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Live Birth Rates in Poor Responders' Group after Previous Treatment with Autologous Platelet-Rich Plasma and Low Dose Ovarian Stimulation Compared with Poor Responders Used Only Low Dose Ovarian Stimulation Before in Vitro Fertilization

Snezhana Stojkovska*, Gligor Dimitrov, Nikoleta Stamenkovska, Makuli Hadzi-Lega, Zoran Petanovski

Department of IVF and Reproductive Medicine, Remedika, Skopje, Republic of Macedonia

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Keywords: Poor ovarian reserves; Platelet-rich plasma; Ovarian rejuvenation; IVF; Live birth rates

***Correspondence:** Snezhana Stojkovska. Department of IVF and Reproductive Medicine, Remedika, Skopje, Republic of Macedonia. E-mail: sstojkovska2000@gmail.com

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Abstract

BACKGROUND: This prospective pilot study determined the efficacy of previous transvaginal intraovarian injection with autologous platelet-rich plasma (PRP) in poor ovarian responders (PORs) fulfilling the Bologna criteria before in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) with low dose ovarian stimulation. Current knowledge of efficient treatment for PORs is limited and often contradictory; also, LBRs of IVF remains disappointingly low.

AIM: We assessed the live birth rates (LBRs) in PORs after previous ovarian treatment with PRP.

METHODS: Overall, 40 patients undergoing IVF/ICSI between June 2017 ending December 2018 were included. A transvaginal intraovarian injection of PRP was performed on 20 patients. Both compered groups were balanced for all basic characteristics, and multivariate analysis was performed to adjust for all known confounders.

RESULTS: Between the groups, a statistical significance in clinical pregnancies and LBR was not found. Clinical pregnancy and live birth rates were 33.33 ± 44.99 and 40.00 ± 50.71 in the PRP group and 10.71 ± 28.95 and 14.29 ± 36.31 in control group retrospectively. However, there is a trend towards higher implantation rates and LBRs in patients with previous treatment with PRP. Anyhow, the number of patients used in the research is insufficient to make a concrete conclusion, and more studies are needed in the future to confirm these results entirely.

CONCLUSION: Even though the treatment of POR responders remains as a therapeutical challenge, the usage of intraovarian injection of autologous PRP in PORs before the IVF performance brings a glimpse of new hope in increasing the success of IVF defined by clinical pregnancy and LBRs.

Introduction

The term poor ovarian responders (PORs) determines a subgroup of in-vitro patients who showcase a decreased response to classical ovary stimulation with gonadotropins, usually as a result of decreased ovary reserves. The IVF procedure results in a decreased number of received oocytes, a decreased clinical pregnancy and LBR [1]. The latest meta-analysis performed by the American Society for Reproductive Medicine (ASRM) in regards to the efficiency of different protocols of ovary stimulation demonstrates an increased cost-benefit with the usage of low dose stimulation using GnRH antagonists [2]. When taking into consideration the need for donating egg cells and different ethical and religious questions that impose themselves, it's clear that there's a need for an additional alternative option that will solve these issues. Therefore, the use of platelet-rich plasma (PRP) is considered as a justified and potentially successful opportunity with which the fertility outcome in PORs may be increased [3], [4].

PRP, as a method in many medical fields, has already demonstrated its beneficial effect on tissue regeneration, angiogenesis activation, inflammation control and anabolism [5]. Unfortunately, there are still insufficient clinical data in the field of ovarian infertility.

PRP has been used for the first time as a medical term in 2007 as: “a preparation consisting of concentrated platelets in a limited plasma volume. It is used in various regeneration procedures of surgical tissues, where growth factors from platelets can affect the speeding up of healing and regeneration of the wounds” [6]. Platelets are cytoplasmic fragments of megakaryocytes, which are formed from the bone marrow and are approximately 2 μm in diameter [7]. Activating the alfa granules from the platelets is one of the most crucial steps which affects the availability of released bioactive molecules, and Ergo, the quality of the PRP. Namely, they contain more than 800 proteins, such as cytokines, hormones, and chemo-attractants of stem cells, macrophages, and neutrophils; these molecules have a fundamental role in the hemostasis and the tissue regeneration [8].

