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AUTOLOGOUS PLATELET-RICH PLASMA (PRP) INTRACORTICAL OVARIAN INJECTION RESTORED OVARIAN FUNCTION AND FOLLICULOGENESIS IN POOR RESPONDERS AFTER ONE MONTH: A CONTROLLED PILOT STUDY

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ABSTRACT

Objective: The aim of our study was to evaluate whether autologous Platelet-Rich Plasma (PRP) injected in the ovarian cortex has therapeutic potency in restoring ovarian function in poor ovarian responders (PORs) after one-month therapy. PRP is beginning to be used in the treatment of ovarian infertility with a possible explanation for its role in improving the follicular microenvironment as well as the influence of growth factors on ovarian stem cells in postnatal oogenesis. Methods: This prospective randomized study included women who contacted the department of IVF in the period from 2017-2018. Women were selected according to the ESHRE consensus on the definition of PORs. A total of 30 women were divided in two groups according to the patient choice: ovarian PRP injection (15 patients) or no intervention (15). PRP was prepared by Regen ACR®-C Kit according to the manufacturer's guidelines. Approximately 3-5 ml of the PRP was injected in ovarian cortex using transvaginal ultrasound guidance. The serum concentration of FSH, LH, estradiol and AMH were determined before the treatment and day-3 of first menstrual cycle after treatment in order to monitor ovarian function. Results: After PRP treatment, the women had significant improvement in ovarian reserve. AMH level in serum was significantly increased, $p<0,05$ ($p=0,02$), FSH level significantly decreased, $p<0,01$($p=0,003$) and number of antral follicles after applying the PRP significantly increased, $p<0,001$ ($p=0,0007$). Conclusions. One-month therapy with PRP has the potency of significantly recovering ovarian function both in its hormonal and follicular development abilities.

Key words: poor ovarian reserves, platelet-rich plasma, Growth Factors

INTRODUCTION

Due to remarks of more authors where the use of PRP improves the function of the target organ, it starts an enthusiastic use of PRP in patients with ovarian insufficiency (1). A possible explanation is the probability of improving the follicular microenvironment as well as the influence of growth factors on ovarian stem cells in postnatal oogenesis. Receptors for growth factors presented on granulose cells confirming their association with the activation process of the primordial follicles. The Growth Factors (GFs) contained in platelet alpha granules are a major part of the PRP. They induce, through appropriate transmembrane receptors in target cells, a whole range of intracellular processes leading to proliferation, differentiation, matrix formation, osteoid production, collagen synthesis, haemostasis, and everything that leads to tissue recovery and regeneration. It is noted, that the mitogen effects of PRP are only limited to augmentation of the normal healing process and is theoretically not mutagenic, as the GFs released do not enter the cell or its nucleus, but only bind to the membrane receptors and induce signal transduction

mechanisms (2).

Oogenesis is the most significant function of the ovarian tissue. For a long time, it was believed that a woman was born with a certain reproductive potential. This dogma has been challenged by evidence supporting postnatal oogenesis in mammals (3). Reports demonstrating formation of new oocytes from newly discovered germline stem cells, referred to as oogonial stem cells (OSCs), has opened new avenues for treatment of female infertility (4). In this context, Tilly's latest review discusses a new concept of how oocyte and their precursor cells can be metabolically altered in order to maintain or increase ovarian function and fertility in a woman. However, if it can be shown that human OSCs are possible precursors of oocytes capable of further fertilization, then they have a potentially high value in the treatment of fertility dependent on aging (5). In addition, it is assumed then OSCs are necessary in the process of haemostasis, regeneration and protecting the integrity of the ovarian tissue. Namely, on the surface single-layer epithelium, "injury" is caused with each ovulation, monthly, and followed by local tissue repair (6).

MATERIALS AND METHODS

The study was approved by the local Ethnics 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. They had normal hysteroscopy and their partners had a normal semen analysis (7). 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 (8). Women with present infection, haemoglobin lower than 11g/L or platelets lower than 150x10⁹/μL were excluded from study (9). In our study, we used a Regen PRP system, (Regen Laboratory, Mont-sur-Lausanne, Switzerland) (10). 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. Intervention was made under propofol intravenous anesthesia following a protocol set by our IVF department. We used a 30 cm single lumen 17G aspiration needles (COOK / Australia). We assessed the PRP benefits through the values of AMH, FSH, estradiol, and AFC. The serum concentration of FSH, estradiol, and AMH was determined before the treatment and day-3 of the first menstrual cycle after treatment to monitor ovarian function.

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

In this study demographic characteristic (age, BMI, infertility duration) and baseline ovarian reserve markers (FSH, AMH, AFC) were similar between the two groups. For H = 3.83 and p> 0.05 (p = 0.15), there was no significant difference in FSH value between the two groups (before intraovarian PRP injection). Namely, the FSH in the group later treated with platelet-rich plasma (PRP) was 17.27 ± 5, 29 IU / L, and in the control group of patients with no intervention was17.64 ± 6.69 IU / L. The value of AMH in the control group, 0.56 ± 0.31 ng/ml for p> 0.05, (p = 0.19) was insignificantly higher than the value of AMH in the study group (before intraovarian PRP injection) 0.35 ± 0.19 ng / ml. For H = 0.96 and p> 0.05 (p = 0.62) there was no significant difference in the number of antral follicles between the study group, before intraovarian administration of PRP, (4.53 ± 0.99) and the control group of patients without intervention (4.53 ± 1.06).

