Live Birth After an Autologous Platelet-Rich Plasma Ovarian In Vitro Activation and Bone Marrow Stem Cells Transplantation in a Premature Ovarian Failure Case Report

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Case report

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Abstract

Background: Patients with premature ovarian failure (POF) exhibit a diminished ovarian reserve and hormonal dysfunction.

Case presentation: We aimed to restore normal hormonal function and folliculogenesis in a 31-year-old patient with POF, who had been amenorrheic for two years. We designed and performed three different ovarian regeneration procedures for three consecutive years, from 2015 to 2017: 1) intraovarian injection of activated autologous platelet-rich plasma (PRP); 2) activated autologous PRP and bone marrow-derived stem cell injection into the ovaries (SEGO); 3) ovarian cortical tissue resection and fragmentation; and *in vitro* ovarian tissue activation with autologous PRP and bone marrow stem cell retransplantation into the ovaries (the SEGOVA). The patient exhibited no improvement following the PRP treatment. The patient regained regular menstrual cycles after the SEGO procedure, although no follicular growth was observed yet. One month after the SEGOVA procedure, follicular growth was detected, and the patient underwent several stimulation protocols without obtaining oocytes. Eight months after the SEGOVA, the patient underwent *in vitro* fertilization (IVF) in a spontaneous cycle, when an oocyte of good quality was retrieved. Following intracytoplasmic sperm injection (ICSI), the oocyte failed to be fertilized. Eleven months after the SEGOVA, the patient reported a spontaneous pregnancy via natural conception. Pregnancy resulted in birth at term by uncomplicated vaginal delivery. After three different ovarian rejuvenation procedures, normal hormonal function and follicular growth were restored in the patient with POF, and the patient had a successful natural pregnancy following the last SEGOVA procedure. Although no ovarian function was detected after the first two procedures, they may have contributed to the outcomes from the SEGOVA procedure, a treatment that showed promising results in recovering ovarian function in patients with POF.

Conclusions: It cannot be ruled out that ovarian rejuvenation with bone marrow-derived stem cells and autologous growth factors, together with ovarian tissue fragmentation, took time to exhibit its effects and contributed to the final result – a successful natural conception and pregnancy.

Background

The principal belief in reproductive medicine of women having a finite ovarian reserve has been challenged recently by the discovery of postnatal new oocyte formation under specific conditions. A population of cells with stem cell-like characteristics and proliferative ability, which act as oocyte precursors, were identified in the ovaries of some species, including humans [1]. Although the presence of oogonial stem cells and somatic ovarian stem cells has been confirmed, along with evidence supporting follicle renewal during the reproductive lifespan, ovarian failure remains only partly treated due to a loss of the supporting ovarian tissue that communicates with oocytes during maturation, the cessation of steroid biosynthesis, and a disruption of the ovarian-hypothalamus endocrine feedback [2]. Thus, strategies to improve fertility and delay or prevent endocrine-related symptoms associated with ovarian failure require renewal and regeneration of not only of oocytes but multiple ovarian cell types [3].

Patients with premature ovarian failure (POF) exhibit a limited or poor ovarian reserve. The emerging technique used for activation of the ovarian tissue and induction of follicles in premature stages, regardless of the influence of gonadotropins, is ovarian fragmentation [4]. Early studies showed that patients who underwent ovarian wedge resection [5] or laparoscopic ovarian laser drilling [6] showed follicle growth and activation of the dormant follicles. Recently, a new method for the activation of dormant follicles was developed using *in vitro* culture of ovarian cortical fragments treated with primordial follicle activators [7]. During the process of *in vitro* activation (IVA), the ovarian cortex is routinely fragmented into small cubes (1—2 mm), leading to the disruption of the Hippo signaling pathway, allowing secondary follicle growth, and subsequently incubated *in vitro* in a culture of ovarian fragments treated with Phosphoinositide 3-kinase (PI3K) stimulators and Phosphatase and tensin homolog (PTEN) inhibitors that act as primordial follicle activators [7, 8]. Successful pregnancies and deliveries with POF and premature ovarian insufficiency (POI) have been reported after applying *in vitro* activation and retransplantation of the ovarian cortex [8–11].

Besides the sufficient follicle numbers, the interplay of molecular signaling events between germ cells and somatic cells appears to be necessary to induce “ovarian rescue” or “re-potentiation”. Maintaining this network is a complex task, and there is no consensus on a definitive therapeutic approach to this problem [12]. In the past few years, intraovarian growth factors were identified to play an important role in the local regulation and modulation of follicular selection and development through paracrine mechanisms [13]. An application of autologous sources of growth factors from activated platelets is one of the expanding techniques for ovarian tissue regeneration, since it is known that growth factors can supply the requisite signal(s) needed for oocyte development. There are several recent reports of *in vitro* fertilization (IVF) pregnancies in patients with a poor prognosis after intraovarian injections of autologous platelet-rich plasma (PRP), followed by IVF, one of which was reported by our team [14–18].