The method of receiving PRP is simple, minimally invasive and at a low cost. The high concentration of factors of growth and cytokines present in the PRP affects the balance between the anabolic and katabolic process, optimising the tissue’s surroundings and favouring the process of tissue regeneration [9].

Material and Methods

Patient selection

In this pilot study, 40 patients were included. A written consent form was received by all patients. Also, all patients were completely informed regarding the case study and the way of stimulation during our research. Patients were divided into two groups, group A and B. The group A consisted of 20 patients, who received a transvaginal intraovarian injection of autologous platelet-rich plasma, before the commencement of an IVF. The choice of this treatment was made after a clear and thorough discussion with the patient/couple. In both groups, there wasn’t a statistically significant difference in regards to age, BMI, hormonal status, number of antral follicles, previous IVF attempts and duration of infertility. Both compered groups were balanced for all basic characteristics, and multivariate analysis was performed to adjust for all known confounders. The timeframe between the application of PRP until ovary stimulation for IVF was 61 ± 18 days.

The study was approved by the local Ethics Committee, and the Institutional Review Board and each patient included in the study signed an informed written consent. The study included PORs who meet at least two of the following three Bologna criteria, published by the European Society of Human Reproduction and Embryology (ESHRE) in 2011 [10]. Only women whose partners’ have normal semen analyses were reviewed [11]. It is important to note that only patients where the IVF process was completed with an embryo transfer were included in the study. The exclusion criteria were ovarian insufficiency due to gonadal dysgenesis and chromosomal abnormalities, immunoglobulin A deficiency, large surgical repairs of pelvic floor leading to the creation of severe pelvic adhesions, the use of anticoagulants, psychotropic medicaments, psychiatric disorders, carcinomas or a history of chronic pelvic pain [12]. Women with present infection, haemoglobin lower than 11 g/L or platelets lower than 150 x 10^3/μL were excluded from the study. Patients who participated in the study were aged from 35 to 42.

Sample preparation

According to the classification proposed by Ehrenfest, 4 different types of PRP are defined, depending on the content of cells and the presence of fibrin [13]. In regards to the Classification of PRP in this case study, it is used as a commercial type of PRP with the lower concentration (2.5 x 3 times) system, Regen PRP, (Regen Laboratory, Mont-sur-Lausanne, Switzerland) [14]. The process was carried out under strict aseptic conditions as well as optimum temperature regulations, i.e., 21-24°C. PRP was prepared according to the manufacturer’s guidelines. In the last step, the volume immediately above the erythrocyte layer was collected. Calcium gluconate was used as an activator. After activation, in a period less than 2 min, approximately 3-5 ml of the PRP was injected into the ovaries under transvaginal ultrasound guidance. The intervention was made under propofol intravenous anaesthesia following a protocol set by our IVF department. The whole intervention lasted about 15 to 20 minutes. We used a 30 cm single lumen 17G aspiration needles (COOK/Australia)

The changes in hormones FSH, estradiol, AMH was closely monitored, both before and after the application of PRP. We observed changes in the number of antral follicles before and after the application of PRP. In both groups, we used the same protocol for ovary stimulation, a low dose stimulation using GnRH antagonists.

Stimulation protocols and IVF procedure

The ovarian stimulation was performed according to the recommendations of the Practice Committee of the American Society for Reproductive Medicine [2]. A mild ovarian-stimulation protocol was performed in all patients. The protocol used 100-mg/day clomiphene citrate on days 2-6 of the cycle, adding the low-dose human menopausal gonadotropin (≤ 150 IU/d) and an antagonist (Cetrotide 0.25) when a lead follicle was ≥ 14 mm. During the controlled ovarian stimulation, we followed a protocol set by our IVF department. We evaluated several parameters such as estradiol (E_2), number

