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Mohamed Rezk
Department of Obstetrics and gynecology , Mansoura New General Hospital , Egypt,
hegazy2014@gmail.com

Mohamed Moustafa
Department of Obstetrics and Gynecology ,Faculty of medicine ,AL-Azhar University ,Cairo ,Egypt,
mohamedkhaled@gmail.com

Hassan Abd-Rabboh
Department of Obstetrics and gynecology , International Islamic Center For population Studies and Research - AL-Azhar University, hassanabdrabboh@gmail.com

Mosaad Ibrahim
Department of Medical Biochemistry, International Islamic Center For Population Studies and Research - Al-Azhar University, elfaroklabm@gmail.com

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Effect of Intraovarian Injection of Autologous Platelet Rich Plasma (PRP) in Premature Ovarian Insufficiency

Obstetrics & Gynecology

Mohamed Ramadan Ibrahem Rezk [1,*] M.B.B.Ch, Mohamed Khaled Moustafa [1] MD,
Hassan Ahmed Abd-Rabboh [2] MD and 3Mosaad Mohamed Ibrahim [3] MD

Corresponding Author:
Mohamed Ramadan Ibrahem Rezk
Dr.mystery.knight@gmail.com

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[1]*Obstetrics and Gynecology Department, Faculty of Medicine, Al-Azhar University Cairo, Egypt.*

[2]*Obstetrics and Gynecology Department, International Islamic Center for Population Studies and Research, Al-Azhar University Cairo, Egypt.*

[3] *Medical Biochemistry Department, International Islamic Center for Population Studies and Research, Al-Azhar University Cairo, Egypt.*

ABSTRACT

Background: The usage of intraovarian platelet-rich plasma (PRP) injections to enhance ovarian functioning in females having poor ovarian response (POR) or primary ovarian insufficiency (POI) has recently been reported.

Aim of the work: To see if intraovarian injections of autologous PRP can lead to ovarian rejuvenation as well as folliculogenesis reactivation in females suffering from premature ovarian insufficiency.

Patients and methods: This prospective controlled study on 50 infertile women suffering from premature ovarian insufficiency was conducted at the obstetrics and gynaecology department of Al Azhar University hospitals. Inclusion criteria: Women under the age of 40 who have at least one ovary, have been infertile for more than a year, have had at least one previous unsuccessful (or cancelled) IVF cycle, or have had amenorrhea for at least 3 months, and have a raised follicle-stimulating hormone (FSH) level (> 25 IU/l), measured at least twice, 4 weeks apart. Exclusion criteria: Women older than 40 years. Ongoing malignancy. Chronic pelvic pain.

Results: The mean total number of follicles at first month was cases was 1(±0.06 SD) with range (0-2), at second month was 1.32(±0.65 SD) with range (0-3) and at third month was 2.5(±0.4 SD) with range (1-3).

Conclusion: Finally, in poor responders, intraovarian injection of autologous PRP may be regarded as an additional therapy strategy. However, before clinical trials can begin, the safety and effectiveness of this new treatment technique, as well as its short- and long-term side impacts, must be examined in more high-quality studies.

Keywords: *Platelet-rich plasma; intraovarian infusion; ovarian rejuvenation; ovarian reserve.*

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INTRODUCTION

In pre-menopausal females, POI is an uncommon but significant reason for sex steroid shortage and infertility. [1]

POI is described as the syndrome of ovarian functioning depletion before 40 years of age, with oligo/amenorrhoea and elevated FSH level (> 25 IU/l), measured at least twice, 4 weeks apart. Approximately 1% of females under the age of 40 are affected by this issue. Premature loss of primordial follicles reduces the ovaries' ability to produce sex hormones significantly. [2]

Infertility and the effects of hypoestrogenism are especially critical for females of reproductive age. Hot flushes, nocturnal sweats, sleeplessness, vaginal dryness, depression, dyspareunia, and reduced libido are all symptoms of POI. Osteoporosis, urogenital symptoms, cardiovascular illnesses, lipid disorders, psychological issues, as well as sexual and cognitive dysfunction are all long-term effects of POI. [3]

While up to 90% of POI instances are idiopathic, the remaining are caused by genetic, enzymatic, or immunological factors.[4]

POI affects 0.01% of females under the age of 20 and 1% of females under the age of 40. [5]

PRP is a natural substance that contains a high concentration of platelets as well as growth factors that are three to five times higher than plasma. Platelet derivate growth factors (PDGFs), transforming growth factor-beta (TGF-β), vessel endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and insulin growth factor (IGF) are some of the growth factors that are kept in granules [5,6]. These

cytokines induce angiogenesis and play a crucial role in cell proliferation, chemotaxis, and differentiation of mesenchymal and other cells. [6]

PRP has recently been employed in ophthalmology, orthopaedics, surgery, and the healing of wounds for a variety of medical disorders. [7]

PATIENTS AND METHODS

This is A prospective controlled study was conducted in the obstetrics and gynaecology department of Al Azhar University hospitals from April 2020 to December 2021.