Another cutting-edge technique recently developed to provide a source of paracrine factors that could contribute to ovarian regeneration is the intraovarian infusion of bone marrow mesenchymal stem cells (BMSCs). BMSCs are known to promote neo-vascularization, reduce granulosa cell apoptosis, and provide a better environment for physiological oocyte maturation [19]. Injections of isolated BMSCs directly into the ovarian tissue or via an artery have been used extensively in the successful restoration of ovarian function in animal models of ovarian failure; however, there are currently very few studies on humans [20, 21]. To the best of our knowledge, there have been only three case reports of autologous BMSCs applied in the clinical treatment of patients with ovarian failure, which resulted in successful pregnancies [22–24]. Both stem cell infusion and PRP treatment allow a partial reversion of aging of the ovary, by providing a better environment for follicle development, while ovarian cortex fragmentation induces folliculogenesis and *in vitro* activation enables the primary follicles to activate and mature, which would otherwise remain dormant in the ovary [7, 23].

These are emerging treatment options for women with ovarian failure desiring pregnancy. The integration of these biotechnological regenerative processes into one procedure in human applications has yet to be attempted. Based on the above studies, we designed and evaluated an approach integrating stem cell

treatment, growth factor incubation, and ovarian *in vitro* activation (SEGOVA). An application has been filed to patent SEGOVA (application number P–2020/1329). This manuscript reports the first successful pregnancy in a patient with POF, after a SEGOVA procedure.

Case Presentation

The patient was a 31-year-old woman who first visited our fertility center (Special Hospital Jevremova, Medigroup, Belgrade) in November 2015, seeking fertility treatment. She was transferred from another fertility clinic (Ars Medica, Montenegro), where she had been a patient since March 2015. She had menarche at 14 years of age. Her body mass index (BMI) was 24. POF diagnosis was made at the age of 29, with hormone levels as follows: Follicle-stimulating hormone (FSH), 44.12 mIU/mL; Luteinizing hormone (LH), 22.9 mIU/mL; Estradiol (E2) < 5 pg/mL; Progesterone (PG), 0.502 ng/mL; and Anti-Müllerian hormone (AMH), 0.1 ng/mL. She had been amenorrheic for two years prior to being referred to our clinic. Her medical history included no systemic disease. She had no known allergies or history of sexually transmitted infections. Genetic analysis showed that she was a carrier of the homozygous C677T mutation (c.665CT, A222V, rs 1801133) in the *MTHFR* gene. Additionally, she was found to have the 4G/5G heterozygous mutant genotype in the *PAI–1* gene.

In March 2015, she started a six-month therapy with Cyclo-Progynova (0.5 mg/2 mg; 2 mg, norgestrel/estradiolvalerat, BAYER WEIMAR GMBH & CO.KG). From March to November 2015, the patient had irregular menstrual cycles and had follicles up to 12 mm in diameter. In November 2015, Estrofem (2 mg estradiol, Novo Nordisk, Denmark) was introduced as an additional therapy. After the initial examination at our clinic, the patient was advised to undergo an ovarian rejuvenation method before IVFcould be attempted. From November 2015 to February 2017, the patient received three different procedures of ovarian rejuvenation: 1) autologous PRP; 2)SEGO; 3)SEGOVA.

Methods

Procedures:

Autologous platelet-rich plasma (PRP) treatment of ovarian tissues

PRP ovarian rejuvenation was performed on the 23rd of November 2015. The FSH, E2, and LH levels before the procedure were 4.99 mIU/mL, 421.7 pg/mL, and 10.26 mIU/mL, respectively. Briefly, 120 mL of whole blood were withdrawn from the cubital vein and anticoagulated using acid citrate dextrose formula A (ACD-a) in a 7:1 ratio. Whole blood was centrifuged using a tabletop blood separation system, and 7 mL of PRP were obtained. After centrifugation, the concentration of platelets was 6.5 × baseline with leucocytes 0.6 × baseline. PRP was activated using autologous thrombin at a ratio of 10:1. After the activation, the volume of PRP (6 mL) was instilled into the ovaries via transvaginal, ultrasound-guided injection using a 17G needle under total anesthesia, 2 mL to the left, and 4 mL to the right ovary. After needle priming, the obtained volume of PRP was injected as multiple subcortical injections, in volumes

between 0.5 to 1 mL in five positions per ovary. The patient suffered no blood loss during the procedure, recovered and was discharged from the hospital the next day.

