The effects of intra-ovarian autologous platelet rich plasma injection on IVF outcomes of poor responder women and women with premature ovarian insufficiency

Fırat Tülek[1,2], Alper Kahraman[3]

[1]Department of Midwifery, Üsküdar University Faculty of Health Sciences, İstanbul, Turkey
[2]Clinic of Obstetrics and Gynecology, Memorial Ataşehir Hospital, İstanbul, Turkey
[3]Clinic of Obstetrics and Gynecology, University of Health Sciences Turkey, Haseki Training and Research Hospital, İstanbul, Turkey

Abstract

Objective: There are controversial results regarding the administrations of platelet rich plasma (PRP) to increase in-vitro fertilization (IVF) success rates in the current literature. The aim of this study was to evaluate the effects of intra-ovarian PRP injections on IVF outcomes of poor responder women and women with premature ovarian insufficiency (POI).

Material and Methods: The medical history and outcome of women receiving intra-ovarian PRP injections performed in a single tertiary center between 2018 and 2021 was retrospectively reviewed.

Results: In total 71 women were included, of whom 21 were diagnosed with POI according to European Society of Human Reproduction and Embryology criteria and 50 were poor responders according to Bologna criteria. Number of retrieved oocytes, number of 2 pronuclear embryos and number of cleavage stage embryos were significantly higher in poor responder women after PRP injections. However clinical pregnancy rates and live birth delivery rates were similar before and after PRP injections in poor responders. In women with POI, 8 embryos were obtained in cycles commenced after PRP injections but no clinical pregnancies were achieved in this group of patients.

Conclusion: Intra-ovarian PRP injections do not appear to increase live birth rates or clinical pregnancy rates in poor responder women or in those with POI, in this cohort. (J Turk Ger Gynecol Assoc 2022; 23: 14-21)

Keywords: Platelet rich plasma, poor responder, in-vitro fertilization, premature ovarian insufficiency

Received: 20 July, 2021 **Accepted:** 21 September, 2021

Introduction

Decreased ovarian reserve and premature ovarian insufficieny (POI) are two entities that dramatically lower the chances of conception with assisted reproductive technologies. The problem stems from the low or absent oocyte yield, that usually cannot be improved by any current techniques.

POI is defined as loss of ovarian functions before the age of 40 years by the European Society of Human Reproduction and Embryology (ESHRE) (1). POI is estimated to have a prevalence of about 1% in the general population and it is a challenging condition for both patients and the physicians (1). Although pregnancies may occur in 5-10% of women with POI, either spontaneously or by in-vitro fertilization (IVF), oocyte donation remains the only treatment option for most patients (2). A range of treatment modalities are suggested to improve ovarian function and to achieve pregnancies without using donor eggs in these patients, including stem cell therapies and ovarian tissue auto-transplantation, although the outcomes have been unsatisfactory (3-6).

Address for Correspondence: *Fırat Tülek*
e.mail: firattulek@yahoo.com ORCID: orcid.org/0000-0003-1668-8746

Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.galenos.2021.2021.0134

For decades, the definition of poor ovarian response was not standardized and studies had been conducted using different criteria. There is now an accepted definition. Low ovarian response is currently defined as ≤3 ovarian follicles on the day of oocyte maturation triggering or ≤3 oocytes obtained in a controlled ovarian stimulation cycle (7). Low ovarian response constitutes 9% to 18% of IVF/embryo transfer cycles (8). These patients have poorer prognosis with live birth rates ranging from 6% to 23% in different studies (9,10). Some of the attempts to improve the oocyte yield by changing the ovarian stimulation protocol, gonadotropin dosage, gonadotropin type, pretreatment use of androgens, and so forth failed to result in better outcomes. Unsuccessful IVF attempts caused by low ovarian response brings additional frustration on already distressed couples.

More recently, innovative approaches, such as in vitro oocyte activation (IVA) which involves harvesting ovarian tissue and treating it with phosphatase and tensin homolog (PTEN) inhibitors in vitro, also seems not to be very efficient, although there have been some miracelous outcomes (6,11). As less labor-intensive approaches, some other treatment alternatives have emerged with yet unproven efficiency. These include ovarian injection of autologous platelet rich plasma (PRP).