and size of follicles, endometrium thickness and gonadotropins administration with ultrasonography and laboratory tests. When one or more leading follicles reached a mean diameter ≥ 18 mm and the estradiol level was ≥ 200 pg/ml, human chorionic gonadotropin (hCG, 5 000 IU; Pregnyl, N.V. Organon, Os, The Netherlands) was administered for triggering the maturation of oocytes. Administration of FSH continued up until the application of HCG. Transvaginal ultrasound-guided oocyte retrieval was performed under short intravenous anaesthesia (Propofol-Lipuro 1%, Braun Melsungen AG, Melsungen, Germany). In both subgroups, transvaginal ovary punctuation followed after 35 to 36 hours of the application of HCG. The ICSI technique was used for all research patients. Embryo-transfers were applied 3 to 5 days later, depending on the condition and the work schedule of the IVF department. The same was performed under ultrasound control. Semen analysis was performed 30 min following liquefaction followed by semen sample preparation by density gradient centrifugation using 90% and 50% PureCeption (SAGE, Trumbull, CT, USA). Embryos were classified according to the scoring system of Hardarson and colleagues [15]. All transfers were performed under abdominal ultrasonography guidance using an embryo catheter (K-SOFT 5000, Cook Medical, Eight Mile Plains, Brisbane, Australia). Intravaginal administered progesterone (Crinone 8%, Merck Serono, Darmstadt, Germany) was used as the luteal phase support. The pregnancy test was considered positive when positive serum hCG levels (> 30 IU/ml) was detected 14 days after embryo transfer (ET). Clinical pregnancy was considered established when at least one visible sac with heart beating was detected by transvaginal ultrasonography.

Statistical analysis

Data analysis is performed in a Statistic program 7.1 for Windows and SPSS Statistics 23.0. For normal distributed data, mean and standard deviation were used. Comparisons across means were evaluated by paired two-tailed Students t-test. The factors with a *P*-value of < 0.1 in the univariate analysis were included in the logistic model. A *P*-value of < 0.05 was considered statistically significant.

Results

Mean patient's age was in the group A 37.47 ± 3.87 years, BMI was 22.63 ± 3.81 kg/m²; infertility duration was 4.0 ± 2.1 year. The mean partner age was 42.40 ± 5.34 years. All patients had multiple previous IVF attempts. Most of the patients had previous diagnostic hysteroscopy and laparoscopy. Differences in serum concentration of FSH, estradiol, AMH and total AFC in both groups were demonstrated in Table 1. Both compered groups were balanced for all basic characteristics, and multivariate analysis was performed to adjust for all known confounders. Between the two groups, there wasn't a statistical significance found in regards to the age, BMI, the basal value of FSH and AMH, length of infertility, previous attempts of IVF. The mean value of platelet concentration was 226.27 ± 82.80/10⁹L. Further analysis was performed to identify factors that might correlate the platelet count in the PRP with the values of FSH, AMH, estradiol and total AFC post-PRP. None of the tests presented statistical significance.

Table 1: Baseline characteristics of patients in both groups

Characteristics	Group A with previous treatment With autologous platelet-rich plasma	Group B	P
Age	37.47 ± 3.87	37.64 ± 3.20	P = 0.99
Body mass index kg/m²	22.63 ± 3.81	24.07 ± 5.01	P = 0.42
Infertility duration	4.0 ± 2.1	4.5 ± 1.2	P = 0.37
Partners age	42.40 ± 5.34	41.26 ± 4.38	P = 0.83
FSH	19.27 ± 2.29	19.22 ± 4.05	P = 0.97
Estradiol on day 3	71.06 ± 31.30	72.54 ± 28.64	P = 0.85
AMH	0.35 ± 0.19	0.72 ± 0.42	P = 0.03

E2, estradiol; FSH, follicle-stimulating hormone; AMH, anti-Müllerian hormone; antral follicle count; p Values pre- vs post-PRP calculated by Students t-test.

Ovarian stimulation parameters such a mean total gonadotropin dose consumed during IVF, mean serum estradiol before HCG trigger, the number of M II oocytes obtained in patients and duration of stimulation are demonstrated in Table 2.