The patient had irregular menstrual cycles following the procedure, and no follicular growth was observed in monthly ultrasound examinations during the next six months. She continued treatment with Cyclo-Progynova (0.5 mg/2 mg; 2 mg, norgestrel/estradiolvalerat, BAYER WEIMAR GMBH & CO.KG) and Estrofem (2 mg estradiol, Novo Nordisk, Denmark) during that period. At that time, the patient decided to undergo a SEGO procedure, which is an upgraded rejuvenation method compared to PRP rejuvenation.

SEGO ovarian rejuvenation: autologous platelet-rich plasma (PRP) and autologous bone marrow stem cell ovarian injection

In June 2016, the patient had FSH, E2, LH, and AMH levels of 11.2 mIU/mL, 391 pg/mL, 2.9 mIU/mL, and < 0.2 ng/mL, respectively. The SEGO procedure was performed on 13th July 2016. PRP preparation was performed as described in section 3.1. A total of 5 mL of activated PRP were obtained for the treatment. Bone marrow (BM) sampling from the tibial bone was performed under general anesthesia, and a small incision (7 mm) was made to penetrate the periosteum. A total of 80 mL of bone marrow was aspirated and centrifuged under sterile conditions, and three layers were obtained: the acellular portion (platelet-poor plasma, PPP), the cells (RBC), and the bone marrow aspirate concentrate (BMAC) containing nucleated cells. The obtained BMAC (3 mL) was diluted to 4 mL with PPP and further used for treatment. Afterwards the procedure, flow cytometry was used to determine the total nucleated cell count (TNC), which was 36.7×10^6 cells/mL, and the cell viability was 96%.

The prepared PRP and BMAC were instilled together into the ovaries via transvaginal, ultrasound-guided injection using a syringe system and a 17G needle, which was performed under total anesthesia. After needle priming, the obtained volume of 3 mL of PRP and 2.5 mL of BMAC were injected into the right ovary, while 2 mL of PRP and 1 mL of BMAC were injected into the left ovary, as multiple subcortical injections, in volumes between 0.5 to 1 mL in five positions per ovary. The patient suffered no blood loss during the procedure, and she recovered and was discharged from the hospital the next day.

One month after the procedure, the patient underwent the first control examination and reported no adverse effects or complications. Ultrasound control performed on 15th August 2016, reported normal menstrual cycling (28/3 days), the endometrium in the periovulatory phase, and an endometrial thickness of 8 mm on the 11th day of the menstrual cycle. Several follicles smaller than 4 mm were observed on the left ovary. The patient continued to receive Cyclo-Progynova (0.5 mg/2 mg; 2 mg, norgestrel/estradiolvalerat, BAYER WEIMAR GMBH & CO.KG), and Estrofem (2 mg estradiol, Novo Nordisk, Denmark) therapy during the next six months. Until January 2017, the patient had regular menstrual cycles with follicles growing up to 10 mm in diameter. In February 2017, the patient was suggested to undergo an advanced intervention of ovarian rejuvenation called SEGOVA, which will be described in the next section.

SEGOVA ovarian rejuvenation: ovarian cortical tissue resection, *in vitro* tissue activation with autologous PRP and BMAC transplantation to the ovaries

PRP preparation

In February 2017, a 33-year-old patient had the following hormonal levels before the procedure: FSH, 4.23 mIU/mL; E2, 18.17 pg/mL; LH, 1.08 mIU/mL; and, Beta Human Chorionic Gonadotropin hormone (Beta-hCG) 0.1 mIU/mL. On 10th February 2017, the patient underwent SEGOVA procedure. Before laparoscopy, PRP preparation was performed. Briefly, whole blood was withdrawn from the cubital vein and was anticoagulated using ACD in a 7:1 ratio. Two 60 mL syringes were used to obtain a total volume of 104 mL of whole blood and 16 mL of ACD-a. Next, the blood was centrifuged using a tabletop blood separation system. After separation, the layers were composed of approximately 53 mL of RBC, 4 mL of PRP, and 47 mL of PPP. Next, platelet count was determined and the PRP was diluted up to 5 mL using PPP. Autologous thrombin was used for activation. Activation was performed in order to release growth factors from the platelets. PRP was activated using autologous thrombin at a ratio of 10:1. Activated PRP (5 mL) was further used for tissue treatment.

