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Case Report

Ovarian Treatment with Autologous Platelet-Rich Plasma Plus Autologous Bone Marrow-Derived Stem Cells: Case Series

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ABSTRACT

Introduction: There are limited treatment options for women with severely Diminished Ovarian Reserve (DOR) who experience repeatedly failed *In Vitro* Fertilization (IVF) cycles. Therefore, most patients resort to using donor oocytes. Ovarian rejuvenation is an innovative procedure intended to reactivate the remaining follicles in the ovary to improve ovarian response in assisted reproduction treatments.

Case presentation: Using two treatments at the same time, we enhance the effects of intraovarian Platelet-Rich Plasma (PRP) injection and autologous bone marrow-derived mesenchymal stem cells on ovarian stimulation outcomes in three women referred to a Uruguayan IVF center.

Conclusion: Data presented herein suggest that autologous intraovarian PRP infusion plus Stem Cells may restore ovarian function. This may enable the achievement of pregnancy. However, the occurrence of spontaneous pregnancy, clinical pregnancy and live birth reflect a significant change. Longer follow-up may detect a functional recovery with improvement of the ovarian reserve hormone markers.

Keywords: Autologous platelet-rich plasma; Mesenchymal stem cells; Pregnancy; Ovarian rejuvenation

INTRODUCTION

Ovarian reserve refers to the quantity of ovarian primordial follicles. DOR indicates a reduction in the quantity of ovarian follicular pool in reproductive-age women and is an important cause of infertility in many couples. It is a phenomenon often noted in women in their mid to late thirties, but it may also affect younger women.

Although these women undergo IVF and other infertility interventions, their pregnancy rates remain low, and they have higher rates of recurrent pregnancy loss.

Ovarian rejuvenation is an innovative procedure for restoring ovarian fertility and development during menopause. It's a regenerative medicine procedure that has been used to enhance fertility in women with premature ovarian insufficiency and has been suggested as a potential solution to those with DOR and failed IVF cycles, provides an alternative to treatments involving donor gametes. In regenerative gynecology, autologous PRP and autologous bone marrow-derived mesenchymal stem cells are novel techniques [1].

A recent meta-analysis shown that intrauterine and subendometrial injections of PRP improved IVF cycles outcomes such as implantation, clinical pregnancy, live birth rates and endometrial thickness in infertile women with previous implantation failure and those with refractory thin endometrium [2].

In terms of mesenchymal stem cells enhancing or restoring fertility potential, a recent systematic review indicates yielding promising results improving fertility potential in various preclinical settings [3].

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PRP is an autologous and highly concentrated solution derived from the patient's own blood. It contains a concentrated source of insulin-like growth factors 1 and 2, fibroblast growth factor, epidermal growth factor, transforming growth factor beta, hormones, and cytokines. In addition to other growth factors, platelets contain different substances, such as fibronectin, vitronectin, and sphingosine 1-phosphate that initiate wound healing. Considering the angiogenic composition of the ovary and the pivotal influence of platelet-derived growth factors on vascular activation and stabilization, treatment with autologous PRP may enable ovarian tissue regeneration [4-6].

Mesenchymal Stem Cells (MSCs) of several stem cell lineages have successfully been used to ameliorate ovarian insufficiency. So far, using mesenchymal stem cells derived from bone marrow is particularly promising. Bone marrow cell's secretory milieu contains many cytokines including the Stem Cell Factor (SCF), mast cell growth factor that signals through the c-kit receptor (Cluster of Differentiation-117). These cytokines are essential in promoting cell proliferation, survival, and angiogenesis [3,7-11].

It is also known that stem cells derived from bone marrow have the potential to give rise to different cellular lineages. The subpopulations of bone marrow-derived cells accommodate c-kit receptors ($CD117^+$ cells) include hematopoietic, endothelial, and mesenchymal lineages [9].

Therefore, multiple IVF centres around the world have embraced the idea of ovarian rejuvenation using stem cells in combination with PRP growth factors.

In this article, we profile some of the first Uruguayan patients who received ovarian rejuvenation therapy.

CASE PRESENTATION

Inclusion criteria

Women under 40 years old who (a) Underwent treatment with reproductive intentions (not to alleviate menopausal symptoms) between November 2021 and March 2023, (b) Attended the corresponding follow-up appointments after treatment.

Case 1

Married 33-year-old woman and 36-year-old male, with primary infertility (2 miscarriages), come for consultation after a year of trying to conceive. The male studies come normal. The female had regular cycles and an Anti-Müllerian Hormone (AMH) of 0.1 ng/ml. Hormonal profile on day 3 (D3): Estradiol (E2) 65 pg/ml; Follicle Stimulating Hormone (FSH) 7.20 mIU/ml; Luteinizing Hormone (LH) 7.12 mIU/ml. The doctor requested several tests for the couple, including karyotype analysis (normal) and *FRM1* gene mutations.