PRP is a blood product containing high concentrations of platelets, a range of cytokines and growth factors, such as platelet derived growth factor, vascular endothelial growth factor (VEG-F), epidermal growth factor, transforming growth factor-beta (TGF-β) and insulin like growth factor-1 and 2 (IGF-1, 2). Source of cytokines in PRP solution could either be platelet degranulations as well as mechanical lysis of other blood cells. PRP is shown to induce angiogenesis, tissue regeneration, activate anabolic pathways for cell proliferation and differentiation, and aids in homing of stem cells (12). This new modality is increasingly used for regenerative purposes in dermatology, orthopedics and aesthetic surgery (13). Owing to the proposed mechanism of action, ovarian injection of PRP is hypothesized to promote ovarian rejuvenation. The rationale for this procedure is based on concentrating the soup of cytokines and growth factors associated with PRP and directly injecting them into ovarian tissue in an attempt to improve ovarian function. Some studies have reported increased ovarian angiogenesis, folliculogenesis, restored menstrual cycles and improved ovarian function tests following ovarian PRP injections (14,15). Although these findings drew attention to ovarian PRP injections in the treatment of infertile patients with poor prognosis, data about the effectiveness of this new modality is scarce, particularly in terms of the ultimate goal of assisted reproduction: live birth delivery rates.

In this study, the outcomes and efficacy of ovarian PRP injections performed for IVF purposes were evaluated retrospectively.

Material and Methods

Patients who underwent ovarian PRP injection due to POI or poor ovarian response in previous cycles in a university affiliated infertility center between 2018 and 2021 were retrospectively evaluated. Data was obtained from hospital records. Ethical approval for this study was obtained from Üsküdar University Faculty of Medicine at 28/05/2021 (approval number: 61351342/MAY 2021-04). The study protocol conformed to the "Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects" and the need for consent was waived by the ethical committee due to the retrospective design.

ESHRE criteria used for the diagnosis of POI are at least four months of amenorrhea and elevated follicle-stimulating hormone (FSH) >25 U/L in patients younger than 40 years of age. The Bologna criteria were adopted for the study definition of poor responders. To be defined as a poor responder by the Bologna criteria, at least two of the following three criteria should be met: 1) age >40 years; 2) poor ovarian response in previous IVF cycles (≤3 oocytes retrieved in a conventional stimulation protocol); and 3) abnormal ovarian reserve tests.

In our institution, documented fixed standards are used to prepare and apply PRP. A total of 20 mL of blood is collected from each patient into two tubes. T-LAB PRP kit (T-Biotechnology, Bursa, Turkey) is used to prepare the PRP. Tubes are centrifuged at 1500 g for eight minutes. Approximately 2 mL of plasma is gathered above the newly formed buffy coat layer from each tube through a 16 G needle into a 5 mL syringe. Plasma obtained from the tubes is transferred into a single re-suspension tube and gently agitated for 30-60 seconds to prepare the PRP solution for use. A total of 4 mL of PRP solution was obtained per patient and divided into two equal portions to inject into each ovary. Patients were sedated for ovarian injection. The procedure was carried on with a 35 cm long 17 G needle under transvaginal ultrasound guidance. 2 mL of solution was injected into the stromal region of each ovary within two hours of PRP preparation.

Women were assessed monthly for menstrual status, antral follicle count and serum hormone levels for at least six months following PRP. Monitoring started at the first mensturation following PRP injection. Controlled ovarian stimulation was initiated in patients that were found eligible within the first five days of the menstrual cycle. Recombinant (rFSH, Gonal-F, Merck Serono S.p.A), human menopausal gonadotropin (hMG, Merional, IBSA Institut Biochimique S.A, Menopur® Ferring Pharmaceuticals) or a combination of recombinant luteinizing hormone and rFSH (Pergoveris, Merck Serono SA) was used for ovarian stimulation, as per practitioner's choice. Patients were monitored during stimulation for follicular growth with serial transvaginal ultrasounds and serum

hormone levels. Adjustments in gonadotropin doses were made in accordance with each patient's follicular growth. Once the leading follicle reached a diameter of 12-14 mm, gondatropin releasing hormone (GnRH) antagonist (Cetrotide 0.25 mg, Pierre Fabre Medicament Production) injections were commenced to suppress premature LH peak and continued to the day of oocyte maturation triggering. A dual-trigger method was used to induce oocyte maturation with a GnRH agonist of 0.2 mg triptorelin acetate, (Gonapeptyl, Ferring Pharmaceuticals) and 250 mcg recombinant human chorionic gonadotropin (Ovitrelle, Merck Serono) when at least one follicle had reached a diameter of 18 mm. Oocytes were retrieved under transvaginal ultrasound guidance 35-36 hours after oocyte maturation trigger. Fertilization was conducted by intracytoplasmic sperm injection. Developing embryos were graded according to İstanbul consensus workshop guidelines (16). Day 3 or day 5 embryos were transferred using an embryo transfer catheter under abdominal ultrasound guidance. A maximum of two embryos were transferred in each attempt. Luteal phase support was initiated in every patient with 200 mg intravaginal progesterone (Lutinus, Ferring Pharmaceuticals) twice a day and continued through the eight to tenth gestational weeks.