Tissue preparation

Laparoscopic resection of ovarian cortex was then performed using a standard procedure, using a laparoscopic technique with an entry through the umbilicus. After making an incision of approximately 2 cm in the umbilical zone, three portals of 5 mm were placed and intra-abdominal pressure between 10 and 12 mmHg was established. A laparoscope of 5 mm in diameter was then introduced, together with auxiliary trocars. After visualization of the ovaries using an adequate instrument, the cortex was fixed, and scissors were used to cut off a part of the cortical tissue. The cortical tissue measured was 254 mg. Hemostasis, port performance, and wound closure were checked. During the further course of the process, the ovary cortex obtained via multiple cutting with scalpel No. 25 was cut into fragments smaller than 1 × 1 mm^2. The tissue prepared in this manner was measured using an analytical scale and placed on a Petri dish and washed using the gammet buffer (Sydney IVF gamete buffer, Cook Medical, USA). After rinsing, the tissue samples were transferred to PRP media and continued to be activated via autologous thrombin mediation. The prepared sample was incubated with the volume of activated PRP (5 mL) for 48 h at 37°C and 5.5% CO_2.

Bone marrow sampling

After 48 h, bone marrow sampling from the proximal tibia was used to obtain stem cells. Biopsy was performed under general anesthesia, and a small cut (7 mm) was used to penetrate the periosteum. Bone marrow (95 mL) was aspirated and centrifuged using a specially automated system under sterile conditions. After separation, the acellular portion and the RBCs were discarded, while 4 mL of BMAC with

nucleated cells were further used for the treatment. In addition, 1 mL of the BM and BMAC sample was used to evaluate cell viability, the TNC, and to perform flow cytometry phenotypic characterization of stem cells from BMAC using a set of specific markers: (CD73, CD90, CD105, CD133, CD 271). Figure 1. presents analyzed CD markers before and after BM concentration. For BMAC the determined TNC was 27 × 10^6 cells/mL, and the viability was 98.7%.

Retransplantation

After BMAC was obtained and ovarian cortex tissue was incubated for 48 h after the the fragmented tissue of the ovary with 5 mL of PRP was injected into the subcortical region of the right and left ovaries, together with 4 mL of BMAC, under the ultrasonic monitoring of 3D color ultrasound GE Voluson 730 Pro, via a transvaginal puncture under general anesthesia using a 16G needle. Injection of PRP, tissue fragments, and BMAC in each ovary was performed as multiple subcortical injections, in volumes between 0.5 and 1 mL in five positions per ovary. The procedure did not involve any complications, and there was no blood loss. The patient was discharged from the hospital the day after the procedure.

All procedures performed in this study were approved by the Institutional Board of Ethics at Special Gynecology Hospital Jevremova, Medigroup, Belgrade, Serbia (IRB No 63/295/2015) and in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. An informed consent was obtained from the participant included in the study.

Monitoring

One month after the procedure, on 9th March 2017, follicular growth was observed and the patient received a short protocol of stimulation with Menopur (menotrofin 75 IU, Ferring GMBH) and Ovitrelle (250 mcg/0.5 mL injection, Merck). On the 15th day of the menstrual cycle, (16th March 2017), the E2 level was 102 pg/mL, the LH level was 31.65 mIU/mL, and the follicles were punctured without retrieving any eggs. In May 2017, the cycle was stimulated with Merional (menotrofin 75 IU, IBSA Institut Biochimique S. A.) and the patient received Klomifen (Clomiphene 50 mg, REMEDICA Ltd., Cyprus). At the end of the stimulation protocol, the E2 level was 65.84 pg/mL and no follicle growth was detected. During June 2017, the patient received a stimulation protocol with Pergoveris (150 IU/75 IU powder and a solvent for injection of Follitropin alfa/Lutropin alfa, Merck Serono Gmbh, but with no follicular growth, and the E2 level was 53.73 pg/mL at the end of the stimulation. In September 2017, the patient had regular menstrual cycles under Cyclo-Progynova therapy (0.5 mg/2 mg; 2 mg, norgestrel/estradiolvalerat, BAYER WEIMAR GMBH & CO.KG) and Estrofem (2 mg estradiol, Novo Nordisk, Denmark). Natural IVF was planned, instead of a stimulated IVF cycle. On the 6th October 2017 (eight months after the procedure), on the 10th day of the menstrual cycle, the endometrial thickness was 7 mm when follicle growth was detected. The patient presented with a 14-mm diameter follicle on the right ovary. On the next day, (7th October 2017), several larger follicles were aspirated, and one Metaphase II (MII) oocyte was obtained.

The hormonal levels were as follows: LH, 49.42 mIU/mL; E2, 114.8 pg/mL; and PG, 0.864 ng/mL. Intracytoplasmic sperm injection (ICSI) was performed, but total fertilization failure occurred. After that, the patient did not undergo any further treatments.

Three months later, in January 2018, the patient reported to be in the 11^{th} week of spontaneous pregnancy. The patient also reported that she was not on therapy at the time of conception. The pregnancy went to term without complications. Birth occurred spontaneously via uncomplicated vaginal delivery, and the Apgar score of the newborn was 9/10.