Fifty infertile women suffering from premature ovarian insufficiency were hospitalized at Al Hussein University Hospital's obstetrics and gynaecology department, and they fulfilled inclusion and exclusion criteria.

Inclusion criteria: Women under the age of 40 who have at least one ovary, have been infertile for more than a year, have had at least one previous unsuccessful (or cancelled) IVF cycle, or have had amenorrhea for at least 3 months, and have a raised FSH concentration (> 25 IU/l), measured at least twice, 4 weeks apart.

Exclusion criteria: Women older than 40 years. Ongoing malignancy. Chronic pelvic pain. A history of major lower abdomen surgeries that resulted in pelvic adhesions. Use of an anticoagulant for which plasma infusion is not recommended

Sample size: 50 women

Sampling technique: This study was performed on systematic random sampling technique.

Methods: The researcher introduced himself to all participants included in this study and requested that they participate after explaining the purpose of the study. All participants were given detailed information about the study's goal and predicted advantages. All participants gave their informed verbal agreement, and the data was kept confidential.

Complete history was taken with special emphasis on: Personal history (age, marital status, parity, address, occupation and any special habits. Complaint of each woman in the study: period of infertility, type of infertility whether primary or secondary, hirsutism and acne. Menstrual history: with emphasis on menstrual dating and regularity. Obstetric history: History of similar condition (recurrent abortion); number of abortions, induced or spontaneous, followed by surgical evacuation or not and if there was any post abortive complications. Contraceptive history :(Type& duration) Past history of any medical problem, allergy to certain drugs and any previous operations . Family history of infertility or consanguinity.

Clinical examination: Physical examination included General examination: Weight, Height, BMI, Abdominal examination , Local (Pelvic) examination

Investigations: General (CBC, urinalysis, Random blood sugar) when needed. Specific: Hormonal profile in all patients of the study, the following were obtained prior to the procedure: Anti-Mullerian hormone (AMH). FSH. Estradiol

On the day of the PRP infusion, 17.5 mL of peripheral venous blood was drawn into a syringe containing 2.5 mL of Acid Citrate A, an anticoagulant solution, and centrifuged at 1200 rpm for 12 minutes to separate RBCs, then plasma was centrifuged at 3300 rpm for 7 minutes to acquire PRP with 4-5 times more platelets than peripheral blood. 1 ml of PRP that contained about (1000,000) platelets was injected into the ovary.

The procedure was done through laparoscopy under general anesthesia. The surgeon created an incision beneath the umbilicus and inserted a cannula to inflate the abdomen with CO2. The doctor was able to see the abdominal organs quite plainly because of the gas. The surgeon put the laparoscope into the incision after the abdomen was inflated. Organs could be examined in real time thanks to a camera linked to the laparoscope that projected images on a screen. One or two incisions between one and two cm in length were made by the surgeon. Other tools could be put through these incisions. Then, through the anterior abdominal wall, a 35 cm single-lumen 17 G needle (used for egg retrieval) was introduced, through which modest volumes of fluid were injected under the capsule of the ovary, where the remaining follicles were located. The needle was withdrawn at the conclusion of the process, which took only 10-15 minutes. The instruments were removed after the process was completed. Stitches or surgical tape were used to close the incisions. The patient was woken after bandages were put over the incisions.

Outcome assessment

After receiving a PRP injection into the ovary, the women were examined via transvaginal ultrasound for at least 2-3 months for the formation of new tiny follicles.

Then, 3 months after the PRP injection, AMH, FSH, and estradiol levels were measured and compared to baseline (before the PRP) levels.

Ultrasound examination: The women were examined in the lithotomy position with an empty bladder using a Philips HD5. On day 2 of the menstrual cycle or withdrawal bleed, a sterile vaginal speculum has been introduced, and a TV ultrasonography probe (7.0-MH endo-vaginal probe) has been placed in the vagina roughly 1 cm away from the cervix to evaluate the volume of the ovary and antral follicle counts (AFC).

Administrative design:

An official written administrative permission letter was obtained from the dean of the Al Azhar Faculty of Medicine, the Al Azhar University hospital manager, and the head of the Obstetrics and gynecology department at the same university. The title and objectives of the study were explained to them to ensure their cooperation.

