters regulatory restrictions in many countries. Thus, interventions that support the regeneration of already aged or damaged gonads and rejuvenate the ovaries are constantly sought for patients who are poor responders.

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Reprint requests: Sonia Herraiz, Ph.D., Fundación IVI, Avenue Fernando Abril Martorell, 106 - Torre A 1ª, 46026 Valencia, Spain (E-mail: Sonia.Herraiz@ivi.es).

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Attempts to overcome a poor response have been thus far focused on stimulating the ovaries to promote follicle growth. Many controlled ovarian stimulation (COS) protocols and other alternatives have been tested, but none have proven to be successful in the clinic (6), probably due to the low number of remaining antral, gonadotropin-dependent, stimulus-responsive follicles within the ovaries (7). Nevertheless, a small pool of quiescent primordial follicles remain, even in the ovaries of patients who are menopausal and have premature ovarian insufficiency (POI), which could potentially contribute to increase the final yield of oocytes. In fact, competent oocytes could be retrieved after the activation and growth of these remaining follicles by several approaches, providing them with an appropriate growth-supporting ovarian niche (8–10).

Bone marrow transplants in patients with POI due to chemotherapy or radiotherapy treatments rescues ovarian function as demonstrated by several spontaneous pregnancies (11–15). Although the molecular autocrine/paracrine mechanisms that control primordial follicle activation remain unknown, it is believed that the local environment (niche) plays a fundamental role. Several studies have used bone marrow-derived stem cells (BMDSC) to activate the niche. We recently described that BMDSC infusion promotes human and mouse follicular growth by increasing ovarian vascularization, stromal cell proliferation, and reducing cell death (16, 17). Furthermore, we have regenerated the endometrium in women with Asherman's syndrome after isolation and infusion of the bone marrow-CD133+ population (18).

Several mechanisms have been proposed to achieve tissue regeneration by adult stem cell therapy (19). In the context of ovarian tissue, paracrine actions should be evaluated for their ability to activate the preexisting quiescent follicles based on the ability of adult stem cells to produce and secrete a variety of cytokines, chemokines, and growth factors, which may be involved in tissue repair (19). Some of these soluble factors are known to be involved in follicular growth, stem cell signaling, angiogenesis, viability, and ovarian response to COS (20–22).

Based on this information, we designed a method to deliver BMDSC directly to human ovaries in an effort to induce ovarian rejuvenation in patients who are poor responders and optimize the recruitment of existing dormant follicles to improve IVF outcomes. The results begin to elucidate the underlying mechanisms of ovarian rejuvenation and point the way for the development of future ART therapies.

MATERIALS AND METHODS

Study Design

This pilot study was designed for autologous stem cell ovarian transplantation (ASCOT) in 17 patients who are poor responders defined according to the European Society of Human Reproduction and Embryology (ESHRE) criteria (23). All participants voluntarily accepted and signed the written consent form.

The study consisted of BMDSC mobilization to peripheral blood by granulocyte colony-stimulating factor (G-CSF) treatment and subsequent collection by apheresis. Cells were delivered into the ovarian artery by the intra-arterial catheter in one side. In each patient, the other ovary was used as a control. The main end points were clinical improvement of ovarian reserve as measured by antral follicle count (AFC) and serum antimüllerian hormone (AMH) levels, monitored up to 5 months after ASCOT, as well as the number of metaphase II (MII) oocytes retrieved after ovarian stimulation. Secondary outcome measures consisted of number of treatment cycles, cancellation rate, number of obtained embryos and euploid embryos assessed by comparative genomic hybridization array, cumulative pregnancy rate (PR), and cumulative live birth rate. Pregnancy could be achieved by fresh or cryo/thawed embryo transfer (ET). Spontaneous pregnancies were also considered.

The study was simultaneously set up to determine the mechanisms involved in a positive ovarian response. Concentrations in each apheresis sample of a number of growth factors released by BMDSC was evaluated and compared with ovarian reserve before and after the ASCOT procedure. The study design is summarized in Supplemental Figure 1 (available online).

Bone Marrow Stem Cell Mobilization and Apheresis

An SC dose of 10 μg/kg/d of G-CSF was administered during 5 days. On the fifth day, stem cell collection was performed if patients reached a threshold of CD34+ circulating cells in peripheral blood $\geq$10 cells/μL. Cell collection was performed using standard procedures, including continuous flow apheresis in an OPTIA cell separator (Caridian). The target was to reach a minimum of 4×10^6 CD34+ cells/kg.

Samples were immediately analyzed by flow cytometry after collection to quantify the CD133+ population. A volume of whole apheresis containing 50×10^6 CD133+ cells was then prepared for infusion.

Intra-arterial Catheterization

Patients were referred to the Interventional Radiology Unit for delivery of the apheresis concentrates to the ovarian artery by intra-arterial catheterization. The time between cell collection and ASCOT was <24 hours in all cases. In each patient, ASCOT was randomly delivered in one ovary. In a digital angiography suite, using the Seldinger technique to approach the common femoral artery with an 4-F port, the ovarian artery was reached with a Cobra 2 catheter and a Terumo's 0.035-inch guide. Then, a 2.5-F microcatheter with a 0.014-inch guide was placed through the Cobra catheter to the opposite end, through which BMDSC were delivered (Fig. 1A).

Patient Follow-Up

The AFC by transvaginal ultrasound and serum AMH levels were considered markers of ovarian response to ASCOT (24). Both were recorded in all patients under basal conditions before G-CSF injection. After the ASCOT procedure, serum AMH and AFC were recorded every 2 days for 2 weeks, then once a week for 4 weeks, and then monthly up to 5 months. The criteria for a positive response by ovarian reserve to