Discussion And Conclusions

It is known that a decrease in the number of primordial follicles is a result of ovarian failure and is associated with cellular and molecular damage of the ovarian tissue due to age or pathogenetic factors. Follicle development in the early preantral phase is gonadotropin-independent. Folliculogenesis during this period can be modulated by intraovarian growth factors through autocrine/paracrine mechanisms [17]. In addition, there are several reports that indicate a potential change in folliculogenesis conditions by using physical methods, autologous PRP growth factor treatments, and stem cell infusions [7, 8, 14–24].

This study is the first report of an integrated approach called SEGOVA, in which autologous platelet/leucocyte-rich plasma activation of fragmented ovarian tissue was performed, followed by orthotopic retransplantation and injection of autologous bone marrow-derived stem cells under sonographic guidance into both ovaries. Eleven months after the procedure, the patient reported a spontaneous pregnancy that resulted in birth. To the best of our knowledge, this is the first report of a pregnancy and live birth in a patient with POF with the heterozygous mutation *PAI–1* 5G/4G and the *MTHFR* C677T genotype, following autologous PRP growth factor treatment, ovarian activation, and stem cell therapy.

The molecular mechanism by which these three methods exhibit their action is still not fully elucidated. The mechanobiological approach has shown that fragmentation of the ovarian cortex to small pieces disrupts the ovarian Hippo signaling pathway and facilitates follicle growth in infertile patients when cortical fragments are grafted back into the patients [7–10]. Furthermore, fragmentation of the ovarian cortex could be enhanced by incubation with growth factors from autologous sources to stimulate follicle growth and minimize apoptosis and ovarian reserve damage [16, 17]. The use of activated platelet products for ovarian tissue regeneration has emerged from the idea that growth factors might supply the requisite signal(s) needed for oocyte development and maturation. These findings have unraveled a novel field of fertility preservation strategies for women with both age-related and POI, who, so far, only had the option either to spontaneously conceive or to use donor oocytes for IVF [25]. Research efforts during the past decade have led to the identification of a novel population of pluripotent stem cells in ovaries, called very small embryonic-like stem cells [26]. These cells are developmentally equivalent to late migratory primordial germ cell-like cells. They are in a default quiescent state but can spontaneously differentiate into gametes and can be targeted to regenerate damaged and non-functional gonads [26]. It has been

suggested that the ovarian stem-cell niche is disrupted with age. Studies in mice showed that transplantation of the non-functional ovarian tissue of aged mice into the ovaries of a young host reactivated the aged tissue [27]. This study indicated that a healthy young niche is crucial for stem cell function and oocyte development. In order to provide sources of growth factors that would activate dormant stem cells in the ovaries, transplantation of bone marrow-derived mesenchymal stem cells has been suggested. Recently, several groups have reported the use of mesenchymal stem cell treatment for ovarian failure. This type of cell is being used clinically for degenerative and immune-related diseases, and can be obtained from several sources, including the bone marrow, adipose tissue, umbilical cord, umbilical cord blood, placenta, amniotic fluid, endometrium, and menstrual blood. There are studies that used *in vitro* expanded BMSCs for ovarian regeneration treatment and others that used fresh cells for transplantation [22, 24]. Our group has chosen the second approach, with minimal manipulation, using freshly obtained BMSCs for retransplantation to avoid a potential loss of pluripotency after *in vitro* expansion. It was shown that BMSCs could stimulate neo-oogenesis *in situ* by inducing the formation of new blood vessels and the population of hormone-secreting cells, by reducing granulosa cell apoptosis and follicular atresia via upregulation of AMH and FSH receptor expression in granulosa cells and providing a better environment for physiological oocyte maturation [19, 21]. This type of treatment could be used in patients with POF who underwent chemotherapy for malignant disease therapy, as well as in perimenopausal women. The additional benefit of using this procedure in aging women would be alleviating menopausal symptoms and improving the general health associated with menopause [1]. Case reports of autologous BMSCs applied for clinical treatment of patients with ovarian failure showed a decline in FSH, increased estrogen and AMH levels, and recovery of menstruation. Pregnancies with live births were reported in one patient with POF and one perimenopausal woman after autologous BMSC transplantation [22–24]. The cases of autologous BMSC application for the treatment of patients with idiopathic POF showed that two cases (20%) recovered menstruation 3 months after transplantation, and one of them (10%) was pregnant and had a successful delivery [22]. Another study showed an increase in estrogen and AMH in 86.7% of the 30 total number of patients with POF, one month after autologous BMSC transplantation, and this change was present throughout the 48-week follow-up period [24]. The third study reported follicular presence and oocyte recruitment 8 weeks after BMSC transplantation into a 45-year-old perimenopausal woman, as well as the subsequent fertilization, viable gestation, and delivery [23]. Similar to these findings, our study also demonstrated an increase in the estrogen levels and the presence of follicular growth one month after the SEGOVA procedure, and the restoration of the regular menstrual cycle after the SEGO procedure in a patient who had been amenorrheic for two years. This trend continued for one year after the procedure. The report by Gabr *et al.* [24] demonstrated that one patient with POF had a spontaneous pregnancy, while three patients were subjected to IVF cycles and became pregnant after BMSC treatment. Our study showed that our patient achieved a spontaneous full-term gestation 11 months after the procedure. It should be noted that the same patient previously received a separate PRP rejuvenation without achieving a reproductive outcome and endocrine restoration, while a PRP and BMSC ovarian rejuvenation combination treatment resulted in the restoration of a normal menstrual cycle. When these technologies were applied together with ovarian *in vitro* activation in the SEGOVA procedure, ovarian rejuvenation was achieved, resulting in follicular growth,