Exclusion criteria included: patients with high (>30 kg/m^2) or low (<18 kg/m^2) body mass indices (BMI); patients with additional endocrine disorders (thyroid dysfunction, hyperprolactinemia, diabetes mellitus, Addison disease, congenital adrenal hyperplasia, Cushing syndrome); patients with corrected or present uterine anomalies; and patients with infertility due to azoospermia. Seventy-one women were recruited for ovarian PRP injection within the selected period of time for POI and poor ovarian response. Twenty-one of them were defined as POI, and two were lost to follow-up and excluded. Fifty women were defined as poor responders by the Bologna criteria. All of the poor responders had a history of previous ovarian stimulation cycle that resulted in ≤3 oocytes being retrieved. Outcomes of IVF cycles before and after PRP administration were compared in poor responders and cycle outcomes following ovarian PRP injection in women with POI were assessed. Our primary outcome was live birth delivery rates. Live birth was defined as live infants delivered after the 24th gestational week. Secondary outcomes were: number of oocytes retrieved; number of metaphase 2 (M2) oocytes; fertilization rates [2 pronuclear embryos (2PN)/M2 oocytes]; number of cleavage stage embryos; and implantation rates (gestational sacs observed/transferred embryos). Outcome parameters were defined in accordance with The International Glossary on Infertility and Fertility Care, 2017 (17).

Statistical analysis

Statistical analysis was done using IBM SPSS, version 23 (Evaluation version; IBM, Armonk, NY, USA). Descriptive statistics are expressed as mean ± standard deviations for normally distributed data and as median (minimum-maximum) for non-normally distributed data. Categorical variables are expressed as numbers and percentages (%). Significance of differences in means and medians among groups were assessed by Student's t-test and Mann-Whitney U test, respectively. Categorical variables were evaluated with Pearson's chi-squared test or Fisher's exact test. A p-value <0.05 was considered significant.

Results

A total of 71 women who underwent ovarian PRP injection within specified period of time were eligible for the study. PRP injection was performed in 50 women because of poor ovarian response in previous IVF cycles and to 21 women due to POI. Two women with diagnosis of POI lost follow-ups and excluded from the study. Mean age and BMI of patients with POI were 37.9±1.9 years and 24.9±3.1 kg/m^2, respectively. In poor responders mean age was 38.1±4.4 years and mean BMI was 25±3.4 kg/m^2.

In 10 (52.6%) of 19 POI cases, menstruation was restored following PRP and controlled ovarian stimulation cycles could be commenced. Mean interval betweeen PRP injections and the start of menstral cycles was 3.1±0.99 months. A total of 16 cycles was performed in these 10 patients. Embryo transfers were canceled due to: failure to retrieve any oocyte at follicle puncture (n=3); lack of follicular growth (n=3); premature ovulation (n=1); and no fertilization achieved (n=1). Embryo transfers were performed in the remaining 8 cycles. Median number of oocytes retrieved in women with POI was 1 (0-2) and the mean number of metaphase 2 oocytes was 0.929±0.82. A total of eight grade 1 and 2 embryos were obtained and transferred. None of embryo transfers resulted in pregnancy. Cycle characteristics of women with POI following ovarian PRP injection is given in Table 1.

Ovarian PRP injection was performed in 50 poor responder women. Following PRP injections, 84 controlled ovarian stimulations were performed in those patients. Cycle outcomes before and after PRP injections were compared. Total gonadotropin doses required and days of stimulation were found to be significantly lower in cycles after PRP injection (p=0.006 and p=0.002, respectively). The number of retrieved oocytes (1.50±1.36 vs 2.18±1.66), number of M2 oocytes (1.16±1.06 vs 1.71±1.32), number of 2PN (0.84±0.89 vs 1.24±1.06), number of cleavage stage embryos (0.50±0.54 vs 1.04±0.96) and rate of top quality (grade 1) embryos obtained [7 (29.2%) vs 32 (59.3%)] were significantly higher in cycles following PRP injection (p=0.026, p=0.02, p=0.029,

p=0.001 and p=0.026, respectively). Frozen-thawed embryo transfers were performed in seven pre-PRP cycles and in 11 post-PRP cycles. Frozen-thawed embryo transfer rates were similar in pre- and post-PRP cycles (14% vs 13%, p=0.872). Cancellation rate of embryo transfer was significantly lower in cycles following PRP injection (p=0.03). One clinical pregnancy was identified in the cycles before PRP injection but resulted in miscarriage. Seven clinical pregnancies were identified in cycles after PRP injection and three of them resulted with miscarriage. There were no live births in pre-PRP cycles but there were four live births in post-PRP cycles. No significant difference was found in live birth rates among pre- and post-PRP cycles (0% vs 4.7%, p=0.296). Comparison of cycle outcomes before and after ovarian PRP injection is summarized in Table 2.