Table 2: Compered ovarian stimulation parameters between groups

Characteristics	Group A with previous treatment with autologous platelet-rich plasma	Group B	P
Number of ampules 75IE	38 ± 13.9	42 ± 12.91	P = 0.08
Estradiol on day of HCG pmol/L	444.53 ± 331.87	528 ± 315.99	P = 0.57
Number of oocytes	1.87 ± 1.13	3.71 ± 2.40	P = 0.20
Duration of stimulation	10 (9.8-10.2)	10.2 (10-10.4)	P = 0.92

*Data presented as Breakdown & one-way ANOVA.

ICSI was used in all cases. All results were made based on IVF/. ET cycle. For all patient's fresh embryo transfers were performed. None of the patients experienced any complications from controlled ovarian stimulation or oocyte retrieval.

Our results of IVF outcome group A such a fertilisation rate 80.67 ± 25.42, implantation rate 33.33 ± 44.99, clinical pregnancy rate 33.33 ± 44.99 and live birth rate 40.00 ± 50.71 and in group B 65.60 ± 25.35, 10.71 ± 28.95, 10.71 ± 28.95, 14.29 ± 36.31 retrospectively were demonstrated in Table 3.

Table 3: Compared parameters of IVF treatment outcomes between groups

Characteristics	Group A with previous treatment with autologous platelet-rich plasma	Group B	P
Fertilization rate	80.67 ± 25.42	65.60 ± 25.35	P = 0.44
Implantation rate	33.33 ± 44.99	10.71 ± 28.95	P = 0.70
Clinical pregnancy rate	33.33 ± 44.99	10.71 ± 28.95	P = 0.69
Live birth rate	40.00 ± 50.71	14.29 ± 36.31	P = 0.71

*Data presented as Breakdown & one-way ANOVA.

Discussion

In this study, we can track a tendency of increasing the rate of clinical pregnancy and the live birth rates in Bologna poor responders, after the usage of the PRP method before the commencement of ovary stimulation during the IVF process.

Currently, there isn't a published randomised controlled study, which researched the effect of intraovarian injection of autologous PRP in poor responders. Available studies by the pioneer of PRP, professor Pantos and Sills [3], [4], showcasing the efficiency of using PRP on a larger indication scale.

The way PRP affects the patient is still not completely evaluated. With the use of platelet-derived growth factors (PDGFs), dysfunctional ovarian tissue is believed to be supplied with essential factors necessary for ovarian regeneration. In this context, it is necessary to mention angiogenesis and follicular vascularisation and their significant role in the ageing of the follicles. Receptors for growth factors are present on granulose cells confirming their association with the activation process of the primordial follicles [16]. Confirmation of all the above statements is also obtained from the Hosseini study [17]. On the other hand, the presence of ovarian stem cells on the surface of the ovarian tissue, under certain conditions, can produce de novo primordial follicles and thus the appearance of new antral follicles [18]. For this reason, it is considered that it can also be a result of the possibility of stimulation of germinative cells from the ovary cortex [19].

Besides, it is also not completely clear the exact reason to which we attribute the trend to increasing LBRs compared to the group b where a PRP was not performed before performing an IVF. Is it an improvement on the number of egg cells and/or their quality?

Anyhow, it is generally accepted that these patients need to be presented with all possible alternative treatments for a successful IVF with their genetic material. With a special emphasis on patients who fulfil the Bologna Criteria for POR and for patients who do not wish to enter a program for donating egg cells.

In conclusion, even though the treatment of POR responders remains as a therapeutical challenge, the usage of intraovarian injection of autologous PRP in PORs before the IVF performance brings a glimpse of new hope in increasing the success of IVF defined by clinical pregnancy and LBRs.

The conclusion in this study has to be reviewed extremely carefully because of the small number of patient participants in this pilot research study. For us to make a conclusion that would have significant statistical value, we would need a larger number of studies with a larger number of patients involved.

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