spontaneous menstrual cycles, and pregnancy. This indicates the synergistic effects of these three procedures and emphasizes the uniqueness of the integrated approach under the SEGOVA procedure.

Although the application of PRP and stem cells represents a new technology in fertility preservation and is still in the early stages, its use is now entering a new chapter in clinical application, with more research efforts directed toward it. Using autologous PRP application and autologous stem cell-based strategies eliminates the need for donor tissue or tissue banking. This case report demonstrates the efficacy of ovarian transplants in recovering follicular growth and restoring hormone production by combining these three bioengineering approaches. The possibility that the effects of former treatments contributed to providing an appropriate environment for a successful spontaneous pregnancy cannot be ruled out. Ovarian rejuvenation of POF with bone marrow-derived stem cells and autologous growth factors, together with ovarian tissue activation, shows promising results for ovarian function recovery in patients with POF. The SEGOVA approach offers a new perspective and possible alternative for women of age who seek to extend their fertility, as well as for women without any ovarian function, who, until now, could only rely on oocyte or embryo donation or adoption.

List Of Abbreviations

Premature ovarian failure (POF), platelet-rich plasma (PRP), stem cell treatment and growth factor ovarian rejuvenation (SEGO), stem cell treatment, growth factor incubation, and ovarian *in vitro* activation (SEGOVA)*, in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), *in vitro* activation (IVA), Phosphoinositide 3-kinase (PI3K), Phosphatase and tensin homolog (PTEN), premature ovarian insufficiency (POI), , bone marrow mesenchymal stem cells (BMSCs), body mass index (BMI), Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Estradiol (E2); Progesterone (PG), Anti-Müllerian hormone (AMH), acid citrate dextrose formula A (ACD-a), red blood cells (RBC), Bone marrow (BM) platelet-poor plasma (PPP), bone marrow aspirate concentrate (BMAC), total nucleated cell count (TNC), Cluster of differentiation 90 (CD90), Cluster of differentiation 105 (CD105), Cluster of differentiation 73 (CD 73), Cluster of differentiation 133 (CD 133), Cluster of differentiation 271 (CD 271), Metaphase II oocyte (MII),

Declarations

Ethics approval and consent to participate:

All procedures performed in this study were approved by the institutional Board of Ethics at Special Gynecology Hospital Jevremova, Medigroup, Belgrade, Serbia (IRB No 63/295/2015) and in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. An informed consent was obtained from the participant included in the study.

Consent for publication:

Available upon request.

Availability of data and materials:

All relevant experimental data, may be available upon request from the authors to any researchers who wish to use them for non-commercial purposes, while preserving any necessary confidentiality and anonymity.

Competing interests:

The authors declare no conflict of interest.

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Authors' contributions:

A.Lj., T.B. and Dž.A conceived and planned the experiments. D.P. and A.P. carried out the experiments. D.P. and DŠ contributed to sample preparation. A.P.C., and E.Dj. contributed to the interpretation of the results. A.Lj., Dž.A and A.P.C took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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References

1. Dunlop CE, Telfer EE, Anderson RA. Ovarian stem cells-Potential roles in infertility treatment and fertility preservation. Maturitas. 2013;76:279-283.
2. Truman AM, Tilly JL, Woods DC. Ovarian regeneration: The potential for stem cell contribution in the postnatal ovary to sustained endocrine function. Mol Cell Endocrinol. 2017;445:74–84. doi:10.1016/j.mce.2016.10.012
3. Ljubić A, Abazović D, Draganić VD, Srbinović M, Perović A, et al. Ultrasound and Biologic Therapy in Reproductive and Perinatal Medicine. Donald Sch J Ultrasound obstetrics gynecology. 2020;14(3):214–9.
4. Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. Endocr Rev. 2015;36(1):1-24. doi:10.1210/er.2014-1020
5. Hendriks ML, Ket JCF, Hompes PGA, Homburg R, Lambalk CB. Why does ovarian surgery in PCOS help? Insight into the endocrine implications of ovarian surgery for ovulation induction in polycystic ovary syndrome. Hum Reprod Update. 2007;13(3):249-264.
6. Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. Cochrane Database Syst Rev.