Ethical committee:

Approval from the college of medicine ethics committee was also acquired, as well as clearance from the Faculty of Medicine's Institutional Research Board (IRB), at AL Azhar University – Cairo

Statistical analysis of the data

The data was loaded onto a computer and analyzed with the IBM SPSS program version 20.0. (Armonk, NY: IBM Corp). Numbers and percentages have been employed to describe qualitative data. The normality of the distribution has been verified by employing the Kolmogorov-Smirnov test. The range (min and max), mean, SD, median, and interquartile range (IQR) have been used to express quantitative

data. The significance of the obtained findings has been determined at the 5% level.

Repeated measures tests were utilized. When two or more periods of normally distributed quantitative data are compared, an ANOVA is employed.

RESULTS

		Cases (n=50)
Age (years)		
Range		24 – 38
Mean ± SD		31.1 ± 4.38
Infertility period (years)		
Range		1 – 5
Mean ± SD		2.66 ± 1.33
Weight (kg)		
Range		65.5 – 103.5
Mean ± SD		84.29 ± 9.89
Height (cm)		
Range		157 – 173
Mean ± SD		164.52 ± 4.88
BMI (kg/m^2)		
Range		25 – 37.6
Mean ± SD		31.11 ± 3.48
Type of infertility	No.	%
Primary	39	78.0
Secondary	11	22.0
Parity		
0	39	78.0
1	5	10.0
2	6	12.0
Amenorrhea		
Primary	2	4.0
Secondary	48	96.0

Table 1: Distribution of demographic data in the cases studied

This table shows that the mean age of the studied cases was 31.1 (±4.38 SD) with range (24-38) years, the average infertility period was 2.66 (±1.33 SD) with range (1-5) years, the average BMI was 31.11 (±3.48 SD) with range (25-37.6) kg/m2, among the studied cases there were 39 (78%) with primary infertility and 11 (22%) with secondary infertility, there were 39 (78%) with nulliparous, 5 (10%) with 1 parity and 6 (12%) with 2 and 48 (96%) had secondary amenorrhea and 2 (4%) had primary.

FSH (mIU/ml)	Cases (n=50)	F	P
Baseline			
Range	25.6 – 95.8	306.7	<0.001*
Mean ± SD	48.51 ± 15.61		
First month			
Range	24.5 – 73.8		
Mean ± SD	41.33 ± 15.64		
Second month			
Range	20.4 – 68.8		
Mean ± SD	38.21 ± 15.64		
Third month			
Range	15.1 – 66.8		
Mean ± SD	34.06 ± 15.74		

F: repeated measures ANOVA test

p: p value for comparing different groups

*: Statistically significant at $p \leq 0.05$

Table 2: Comparison between FSH at different periods

This table reveals that the difference in FSH between periods was statistically significant.

AMH (ng/ml)	Cases (n=50)	F	P
	Baseline		
Range	0.03 – 0.9	9.34	0.041*
Mean ± SD	0.39 ± 0.09		
	First month		
Range	0.03 – 0.95		

Mean ± SD	0.63 ± 0.1
	Second month
Range	0.1 – 1.0
Mean ± SD	0.71± 0.12
	Third month
Range	0.2 – 1.1
Mean ± SD	0.8 ± 0.11

F: repeated measures ANOVA test
p: p value for comparing different groups
*: Statistically significant at $p \leq 0.05$

Table 3: Comparison between AMH at different periods
This table shows that AMH varied statistically significantly at different periods.

Estradiol (pg/mL)	Cases (n=50)	F	P
Baseline			
Range	14.4 – 22	96.486	<0.001*
Mean ± SD	18.04 ± 2.31		
First month			
Range	15.3 – 22.9		
Mean ± SD	18.6 ± 2.25		
Second month			
Range	15.3 – 24.2		
Mean ± SD	19.16 ± 2.34		
Third month			
Range	15.3 – 24.3		
Mean ± SD	19.43 ± 2.36		

F: repeated measures ANOVA test
p: p value for comparing different groups
*: Statistically significant at $p \leq 0.05$

Table 4: Comparison between Estradiol at different periods
This table reveals that the difference in estradiol at different periods was statistically significant..