The couple underwent 2 inseminations (unsuccessful) and 1 IVF cycle. During the IVF, the woman had a poor response, only 2 eggs were obtained, to whom Intracytoplasmic Sperm Injection (ICSI) was performed. Conception did occur, but development stopped after Day 1 (D1).

Afterward, the doctor explained the possible paths forward, including ovarian rejuvenation. The couple decided to proceed with the treatment.

A month after the ovarian injection of PRP and stem cells, the AMH was 0.8 ng/ml and the couple came for an insemination (unsuccessful). 2 months after treatment, they came for a second insemination, and the woman became pregnant.

The pregnancy was normal and without any complications. A healthy baby boy was born at 38 weeks.

Case 2

Married 36-year-old woman and 39-year-old male, with primary sterility, come for a consultation after 2 years of attempting to conceive. The woman has celiac disease, hypothyroidism, and primary dysmenorrhea.

The male studies come out normal. For the female, an AMH of 0.29 ng/ml was found. Hormonal profile on day 3 (D 3): E2 59 pg/ml; FSH 4.82 mIU/ml; LH 3.10 mIU/ml. The couple decided to do 4 inseminations, not successful. They tried IVF, but the cycle was cancelled due to poor response.

The doctor explained the possible paths forward, including ovarian rejuvenation. The couple decided to proceed with ovarian rejuvenation. A month after the treatment, AMH value was 0.77 ng/ml. Two months after the treatment AMH was 0.9 ng/ml. A couple of weeks later, the couple informed the clinic that they achieved pregnancy spontaneously. The pregnancy was normal and without any complications. A healthy baby boy was born.

Case 3

Married 39-year-old woman and 43-year-old male, with primary sterility, come for a consultation after 2 years of trying to conceive. The doctor requested several tests for the couple, including genetic testing: Karyotype analysis and cystic fibrosis (normal). The male has severe oligoasthenoteratozoospermia, bilateral varicocele and admits to consuming recreational marihuana regularly. The woman has primary dysmenorrhea and an AMH of 0.5 ng/ml. Hormonal profile on day 3 (D 3): E2 33 pg/ml; FSH 12.1 mIU/ml; LH 4.88 mIU/ml. The couple did IVF-ICSI, only 3 ovocytes were yielded, one did not fertilize and the other two were transferred on D3 (no pregnancy). The doctor explained the possible paths forward, including using donors and ovarian rejuvenation. The couple decided to proceed with ovarian rejuvenation.

A month after treatment, the AMH was still 0.5 ng/ml. Three months after the ovarian rejuvenation, the couple did IVF-ICSI, with a poor response. Three oocytes were obtained. One of the embryos was arrested after D1. Two D3 embryos were transferred (the patient did not achieve pregnancy).

Eight months later, the couple decided to do an IVF cycle with donor oocytes, they did not accept a sperm donor. The cycle was performed. The fertilization rate was 75%, and two embryos survived to D5. The embryo transfer has not yet been accomplished because of high progesterone levels (Table 1).

Table 1: Results of ovarian rejuvenation therapy using stem cells.

AMH (ng/ml)	Hormonal profile on day 3			AMH values after treatment (ng/ml)
	E2 (pg/ml)	FSH (mIU/ml)	LH (mIU/ml)	
0.1	65	7.2	7.12	0.8
0.29	59	4.82	3.1	0.77
0.5	33	12.1	4.88	0.5

Note: AMH: Anti-Müllerian Hormone; E2: Estradiol; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone.

Prior to the intervention, clinical and paraclinical evaluation, including a complete blood count and coagulation profile, was conducted following informed consent.

PRP preparation method

To perform a PRP, 8 citrate tubes containing 9.5 ml of patient's peripheral blood were collected. The samples were centrifuged. It was done in 4 steps.

Step 1: First centrifugation of 20 min at 830 g

Step 2: PRP aspiration

Step 3: Second centrifugation of 10 min at 830 g

Step 4: PRP aspiration, to get 5 ml of PRP. No platelet activator is added to the sample

In our experience, commercial PRP kits have failed to yield an adequate quantity of platelets, as measured by a hematology counter.

Stem cells preparation method

In prone position, bone marrow was extracted from the Posterior Superior Iliac Spine (PSIS). Subsequently, work is carried out in a laminar flow chamber, preparing the material for centrifugation to obtain Bone Marrow Aspirate Concentrate (BMAC). BMAC is a biological preparation with a high concentration of mesenchymal stem cells. The volume of BMAC containing bio factors and mesenchymal stem cells was delivered in sterile syringes to the gynecological/obstetric team.