2012;13(6):CD001122. doi: 10.1002/14651858.CD001122.pub4

7. Kawamura K, Cheng Y, Suzuki N, Deguchi M, Sato Y, Takae S, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. Proc Natl Acad Sci U S A. 2013;110(43):17474-17479. doi:10.1073/pnas.1312830110
8. Kawamura K, Kawamura N, Hsueh AJ. Activation of dormant follicles: a new treatment for premature ovarian failure? Curr Opin Obstet Gynecol. 2016;28(3):217-222. doi:10.1097/GCO.0000000000000268
9. Kawashima I and Kawamura K. Disorganization of the germ cell pool leads to primary ovarian insufficiency. Reproduction. 2017;153(6):R205-R213. doi: 10.1530/REP-17-0015. Epub 2017 Mar 13.
10. Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, Morimoto Y, Kawamura K. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. Hum Reprod. 2015;30(3):608-615.
11. Zhai J, Yao G, Dong F, Bu Z, Cheng Y, Sato Y, et al. *In Vitro* Activation of follicles and fresh tissue autotransplantation in primary ovarian insufficiency patients. J Clin Endocrinol Metab. 2016;101(11):4405-4412. doi:10.1210/jc.2016-1589
12. Sills ES and Wood SH. Autologous activated platelet rich plasma injection into adult human ovary tissue: Molecular mechanism, analysis, and discussion of reproductive response. Bioscience Rep. 2019;39(6):BSR20190805; DOI: 10.1042/BSR20190805
13. Emori C and Sugiura K. Role of oocyte-derived paracrine factors in follicular development. Anim Sci J. 2014;85(6):627–633.
14. Sills ES, Rickers NS, Li X, Palermo GD. First data on *in vitro* fertilization and blastocyst formation after intraovarian injection of calcium gluconate-activated autologous platelet rich plasma. Gynecol Endocrinol. 2018:1-5.
15. Sfakianoudis K, Simopoulou M, Nitsos N, Rapani A, Pantou A, Vaxevanoglou T, et al. Case series on platelet-rich plasma revolutionary management of poor responder patients. Gynecol Obstet Invest. 2018;22:1-8.
16. Ljubić A, Abazović Dž, Vučetić D, Ljubić D, Pejović T, Božanović T. Autologous ovarian *in vitro* activation with ultrasound-guided orthotopic re-transplantation. Am J Clin Exp Obstet Gynecol. 2017;4(5):51-57.
17. Farimani M, Heshmati S, Poorolajal J, Bahmanzadeh M. A report on three live births in women with poor ovarian response following intra-ovarian injection of platelet-rich plasma (PRP). Mol Biol Rep. 2019;46:1611. https://doi.org/10.1007/s11033-019-04609-w
18. Pantos K, Nitsos N, Kokkali G, Vaxevanoglou T, Markomichali C, Pantou A, et al. Ovarian rejuvenation and folliculogenesis reactivation in peri-menopausal women after autologous platelet-rich plasma treatment. In: Abstracts, ESHRE 32nd Annual Meeting; 2016. pp. 3-6.
19. He Y, Chen D, Yang L, Hou Q, Ma H, Xu X. The therapeutic potential of bone marrow mesenchymal stem cells in premature ovarian failure. Stem Cell Res Ther. 2018;9(1):263. Published 2018 Oct 4. doi:10.1186/s13287-018-1008-9.

20. Tinjić S, Abazović D, Ljubić D, Vujović S, Vojvodić D, Božanovć T, et al. Ovarian Rejuvenation. Donald Sch J Ultrasound obstetrics gynecology. 2019; 13:64–8.
21. Yoon SY. Mesenchymal stem cells for restoration of ovarian function. Clin Exp Reprod Med. 2019;46(1):1-7. doi:10.5653/cerm.2019.46.1.1.
22. Edessy M, Hosni HN, Shady Y, Waf Y, Bakr S, Kamel M. Autologous stem cells therapy, The first baby of idiopathic premature ovarian failure. Acta Med Int. 2016;3:19-23.
23. Gupta S, Lodha P, Karthick MS, Tandulwadkar SR. Role of autologous bone marrow-derived stem cell therapy for follicular recruitment in premature ovarian insufficiency: Review of literature and a case report of world's first baby with ovarian autologous stem cell therapy in a perimenopausal woman of age 45 year. J Hum Reprod Sci. 2018;11(2):125-130. doi:10.4103/jhrs.JHRS_57_18
24. Gabr H, Elkheir WA, El-Gazzar A. Autologous stem cell transplantation in patients with idiopathic premature ovarian failure. J Tissue Sci Eng. 2016;7(3 Suppl):27.
25. Goswami D, Conway GS. Premature ovarian failure. Horm Res. 2007;68:196-202. doi: 10.1159/000102537
26. Bhartiya D, Patel H, Parte S. Improved understanding of very small embryonic-like stem cells in adult mammalian ovary. Hum Reprod. 2018;33:978–9.
27. Niikura Y, Niikura T, Tilly JL. Aged mouse ovaries possess rare premeiotic germ cells that can generate oocytes following transplantation into a young host environment. Aging (Albany NY) 2009;1:971–8.