Outcomes of cycles performed in poor responders after PRP injection were subjected to a subgroup analysis stratified by time interval between PRP injection and initiation of the cycle. All of the clinical pregnancies and live births in our study population were achieved in patients when ovarian stimulation cycles commenced within 90 days following PRP injection (Table 3). Gonadotropin requirements tended to decrease in cycles initiated within the first 90 days following PRP injections. However none of these findings were statistically significant. Stratification of cycle outcomes with respect to interval between cycle starting day and PRP injection is given in Table 3.

Discussion

In this study IVF cycles were evaluated following ovarian PRP injection in patients with POI and poor ovarian response. The main outcome measure was live birth rate while other main cycle outcomes were also assessed.

Table 1. Outcomes of IVF cycles in patients with POI following ovarian PRP injection

Number of cycles	16
Median estradiol levels (pg/mL)	265 (59-894)
Median progesterone levels (ng/mL)	0.45 (0.1-1.5)
Median endometrial thickness (mm)	8.2 (7.2-9.5)
Median number of retrieved oocytes	1 (0-2)
Mean metaphase 2 oocytes	0.93±0.82
Fertilization rate	0.77±0.72
Number of day 3 embryo transfers	8
Number of grade 1 embryo	3 (37.5%)
Number of grade 2 embryo	5 (62.5%)
Mean number of transferred emryos	0.43±0.62

IVF: In-vitro fertilization, POI: Premature ovarian insufficiency, PRP: Platelet rich plasma

In poor responder women significantly increased numbers of oocytes, M2 oocytes, 2PN embryos, grade 1 embryos and cleavage stage embryos were obtained from cycles following ovarian PRP injection. These findings are consistent with previous studies (18-20). Although the effective mechanisms are not clear, it has been suggested that these findings may be due to the effect of platelet-derived cytokines which may improve the ovarian microenvironment, enhance ovarian vascular activation and stabilization or even result in de novo oocyte development from precursor stem cells (21-23).

Some case series and studies have reported pregnancies in women with POI following ovarian PRP injections, either spontaneously or via IVF (20,24-26). However, in the present study no live births occurred in women with POI after ovarian PRP injection. There was an increasing trend in live births following PRP injections in women with poor response but this increase was not significant, which again is in line with the studies conducted by Melo et al. (18) and Stojkovska et al. (27). This might be due to small sample sizes. However, in a previous study, general cumulative live birth rates were estimated to be approximately 13.7% in poor responders after two IVF cycles without PRP injections and this rate ranged between 4.4% and 17.2% when patients were stratified with respect to age (28). For poor responder women, live birth delivery rate following PRP injection was estimated as 4.7% in our study, lower than the reported cumulative live birth rates in poor responders as a whole in earlier studies. There does not seem to be any increase in live birth rate in poor responders when using ovarian PRP injection following the technique we used, possibly due to specific preparation techniques on the composition and thus the resultant effects of the PRP preparations. Different centrifugation processes are known to change the final composition of PRP solutions. For example, forces applied to samples exceeding 800 g in centrifugation has been shown to decrease the concentration of TGF-β in PRP preparations by disruption of platelets and granules containing growth factors (29). TGF-β mediates follicular development through effects on cellular differentiation, proliferation and chemotaxis and activation of various regulatory proteins (21). In animal models, inhibition of TGF-β pathways have been shown to reduce fertility by disrupting multiple ovarian processes, such as follicular development and cumulus-oocyte complex expansion and provokes premature luteinization of granulosa cells leading to ovulation failures (30,31). High TGF-β concentration in orthopedic studies is associated with bone deterioration and fibrocartilage calcifications (32). In the present study the centifugal force was equivalent to 1500 g, in accordance with PRP kit manufacturer's instructions. It should be noted that PRP preparation techniques that are suitable for extra-ovarian

applications might not be optimal for ovarian injection. Further research is needed in this area.

The effects of PRP preparations are entirely dependent on their exact composition. The presence of different proportions of other leukocytes, all of which are capable of secreting a broad range of cytokines, such as VEG-F and other proteins and may directly induce platelet degranulation (33). The protein contents of platelet granules may be both pro- and anti-inflammatory. Inhibition of the nuclear factor-kappa b (NF-kb) pathway by platelets is associated with suppression of inflammation and this effect is more prominent in leukocyte-poor rather than leukocyte-rich PRP preparations (34).

There are a wide range of variables that may affect the final composition of PRP preparations, including the donor hematological status and preparation technique. Weibrich et al. (35), using an animal model, demonstrated that PRP preparations with platelet concentrations between 1-6 fold of the donor whole blood platelet count enhanced peri-implant bone regeneration. This effect disappeared when the final PRP platelet count was either <1 or >6 times the whole blood platelet count. A study by Sills et al. (36) in reproductive medicine showed that the increase in anti-mullerian hormone levels in women following ovarian PRP injection was greater in women with higher whole blood platelet counts.

