

Intra-Ovarian Platelet-Rich Plasma Injections: Single-Centre Experience

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1. Abstract

Objective: To describe the effects of intra-ovarian platelet-rich plasma injection on the ovarian stimulation outcomes in women referring to an in vitro fertilization center.

Method: We conducted a single-center retrospective study on 179 women that underwent intra-ovarian platelet-rich plasma injection over the last three years. Inclusion criteria included women over age 35 with at least one ovary with a history of infertility, hormonal abnormalities, absence of menstrual cycle and premature ovarian failure.

Results: Mean (\pm SD) patient age was 43 ± 4 years. Both serum FSH levels and serum E2 significantly reduced after treatment from 29.0 pg/ml to 18.0 pg/ml; $p < 0.0001$ and from 65.6 pg/ml to 47.2 pg/ml; $p = 0.034$ respectively. None of the 179 women reported any complications post operatively. After PRP, 17/179 (9.49%) women became pregnant.

Conclusion: The results of our observational study revealed that PRP intra-ovarian injection is associated with improved function of ovarian tissue. Future further randomized clinical trials in this field are needed to shed light in the use of PRP in ovarian rejuvenation.

2. Keywords: Intra-ovarian Platelet-rich plasma

injections; Ovarian rejuvenation; Premature ovarian failure; Infertility

3. Introduction

Platelet-rich plasma (PRP) is becoming more popular as a nonoperative treatment option for a broad spectrum of gynecological disorders [1]. Ovarian rejuvenation is an innovative procedure intended to restore ovary fertility and development during the climacteric and has been used to enhance fertility in women with Premature Ovarian Insufficiency (POI). The use of PRP for ovarian “rejuvenation” was first outlined only a few years ago, when a group of poor prognosis infertility patients received intra-ovarian injection of PRP followed by In Vitro Fertilization (IVF) with their own oocytes [2].

PRP is a natural product containing high concentrated platelets with growth factors in concentration three to five times higher than plasma [2]. PRP is an autologous and highly concentrated solution of plasma, which is prepared from the patient’s own blood and contains a concentrated source of insulin-like growth factor-1 and 2 (IGF-1, IGF-2), fibroblast

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growth factor (FGF), epidermal growth factor (EGF), transforming growth factor beta (TGF- β), hormones and cytokines [3,4].

In addition to other growth factors, platelets contain other substances, such as fibronectin, vitronectin and sphingosine 1-phosphate, that initiate wound healing [1,5,6]. Growth factors promote wound healing by initiating the following stages: tissue necrosis resolution, chemotaxis, cell regeneration, cell proliferation and migration, extracellular matrix synthesis, remodeling, angiogenesis and epithelialization [1,7]. Considering the angiogenic composition of the ovary and the pivotal influence of platelet-derived growth factors on vascular activation and stabilization, treatment with autologous PRP may be viewed as the enabler of ovarian tissue regeneration [8]. PRP also contains a member of the TGF- β superfamily, growth differentiation factor 9 (GDF-9) [9]. The *gdf-9* gene expression is regarded as a biomarker of oocyte maturation potential [10,11] and its mutations have been linked to premature ovarian dysfunction [3].

As ovarian PRP treatment is a new practice, the clinical evidence on its benefits is still preliminary. Thus, this retrospective study was conducted to determine the effects of intra-ovarian PRP injection on the ovarian stimulation outcomes in women referring to an IVF center.

4. Methods

Inclusion criteria included women over the age of 35 with at least one ovary with a history of infertility, hormonal abnormalities, absence of menstrual cycle and premature ovarian failure. During the patients' first consultation, a detailed reproductive history was recorded and pelvic scan for the ovarian size, hormonal analysis for follicle stimulation hormone (FSH), anti-Müllerian (AMH), estradiol (E2) and luteinizing hormone (LH) were measured. In case of amenorrhea, hormone evaluation was obtained independently of a menstrual cycle. FSH, estradiol (E2), luteinizing hormone (LH) and anti-Müllerian

hormone (AMH) levels were determined on an unspecified day prior to initiating PRP treatment. The option of PRP treatment was presented to the patient based on previous published literature [2,12].