	Cases (n=50)		
	Number of follicles 14-18 mm	**Number of follicles ≥ 18 mm**	**Total number of follicles**
First month			
Range	0 – 2	0	0 – 2
Mean ± SD	1 ± 0.06	0	1 ± 0.06
Second month			
Range	0 – 3	0	0 – 3
Mean ± SD	1.32 ± 0.65	0	1.32 ± 0.65
Third month			
Range	1 – 3	0-1	1 – 3
Mean ± SD	2.04 ± 0.2	0.71±0.2	2.5 ± 0.4

Table 5: Distribution of studied cases as regard Size of follicles
This table shows that the mean total number of follicles at first month of the studied cases was 1 (±0.06 SD) with range (0-2), at second month was 1.32 (±0.65 SD) with range (0-3) and at third month was 2.5 (±0.4 SD) with range (1-3).

DISCUSSION

Ovarian ageing is a physiological phenomenon characterised by a decrease in the number and quality of oocytes preserved inside the follicular cohort. The physiological fall in the number of follicles that occurs as women age has serious consequences for fertility and is becoming a more common reason for females to look for fertility therapy. Actually, in the United States, the number of females receiving assisted reproductive technologies (ART) for infertility therapy with a prognosis of decreased ovarian reserve (DOR) increased from 22,089 in 2010 to 40,883 in 2017. [8]

Primary ovarian insufficiency (POI), the most severe type of DOR, impacts 1% of reproductive-age women and is defined by a significant loss in ovarian reserve before the age of 40, leading to menopausal serum gonadotropin hormone concentrations and monthly irregularity or amenorrhea. POI is defined by the European Society of Reproductive Medicine and Embryology as the existence of oligomenorrhea-amenorrhea for a minimum of four months plus serum follicle stimulating hormone (FSH) concentrations of ≥ 25 IU/ml assessed at least twice in a four-week interval, with a start prior to the age of 40. [9]

Females having POI as well as PORs and poor fertility results are regarded as the major challenges of reproductive research. POI impacts 1% of females of reproductive age and is characterised by markedly diminished ovarian reserve, menstrual irregularities, or amenorrhea before the age of 40.[10]

In spite of numerous methods to improve the results of ART in PORs, the rate of pregnancy in such individuals

stays low. PRP has lately become popular in regenerative medicine in a variety of sectors, including orthopaedics, dermatology, dentistry, and cosmetic operations. [11]

Platelet-rich plasma (PRP) is platelet-rich blood plasma that has been supplemented with growth factors and cytokines. Platelet accumulation in a tissue promotes cell growth and tissue regeneration via protein secretion in response to cytokines, while growth factors lead to cellular injury reversal and tissue renewal. PRP was widely used in the field of infertility to treat thin endometrium and failed implants. It has recently been looked into in patients with ovarian insufficiency. [12]

The goal of this research is to see if intraovarian injections of autologous PRP can lead to ovarian rejuvenation as well as folliculogenesis reactivation in females suffering from premature ovarian insufficiency in assisted reproduction units.

The average age of the patients studied was 31.1 (±4.48 SD) with a range of 24–38 years, the average infertility period was 2.66 (±1.33 SD) with a range of 1–5 years, and the average BMI was 31.11 (±3.48 SD) with a range of 25–37.6 kg/m2, with 39 (78%) cases having primary infertility and 11 (22%) having secondary infertility. There were 39 (78%) with nulliparous women, 5 (10%) with 1 parity, and 6 (12%) with 2, and 48 (96%) had secondary amenorrhea, and 2 (4%) had primary.

Stojkovska et al. [13] conducted the first study, which compared the live birth rate (LBR) of 20 poor responders who received 3–5 mL of autologous PRP under transvaginal ultrasonography surveillance to 20 control subjects. Other basic parameters like age, BMI, partner's age, and baseline FSH were balanced in both groups, and multivariable analysis revealed no statistical differences when all known confounders were taken into account. Following 61 ± 18 days, the same procedure of low-dose activation with GnRH antagonist was employed in both groups when PRP was applied. According to the findings, there was no statistical significance in clinical pregnancy as well as LBR. In the present study we found that mean ovarian volume of the studied cases was 6.15 (±2.11 SD) with range (1.9-2.11) cm^3 and all the studied cases had normal ovary with no endometriosis.

According to the findings of this investigation, there was high statistically significant difference FSH at different periods.

Injecting 4 mL of PRP into each ovary using 30 mL of peripheral blood, Sfakianoudis et al. [14] noticed a significant decrease in FSH concentrations and an increase in AMH values from 0.02 to 0.08 ng/mL in a POI woman 40 years old with 19 months of amenorrhea. A pregnancy was achieved following 8 weeks of PRP therapy and natural IVF, but it terminated in a biochemical loss miscarriage.