Whether the observed effects after PRP injection is a consequence of ovarian trauma caused by procedure is a matter of debate. The hippo signaling pathway is a tumor suppressor cascade that regulates cell proliferation, apoptosis and stem cell regeneration and is known to impede folliculogenesis by preventing progression of pre-antral follicles

Table 2. Comparison of outcomes of IVF cycles applied before and after ovarian PRP injection in poor responder patients

	Cycles before ovarian PRP injection	Cycles after ovarian PRP injection	p
Number of cycles	50	84	-
Total dose of gonadotropin (IU)	3907.5±990.15	3507.14±1076.94	**0.006**
Mean days of stimulation	10.76±1.83	9.73±1.82	**0.002**
Fertilization rate (2 pronuclear embryo/M2 oocytes)	42/58 (0.724)	104/144 (0.722)	0.976
Implantation rate (gestational sacs/transferred embryo)	1/28 (3.6%)	7/79 (8.8%)	0.357
Mean estradiol levels (pg/mL)	384.08±227.22	589.40±449.17	**0.014**
Mean progesterone levels (ng/mL)	0.62±0.49	0.60±0.48	0.786
Mean endometrial thickness (mm)	8.38±1.53	8.44±1.42	0.487
Mean number of retrieved oocytes	1.50±1.36	2.18±1.66	**0.026**
Mean number of metaphase 2 oocytes	1.16±1.06	1.71±1.32	**0.020**
Mean number of 2 pronuclear embryos	0.84±0.89	1.24±1.06	**0.029**
Mean number of cleavage stage embryo	0.50±0.54	1.04±0.96	**0.001**
Number of day 3 embryo transfers	21 (87.5%)	48 (85.7%)	1
Number of day 5 embryo transfers	3 (12.5%)	8 (14.3%)	
Mean number of transferred embryos	0.56±0.64	0.94±0.78	**0.006**
Number of grade 1 embryos	7 (29.2%)	32 (59.3%)	**0.026**
Number of grade 2 embryos	17 (70.8%)	22 (40.7%)	
Clinical pregnancies %, (n)	2% (1)	8.3% (7)	0.16
Cancellation rate %, (n)	52% (26/50)	33% (28/84)	**0.03**
Live birth delivery rates	0% (0/50)	4.7% (4/84)	0.296

IVF: In-vitro fertilization, PRP: Platelet rich plasma, M2: Metaphase 2

Table 3. Distribution of cycle outcomes due to interval between commencement and PRP injection

Interval between PRP injection and cycle initiation	<30 days	30-60 days	60-90 days	>90 days	p
Number of cycles	13	29	33	9	-
Gonadotropin doses required (IU)	3848.1±1908.54	3587.0±1033.3	3243.2±700.72	3725.1±685.3	0.427
Clinical pregnancies	0	4	3	0	0.696
Live births	0	2	2	0	0,724

to early antral follicles (37). This pathway is involved in a cell-contact type inhibition and polymerization of globular actin to filamentous actin inactivates the hippo signaling pathway (3). In light of this investigations into IVA techniques have resected, fragmented and re-transplanted ovaries in the presence of hippo inhibiors, protein kinase B (Akt) stimulators or by experimental direct trauma to disrupt the hippo pathway, with some success (3,5,6,11). Zhang et al. (4) conducted a study to observe the effects of ovarian biopsy and scratching on ovarian function. They took a 5 mm biopsy and inflicted three superficial scratches of 2-4 mm on each ovary. The observed improvement in ovarian functions were less than in IVA studies and the authors suggested that this may be due to insufficient disruption of hippo pathway, posibly due to insufficient ovarian trauma. Thus it is doubtful that inserting a 17G needle will inflict adequate damage to the ovary to disrupt the hippo pathway. The Yes-associated protein/transcriptional co-activator with PDZ binding motif (YAP/TAZ) system is an oncogenic component of the hippo pathway and its activation stimulates follicular growth (3). This system is regulated by mechanical factors. The YAP/TAZ system is activated by increased tensile forces within the cytoplasm and inhibited by decreased tensile forces (38). The exact mechanical forces applied on follicles that occur when injecting a fluid bolus into ovarian stroma, as well as its effects on the YAP/TAZ system, are hard to predict. Placebo-controlled trials involving ovarian PRP injections are lacking. However, the findings of Sills et al. (36) showed a correlation between patients' platelet counts and ovarian functions after PRP injections and this finding indicates at least some effects of ovarian PRP injection are not solely results of mechanical effects of injection.