Exclusion criteria included current or previous IgA deficiency, ovarian insufficiency secondary to sex chromosome abnormality, prior major lower abdominal surgery resulting in pelvic adhesions, anticoagulant use for which plasma infusion is contraindicated, mental health disorder including active substance abuse or dependence, ongoing malignancy, or chronic pelvic pain [13,14].

The volume of peripheral blood required to prepare 6-8 mL of PRP for administration was 40-60 mL. The initial concentration of platelets in the peripheral blood sample was 25000/ μ L, while the prepared PRP had a concentration of 900.000/ μ L. A volume of approximately 2-4 mL per ovary, depending on ovarian volume, was used for the intra-ovarian injection. The essential parts of the technique consisted of a non-surgical, transvaginal, ultrasound-guided, multifocal, needle injection and diffusion in the subcortical layers. The injection included multiple sites; two-three punctures being performed per ovary using a 17-gauge needle. After activated PRP injection was bilaterally completed, careful ultrasound assessment of the pelvis was performed to observe vascular integrity and absence of free pelvic fluid. Sedation with propofol was used for the ovarian PRP injection. For all patients, the procedure was completed in less than seven minutes. Following the procedure, each patient was asked to remain supine and rest for 15 min; vital signs were rechecked before home discharge.

During our study, follow-up of all cases was performed during the first menstrual cycle post PRP treatment. This was reported for all cases within the subsequent calendar month. Hormone levels following PRP treatment were determined on the second or the third day of the subsequent menstrual cycles, consecutively for six months. In case of amenorrhea,

the hormone levels were tested every 31-32 days; normal values being FSH<10 and 30 <E2 <60 pg / ml. The data were analyzed using GraphPad Prism 8.3, presented as mean \pm SD. Normality tests (D'Agostino & Pearson test, Shapiro-Wilk test, Kolmogorov-Smirnov test) did not show Gaussian distribution and thus paired and non-parametric models (Wilcoxon test) were used. Finally, the value of $p < 0.05$ was considered significant. The research protocol was approved by the Ethics Committee of Crete Fertility Centre and all participants gave verbal informed consent before the study began.

5. Results

Of the 500 women who had undergone PRP treatment over the last three years in our centre (January 2017-January 2020), 179 women with a history of infertility, hormonal abnormalities, absence of menstrual cycle, premature ovarian failure had hormonal levels recorded up to four months after treatment and these were included in the study. Mean (\pm SD) patient age in this group was 43 ± 4 years. The results were mainly analyzed in relation to the initial FSH and E2 profile.

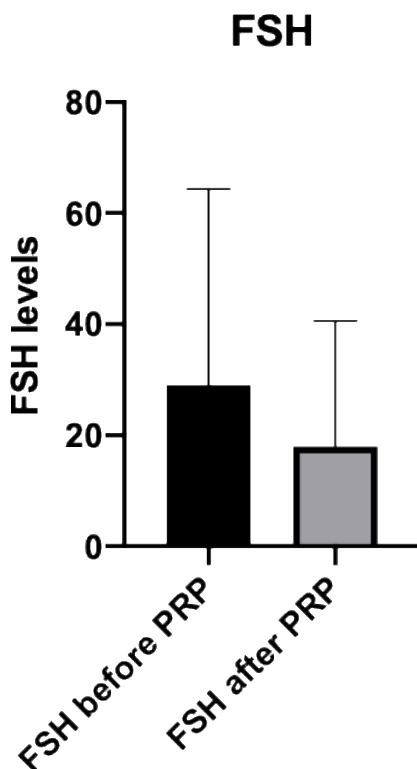


Figure 1: Comparison of mean FSH pre- and post PRP treatment.

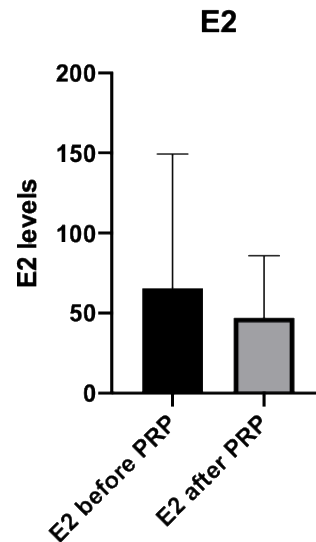


Figure 2: Comparison of mean E2 pre- and post PRP treatment.