Pacu et al. [15] showed that FSH (UI/ml) seemed to decline more during the second menstrual cycle (7.05±1.43) than the first cycle (8.30±2.13) , with a significant difference reaching $P < 0.001$ when compared to the level prior to PRP therapy (11.50±4.05). Nevertheless, six months after therapy, the level (11.28±3.23) has returned to pre-treatment levels. Although LH (UI/ml) followed a comparable pattern to FSH, the difference between the first and second menstruation cycles (5.10±1.29 and 5.20±1.44, respectively) and the later recovery to pre-PRP levels was less pronounced (pre-PRP, 7.25±1.92 and at 6 months, 6.00±2.36).

Our findings regarding Comparison between AMH at baseline, first month, second month, third month revealed statistically significant difference between them.

Pacu et al. [15] showed that after PRP therapy, the AMH level (ng/ml) improved in both the first (0.82±0.33) and second (0.99±0.36) menstrual periods, with the second cycle showing a significant rise ($P<0.05$). Its level had fallen significantly ($P < 0.05$) by the 6th month after therapy (0.7±0.33) compared to the amount at the second menstrual cycle after therapy, and while it remained slightly greater than pre-PRP therapy (0.69±0.32), the difference has not been deemed significant.

Petryk et al. [16] documented that 38 infertile females having low ovarian reserve and at least two failed IVF cycles got PRP intraovarian injections via an ultrasound-guided technique or a laparoscopic-assisted method. Following the procedure, FSH and LH concentrations both dropped significantly. In the meantime, AMH levels increased from 0.08 to1.1 ng/mL. Four of the 38 females who conceived naturally had healthy babies. Twenty ladies had IVF, and fifteen of them successfully retrieved 1 to 3 eggs. In eight females, the embryos were transferred during the same menstrual period. Due to poor endometrial quality or other factors, another seven patients might be able to obtain frozen embryos for eventual transfer. There have been two live, healthy deliveries and four continuing conceptions following both fresh and frozen embryo transfers, resulting in a pregnancy success percentage of 26% (10/38) in all cases treated with PRP. Nonetheless, two instances of genetic anomalies have been discovered in seven frozen nuclei.

Our results regarding Comparison between Estradiol at baseline, first month, second month, third month revealed high statistically significant difference.

In research by Barad et al. [17], 21/43 women still menstruated regularly (subgroup A, age 43.9 ± 5.1 years), whereas 23/43 (subgroup B, age 42.6 ± 6.2 years) had been amenorrheic for a mean of 6 months. AMH, FSH, and estradiol were 0.18 ± 0.20 ng/mL, 37.5 ± 47.6 mIU/mL, and 100.2 ± 73.4 pg/mL in A, and 0.06 ± 0.11 ng/mL, 73.0 ± 44.8 mIU/mL, and 66.7 ± 57.6 pg/mL in B, respectively. After APRP, A-patients' estradiol levels have risen to 211 ± 193.7 pg/mL ($P = 0.029$), whereas B-patients only showed a tendency to 98.1 ± 86.5 ($P = 0.09$). 14/21 (66.7%) of A women began IVF cycles, with 5/21 (23.8%) completing the process. So far, just one woman has produced a clinical pregnancy that is still ongoing. Among B women, 8/23 (34.8%) started IVF cycles, but only 2/23 (8.7%) completed retrieval and none became pregnant.

In the current study we found that mean total number of follicles at first month was 1 (±0.06 SD) with range (0-2), at second month was 1.32 (±0.65 SD) with range (0-3) and at third month was 2.5 (±0.4 SD) with range (1-3).

Cakiroglu et al. [10] reported 311 females diagnosed with POI based on oligo/amenorrhea lasting a minimum of four months, an increased serum FSH of > 25 IU/l on two occasions at a four-week interval, and beginning prior to the age of 40 years. These individuals received 2–4 mL of autologous PRP injections produced using 20

mL of peripheral blood. While the retrieved oocyte numbers were not observed prior to and following PRP injection, PRP therapy enhanced AMH and antral follicle count. There were 16 live births from 23 patients who had spontaneous pregnancies. 13 pregnancies and 9 live deliveries have been recorded among IVF women. Twenty-five women (8.0%) had live births; while another 25 (8.0%) had cryopreserved embryos. Because the average AMH concentration prior to PRP therapy was quite high at 0.13 ± 0.16 ng/mL, the recruited POI individuals may be at an early stage.

CONCLUSION

In poor responders, an intraovarian injection of autologous PRP could be deemed as an alternative therapy approach. Nevertheless, before clinical implementation, the safety and effectiveness of this unique therapeutic technique, as well as its short-and long-term adverse impacts, must be explored in more high-quality studies.

Conflict of interest : none

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