Currently, PRP preparation techniques for ovarian PRP injections lack standardization. A wide range of PRP preparation techniques have been used in published studies, often without giving fine detail. In addition, final PRP preparations are also dependent on the hematological status of the donor women. Lack of standardization of these preparations means that comparison between studies is unreliable. Many PRP classification systems have been proposed to provide uniformity but none have been widely accepted (39). Among these, Magalon et al. (40) described a comprehensive classification system, the "DEPA classification", that has the advantage of retrospective application. However, to use DEPA precise cell counts for whole blood and the final PRP preparation should be known, together with volume of collected blood and injected PRP volume. When using commercial PRP preparation kits some of these data are not readily available without manufacturer co-operation. Rossi et al. (39) suggested that an ideal classification for PRP preparations to provide a degree of reproducibility and uniformity should include at least platelet counts, leukocyte count (with percentage of neutrophils), red blood cell count and concentration and dose of PRP preparation used. A limitation of the present study is the lack of these data. Apart from molecular research, inclusion of these parameters in future studies would help standardization and comparability of studies.

To date there is no consensus about optimal timing for intiation of IVF cycles following ovarian PRP injections. In the present study, IVF outcome was assessed in relation to the period between PRP injection and cycle initiation. There was a non-significant trend in required gonadotropin doses in cycles commenced within 90 days of PRP injection, with the lowest doses in cycles initiated between 60-90 days after PRP injection. Although there is no direct quantification of ovarian reserve, lower gonadotropin dose might suggest improved ovarian functions, peaking between 60-90 days after PRP injections. Earlier studies showed improved results of tests of ovarian reserve following PRP injection and it was suggested that the effect of PRP injection may be to enhance pre-antral follicular growth or prevent their atresia (18,25,36). Besides hormones and other gonadotropins, some as yet poorly understood paracrine factors are shown to regulate ovarian folliculogenesis. One of these is growth differentiating factor-9 (GDF-9). GDF-9 is an oocyte-derived local factor that is thought to act synergistically with bone morphogenetic protein-15 (BMP-15) to stimulate follicular development. GDF-9 enhances follicular growth beyond pre-antral stages of follicles and it is known to be secreted throughout folliculogenesis (37). Both GDF-9 and BMP-15 are members of TGF-β super family and their actions are known to overlap with other members of this group of proteins (41). There is evidence that GDF-9 stimulates progression of primary follicles to small pre-antral follicles (42). Under physiological conditions, progression of primary follicles to pre-antral follicles takes approximately 120 days (43). However supra-physiologic local ovarian TGF-β levels after PRP injection might hasten this process or trigger the shift from primary to small pre-antral follicles. Besides stimulation of pre-antral follicle growth, an increased number of hormone-responsive pre-antral follicles could be one of the possible reasons of reduced gonadotropin requirements observed in our study.

Moreover triggering of the shift from primary to pre-antral follicles might explain the delayed effects of PRP that were observed two to three months after injection, long after the degradation of injected cytokines. However there are still many uncertainties concerning the paracrine regulation of folliculugenesis, as well as in the composition of PRP.

Platelets are known to contain more than 800 types of proteins and more than 30 types of bioactive molecules that could be released into PRP preparations at various rates and concentrations upon degranulation or degradation (25,44).

One of the aims of future research in this field should be to identify which of these proteins and at what doses actually benefits outcome. In this way, a procedure which currently consists of the injection of a non-standardized soup of pro- and anti-inflammatory cytokines, differently affecting various target tissues may evolve into groundbreaking therapies.

Study limitation

Some limitations should be noted. This study lacked a control group. Cycle outcomes were compared in the same group of poor responder women before and after PRP injections. Therefore one should keep in mind the "regression to the mean" bias when interpreting our results. Larger studies with control groups would provide more precise data.

There are no reports of any serious adverse effects associated with ovarian PRP injections and no adverse side-effects were observed in our cohort. However, it should be noted that long term effects of this procedure are not known and administering highly concentrated growth factors to tissues carries the theoretical risk of inducing malignant transformation.

Conclusion

Intra-ovarian PRP injections do not appear to increase live birth rates or clinical pregnancy rates in poor responder women, at least using the techniques described herein. The heterogeneity of current methods used in the literature and inadequate understanding of paracrine mechanisms involved in folliculogenesis are barriers to improvement of this therapy. Further research is required to improve outcomes of intra-ovarian PRP injections.