Figures

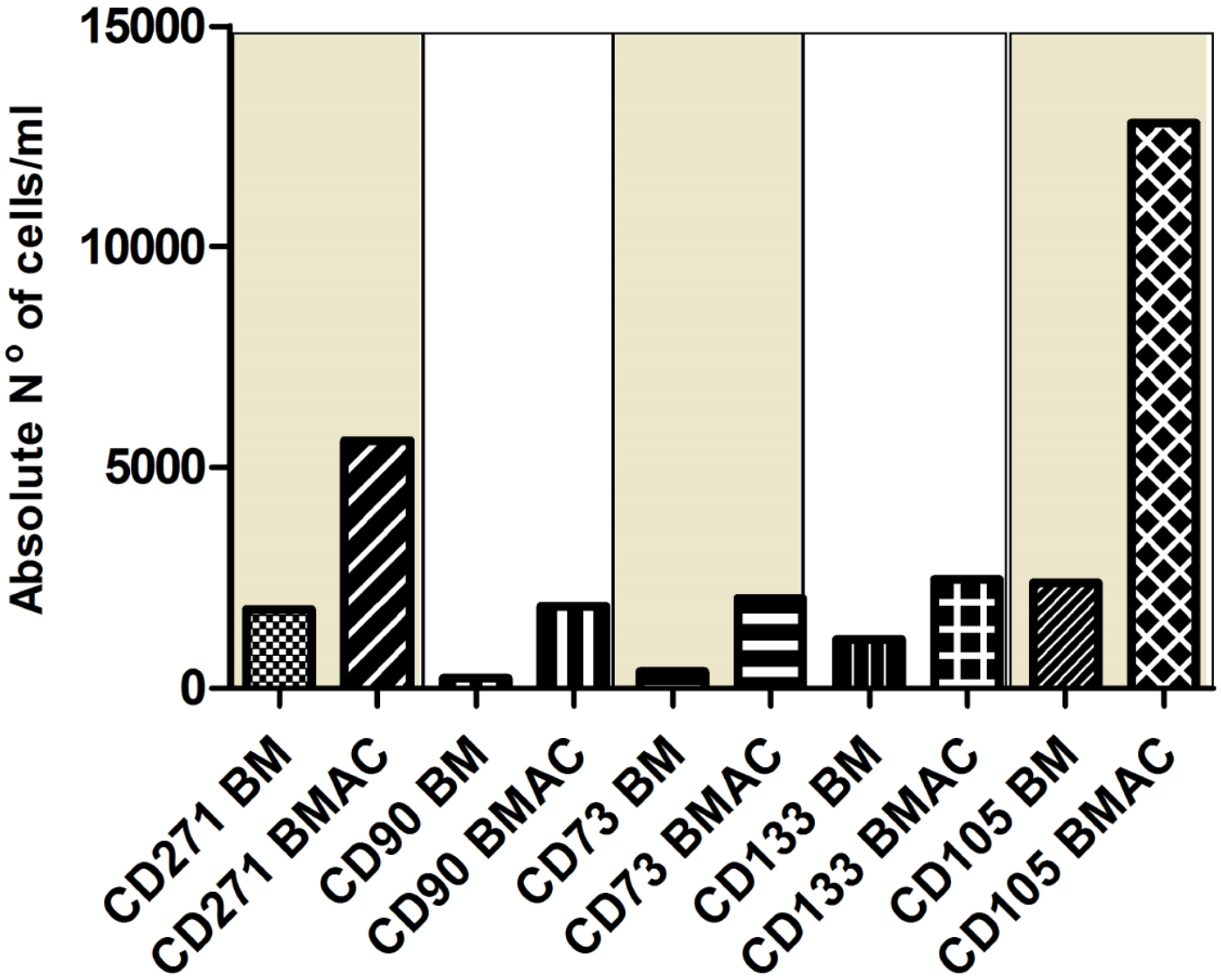

Figure 1

CD marker analyzed before and after BM concentration