Intraovarian injection

The procedure was performed in surgical block with the patient sedated and pain control through IV access by an anesthesiologist. The procedure was performed by a gynecologist who specialized in assisted reproduction, with more than 10 years of experience in our IVF center. Initially, autologous platelet-rich plasma is injected, followed by the injection of stem cells.

RESULTS AND DISCUSSION

In our study, we analysed three cases involving women in their 30s who sought fertility assistance at our center to achieve pregnancy.

In case 1, the couple's primary infertility was attributed to the female partner's extremely low Anti-Müllerian Hormone (AMH) levels, indicating diminished ovarian reserve. Despite initial poor response to IVF treatment, which resulted in only two eggs retrieved and subsequent failed embryo development, the couple achieved successful pregnancy following ovarian rejuvenation. This suggests a positive outcome associated with the rejuvenation procedure, as evidenced by the subsequent successful insemination and pregnancy without complications. This case highlights the potential of ovarian rejuvenation in improving fertility outcomes, particularly in cases of diminished ovarian reserve.

Case 2 presents a different scenario, with the female partner also experiencing decreased ovarian reserve as indicated by her AMH levels. However, the couple faced additional challenges, including the female partner's comorbidities such as celiac disease and hypothyroidism. Despite failed insemination attempts and the cancellation of an IVF cycle due to poor response, the couple opted for ovarian rejuvenation, leading to a significant increase in the female partner's AMH levels. Remarkably, the couple achieved spontaneous pregnancy shortly after the treatment, suggesting a potential link between ovarian rejuvenation and improved fertility outcomes, even in cases complicated by underlying health conditions.

Contrastingly, case 3 presents a more challenging scenario characterized by both male and female factors contributing to infertility. While the female partner had diminished ovarian reserve, the male partner exhibited severe oligoasthenoteratozoospermia and bilateral varicocele, aggravated by recreational marijuana use. Despite undergoing ovarian rejuvenation, the female partner's AMH levels remained unchanged, and subsequent IVF cycles yielded poor outcomes, including failed embryo development and unsuccessful pregnancy following embryo transfer. Ultimately, the couple resorted to using donor oocytes but faced further setbacks with failed embryo development, highlighting the complexities and limitations of treatment options in cases of severe male and female infertility.

In conclusion, the comparison of these three cases underscores the variability in treatment outcomes and challenges encountered in managing infertility, particularly when employing ovarian rejuvenation techniques. While cases 1 and 2 demonstrate potential results with improved fertility outcomes following rejuvenation, case 3 highlights the complexity and limitations of such interventions in cases of severe male factor infertility. Further research is needed to elucidate the mechanisms underlying ovarian rejuvenation and its efficacy in improving fertility outcomes across different patient populations. Additionally, comprehensive evaluation and personalized

treatment strategies are essential in optimizing outcomes for couples facing infertility challenges.

CONCLUSION

The primary objective of ovarian rejuvenation treatment is to enhance the ovarian reserve and facilitate the retrieval of sufficient oocytes for IVF in women with severely diminished ovarian reserve. Data presented herein suggest that autologous intraovarian PRP infusion plus stem cells may restore ovarian function, enabling the reactivation of the folliculogenesis process and the enhancement of the hormonal profile. This may enable the achievement of pregnancy. However, the evidence for the clinical application is novel and has not yet been sufficiently elucidated. The effects of this treatment cannot be properly assessed on clinical, chemical, and live birth rates, however, the occurrence of spontaneous pregnancy, clinical pregnancy and live birth reflect a significant change. The effects of a PRP plus stem cells are linked to its high regenerative and anti-inflammatory properties. Longer follow-up may detect a functional recovery with improvement of the ovarian reserve hormone markers.

CONFLICTS OF INTEREST

All authors report no relationships that could be construed as a conflict of interest.

CONSENT TO PARTICIPATE

Written informed consent was obtained from the patients to participate in this case series.

CONSENT TO PUBLICATION

Written informed consent was obtained from the patients for the publication of this case series.

AVAILABILITY OF DATA AND MATERIAL

Raw data were generated at Center for Human Reproduction of the Interior, Uruguay. Derived data supporting the findings of this study are available from the corresponding author V.A on request.

AUTHOR'S CONTRIBUTION

Conceptualization: Victoria Alzogaray; Formal analysis, investigation and methodology: Victoria Alzogaray1, Ana Orihuela, Milton Mazza, Rita Vernocchi, Martha Guidobono, Juan Rodriguez Buzzi, and Wanda Novick; supervision: Ana Orihuela; Validation: Victoria Alzogaray, Ana Orihuela; writing-original draft: Victoria Alzogaray; writing-review and editing: Ana Orihuela

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