Ethical Committee Approval: *Ethical approval for this study was obtained from Üsküdar University Faculty of Medicine at 28/05/2021 (approval number: 61351342/MAY 2021-04, date: 28.05.2021).*

Informed Consent: *Retrospective study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: F.T.; Concept: F.T., A.K.; Design: F.T., A.K.; Data Collection or Processing: F.T.; Analysis or Interpretation: F.T., A.K.; Literature Search: F.T., A.K.; Writing: F.T., A.K.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

Financial Disclosure: *The authors declared that this study received no financial support.*

References

1. European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI, Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Hum Reprod 2016; 31: 926-37.
2. Fraison E, Crawford G, Casper G, Harris V, Ledger W. Pregnancy following diagnosis of premature ovarian insufficiency: a systematic review. Reprod Biomed Online 2019; 39: 467-76.
3. Diminished Ovarian Reserve and Assisted Reproductive Technologies. Current Research and Clinical Management. In: Orhan Bukulmez, (eds). Springer International Publishing; 2020.
4. Zhang X, Han T, Yan L, Jiao X, Qin Y, Chen ZJ. Resumption of ovarian function after ovarian biopsy/scratch in patients with premature ovarian insufficiency. Reprod Sci 2019; 26: 207-13.
5. Kawamura K, Ishizuka B, Hsueh AJW. Drug-free in-vitro activation of follicles for infertility treatment in poor ovarian response patients with decreased ovarian reserve. Reprod Biomed Online 2020; 40: 245-53.
6. Suzuki N, Yoshioka N, Takae S, Sugishita Y, Tamura M, Hashimoto S, et al. Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. Hum Reprod 2015; 30: 608-15.
7. Ovarian Stimulation TEGGO, Bosch E, Broer S, Griesinger G, Grynberg M, Humaidan P, et al. ESHRE guideline: ovarian stimulation for IVF/ICSI. Hum Reprod Open 2020; 2020: hoaa009.
8. Garcia-Velasco JA, Isaza V, Requena A, Martínez-Salazar FJ, Landazábal A, Remohí J, et al. High doses of gonadotrophins combined with stop versus non-stop protocol of GnRH analogue administration in low responder IVF patients: a prospective, randomized, controlled trial. Hum Reprod 2000; 15: 2292-6.
9. Busnelli A, Papaleo E, Del Prato D, La Vecchia I, Iachini E, Paffoni A, et al. A retrospective evaluation of prognosis and cost-effectiveness of IVF in poor responders according to the Bologna criteria. Hum Reprod 2015; 30: 315-22.
10. Chai J, Lee VC, Yeung TW, Li HW, Ho PC, Ng EH. Correction: live birth and cumulative live birth rates in expected poor ovarian responders defined by the bologna criteria following IVF/ICSI treatment. PLoS One 2015; 10: e0131334.
11. 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: 17474-9.
12. Tandulwadkar S, Karthick MS. Combined use of autologous bone marrow-derived stem cells and platelet-rich plasma for ovarian rejuvenation in poor responders. J Hum Reprod Sci 2020; 13: 184-90.
13. Urman B, Boza A, Balaban B. Platelet-rich plasma another add-on treatment getting out of hand? How can clinicians preserve the best interest of their patients?. Hum Reprod 2019; 34: 2099-103.
14. Bos-Mikich A, de R, Frantz N. Platelet-rich plasma therapy and reproductive medicine. J Assist Reprod Genet 2018; 35: 753-6.
15. Hosseini L, Shirazi A, Naderi MM, Shams-Esfandabadi N, Borjian Boroujeni S, Sarvari A, et al. Platelet-rich plasma promotes the development of isolated human primordial and primary follicles to the preantral stage. Reprod Biomed Online 2017; 35: 343-50.
16. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011; 26: 1270-83.
17. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril 2017; 108: 393-406.