The mean value of platelet concentration was 226,27±82,80/10⁹L. We evaluate day-3 baseline ovarian values of AMH, FSH, and AFC in the cycle before intraovarian cortical PRP injection and in 41±18 days after PRP. After one treatment with PRP, the women in group I had significant improvements in ovarian reserve (table 1 and 2). AMH level in serum was significantly increased, p<0,05 (p=0,02), and FSH level significantly decreased, p<0,01(p=0,003). In our study we noticed an increased

number of antral follicles after applying the PRP, p<0,001 (p=0,0007). There is no change in the control group.

Table 1. FSH values in group I before & after intraovarian PRP injection

Variable	Mean	Std. Dv.	N	Diff.	Std. Dv. Diff.	t	df	p
FSH (before intraovarian PRP injection)	17,27	5,29						
FSH (after intraovarian PRP injection)	12,38	4,26	15	4,89	5,29	3,58	14	0,003

Table 2. Differences between AMH and antral follicular counts in group I before & after intraovarian PRP injection

Pair of Variables	Valid	T	Z	p-level
antral follicular counts before & after PRP injection	15	0,00	3,41	0,0007
AMH before & after PRP injection	15	16,00	2,29	0,02

We can notice that the PRP method objectively has the potential of recovering ovarian function and increases the chances of clinical pregnancy in PORs. After treatment with PRP, AMH level in serum was significantly increased, p<0,05 (p=0,02), and FSH level significantly decreased, p<0,01(p=0,003). In our study we noticed an increased number of antral follicles after applying the PRP, p<0,001 (p=0,0007). It seems this fact is achieved mostly by the paracrine effects of Growth Factors contained in platelet alpha granules as a major part of PRP.

A further analysis was carried out to identify factors that could correlate or predict the response of the PRP injection in ovarian tissue. We took into account the previous investigations carried out by E. Scott Sills but our tests were not significant at the 95% confidence level. Further analysis was performed to identify factors which 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.

DISCUSSION

The use of PRP in reproductive medicine was first reported in 2017 by Pantos at a medical conference at ESHRE (1). During the following year the next pilot study was published. This pilot study was focused on intraovarian injection of autologous platelet-rich plasma before in vitro fertilization (11). It remains essential to understand the physiological basis of the aging of the ovaries in order to interpret the mechanisms of action. 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 vascularization and their significant role in the aging of the follicles. Receptors for growth factors are present on granulose cells confirming their association with the activation process of the primordial follicles. The most important component in PRP is the transforming growth factor beta family (TGF beta) that plays a significant role during the developmental phases of the follicle (12). A confirmation of all the above statements is also obtained from the Hosseini study (13). This study evaluates the effects of platelet-rich plasma (PRP) on growth and survival of isolated early human follicles in a three-dimensional culture system. The conclusion was that media supplementation with PRP can better support viability and growth of isolated human early preantral follicles in vitro.

On the other hand, the presence of OSCs on the surface of the ovarian tissue, under certain conditions, are able to produce de novo primordial follicles and thus the appearance of new antral follicles. It is noteworthy to mention that only a fraction of the OSCs in culture undergo meiosis to form oocytes. Why only a few cells express Stra8 and undergo differentiation remains unknown (5). Elucidating the mechanisms that cause OSCs to age could lead to new treatments that could delay ovarian aging and slow infertility. In addition, several questions about the mechanism of action of the PRP remain unanswered.

It is clear that several challenges exist when trying to interpret the efficacy of PRP in PORs. There are no standard protocols or standard definitions of poor responders, which can make it challenging to compare studies and perform a meta-analysis (14). Many efforts need to be made before determining the best approach and method for prevention and treatment of PORs.

Limitations, reasons for caution: There are still insufficient controlled clinical trials in the field of ovarian infertility. The limitation of this format should be taken into account i.e. sample size, design, the absence of previous data attesting the safety of PRP injection into ovarian cortex, etc. Future studies are needed to corroborate our results.

CONCLUSION

Because the diagnosis of PORs often leaves limited time for treatment, patients should be given the choice of possible treatments with appropriate information consent. High concentrations of growth factors and cytokines in the PRP in damaged tissue affect the balance between anabolic and catabolic processes, optimizing the tissue environment, and favouring the process of tissue healing.

One-month therapy has the potency of significantly recovering ovarian function both in its hormonal and follicular development abilities. Furthermore, intraovarian injection of autologous PRP in PORs before IVF gives the impression of being a new promising method for achieving better IVF outcome.

Wider implications of the findings: Treatment whit PRP is simple, safe, minimally invasive and with potential cost-effectiveness. In this respect, a continuous interaction between biological and clinical research is vital to develop a proper clinical treatment for a selected group of patients who fulfilled the criteria of poor ovarian responders.

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Disclosure statement

None of the authors report any conflict of interest with this research.

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