Both serum FSH levels and mean serum E2 significantly reduced after treatment from 29.0pg/ml to 18.0pg/ml; $p < 0.0001$ (Figure 1) and from 65.6 pg/ml to 47.2 pg/ml; $p = 0.034$ (Figure 2) respectively. After PRP, 17/179 (9.49%) women became pregnant. Menopausal women from three to eleven months, who were not seeking for pregnancy, cited that they had a feeling of wellness, improved or halted hot flushes and vaginal dryness for the following eight to ten months post treatment.

During the procedure, except for a light vaginal bleeding which lasted five minutes at most and halted under local pressure, no other complications were reported. Furthermore, it should be mentioned that no painkillers were administered. None of the 179 women have reported any complications post operatively.

6. Discussion

To our knowledge, this is the largest cohort in the literature evaluating the efficacy of PRP intra-ovarian infusion on ovarian rejuvenation. The results of our observational study revealed that PRP intra-ovarian injection is associated with improved function of ovarian tissue.

During the first menstrual cycle post PRP treatment a decrease of a previously high FSH level and high E2 was recorded. The decrease in FSH level was in

agreement with that pinpointed in a case series report published recently reporting the use of PRP in menopausal and prematurely menopausal women [15] as well as in case series reporting the use of PRP in poor responders to IVF [2,12], with one-month post PRP hormonal levels influenced similarly, i.e., there was a decrease in FSH levels. The positive effect of PRP on ovarian tissue and function may be viewed as being mirrored by the decrease in FSH as previously documented, albeit on a case series level, by other researchers [2,12,15].

Pantos et al. studied a group of eight infertile menopausal women (with amenorrhea of 12-96 months) [2]. In approximately 40% of the women, menstrual cycles were restored within 1-3 months after the injection, while 18.5% of them experienced resumption of ovulation cycles with 1-5 oocytes obtained from the IVF cycles [2]. Sills et al. investigated the effects of the intra-ovarian injection of activated PRP in four cases in 2018 and observed increased AMH and significantly decreased FSH levels with at least one embryo obtained from the IVF cycles in all patients [13]. However, the precise mechanism of PRP in ovarian rejuvenation has not been known yet. A proposed hypothesis is that the cell growth factors present in PRP may stimulate the remaining stem cells in the ovaries and thus provide necessary conditions for the differentiation of those cells to be strengthened [16].

It is noteworthy that no adverse side effects were reported by any of our patients which is in agreement with the current literature [2,12,15]. There are no studies reporting any side effects from the PRP application in the reproductive system [15]. Various studies highlight that PRP growth factors do not present with risk, are non-mutagenic and are incapable of inducing tumor generation [17,18].

Despite the strength of this study which is the largest cohort of patients that underwent PRP for ovarian rejuvenation, it is limited by several factors, including its retrospective nature as well as the absence of a

placebo control group. The present research was an uncontrolled longitudinal study with all the patients receiving the same pre- and post-interventions without a control group. Therefore, for the best valid comparison homogeneity, a control group can be included in future studies.

The evidence on the clinical application of intra-ovarian PRP injection is very novel and has not been sufficiently elucidated yet. Future further randomized clinical trials in this field are needed to shed light in the use of PRP in ovarian rejuvenation.

7. Author Contributions

Fraidakis Matthaios: As the Head and Founder of Crete Fertility Centre (CFC), conceived the idea of this study, was the treating doctor of the patients, contributed to the interpretation of the data, co-drafted the manuscript and reviewed its final version.

Anifantaki Alik: Senior embryologist of Crete Fertility Centre collected the pz blood and prepared the plasma in CFC labs, collected the information and reviewed the manuscript.

Skouradaki Meltini and Tsakoumi Vivi: Collected the pz blood and prepared the plasma in CFC labs, collected the information and reviewed the manuscript.

Bitzopoulou Popi: Collected the information and reviewed the manuscript.

Prombona Nefeli: As Midwife, assisted **Dr. Fraidakis** to his clinical work and reviewed the manuscript.

Kakouri Persefoni: Collected the data and drafted the manuscript.

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