18. Melo P, Navarro C, Jones C, Coward K, Coleman L. The use of autologous platelet-rich plasma (PRP) versus no intervention in women with low ovarian reserve undergoing fertility treatment: a non-randomized interventional study. J Assist Reprod Genet 2020; 37: 855-63.
19. Sfakianoudis K, Simopoulou M, Grigoriadis S, Pantou A, Tsioulou P, Maziotis E, et al. Reactivating ovarian function through autologous platelet-rich plasma intraovarian infusion: pilot data on premature ovarian ınsufficiency, perimenopausal, menopausal, and poor responder women. J Clin Med 2020; 9: 1809.
20. Panda SR, Sachan S, Hota S. A Systematic review evaluating the efficacy of ıntra-ovarian ınfusion of autologous platelet-rich plasma in patients with poor ovarian reserve or ovarian ınsufficiency. Cureus 2020; 12: e12037.
21. Sills ES, Wood SH. Autologous activated platelet-rich plasma injection into adult human ovary tissue: molecular mechanism, analysis, and discussion of reproductive response. Biosci Rep 2019; 39: BSR20190805.
22. Sfakianoudis K, Simopoulou M, Nitsos N, Rapani A, Pantou A, Vaxevanoglou T, et al. A Case series on platelet-rich plasma revolutionary management of poor responder patients. Gynecol Obstet Invest 2019; 84: 99-106.
23. 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; 34: 756-60.
24. Pantos K, Simopoulou M, Pantou A, Rapani A, Tsioulou P, Nitsos N, et al. A Case series on natural conceptions resulting in ongoing pregnancies in menopausal and prematurely menopausal women following platelet-rich plasma treatment. Cell Transplant 2019; 28: 1333-40.
25. Cakiroglu Y, Saltik A, Yuceturk A, Karaosmanoglu O, Kopuk SY, Scott RT, et al. Effects of intraovarian injection of autologous platelet rich plasma on ovarian reserve and IVF outcome parameters in women with primary ovarian insufficiency. Aging (Albany NY) 2020; 12: 10211-22.
26. Hsu CC, Hsu L, Hsu I, Chiu YJ, Dorjee S. Live birth in woman with premature ovarian ınsufficiency receiving ovarian administration of platelet-rich plasma (PRP) in combination with gonadotropin: a case report. Front Endocrinol (Lausanne) 2020; 11: 50.
27. Stojkovska S, Dimitrov G, Stamenkovska N, Hadzi-Lega M, Petanovski Z. 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. Open Access Maced J Med Sci 2019; 7: 3184-8.
28. Xu B, Chen Y, Geerts D, Yue J, Li Z, Zhu G, et al. Cumulative live birth rates in more than 3,000 patients with poor ovarian response: a 15-year survey of final in vitro fertilization outcome. Fertil Steril 2018; 109: 1051-9.
29. Landesberg R, Roy M, Glickman RS. Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. J Oral Maxillofac Surg 2000; 58: 297-301.
30. Li Q, Pangas SA, Jorgez CJ, Graff JM, Weinstein M, Matzuk MM. Redundant roles of SMAD2 and SMAD3 in ovarian granulosa cells in vivo. Mol Cell Biol 2008; 28: 7001-11.
31. Pangas SA, Li X, Robertson EJ, Matzuk MM. Premature luteinization and cumulus cell defects in ovarian-specific Smad4 knockout mice. Mol Endocrinol 2006; 20: 1406-22.
32. Wang X, Xie L, Crane J, Zhen G, Li F, Yang P, et al. Aberrant TGF-β activation in bone tendon insertion induces enthesopathy-like disease. J Clin Invest 2018; 128: 846-60.
33. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009; 27: 158-67.
34. Sundman EA, Cole BJ, Karas V, Della Valle C, Tetreault MW, Mohammed HO, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. Am J Sports Med 2014; 42: 35-41.
35. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet concentration in platelet-rich plasma on peri-implant bone regeneration. Bone 2004; 34: 665-71.
36. Sills ES, Rickers NS, Petersen JL, Li X, Wood SH. Regenerative effect of intraovarian injection of activated autologous platelet rich plasma: serum anti-mullerian hormone levels measured among poor prognosis in vitro fertilization patients. Int J Regenr Med 2020; 1: 2-5.
37. Hsueh AJ, Jones PB, Adashi EY, Wang C, Zhuang LZ, Welsh TH Jr. Intraovarian mechanisms in the hormonal control of granulosa cell differentiation in rats. J Reprod Fertil 1983; 69: 325-42.
38. Low BC, Pan CQ, Shivashankar GV, Bershadsky A, Sudol M, Sheetz M. YAP/TAZ as mechanosensors and mechanotransducers in regulating organ size and tumor growth. FEBS Lett 2014; 588: 2663-70.
39. Rossi LA, Murray IR, Chu CR, Muschler GF, Rodeo SA, Piuzzi NS. Classification systems for platelet-rich plasma. Bone Joint J 2019; 101-B: 891-6.
40. Magalon J, Chateau AL, Bertrand B, Louis ML, Silvestre A, Giraudo L, et al. DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. BMJ Open Sport Exerc Med 2016; 2: e000060.
41. Peng J, Li Q, Wigglesworth K, Rangarajan A, Kattamuri C, Peterson RT, et al. Growth differentiation factor 9:bone morphogenetic protein 15 heterodimers are potent regulators of ovarian functions. Proc Natl Acad Sci U S A. 2013; 110: E776-85.
42. Vitt UA, McGee EA, Hayashi M, Hsueh AJ. In vivo treatment with GDF-9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. Endocrinology 2000; 141: 3814-20.
43. Encyclopedia of Reproduction. Second Edition. In: Michael K Skinner (eds). Amsterdam; Boston: Elsevier, Academic Press; 2018.
44. Schilephake H. Bone growth factors in maxillofacial skeletal reconstruction. Int J Oral Maxillofac Surg 2002; 31: 469-84.