
Effect of Intraovarian Injection of Platelet-Rich Plasma on Ovarian Rejuvenation in Patients with Poor Ovarian Reserve

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Abstract

Background:

Objectives: Assess the effects of intraovarian injection of autologous PRP on ovarian reserve measures and pregnancy prognosis in women with a history of low ovarian reserve.

Patients & Methods: At the Faculty of Medicine, Obstetrics and Gynecology department, the outcomes of 28 infertile patients with poor ovarian reserve was injected with autologous platelet-rich plasma (PRP) into her ovary, a procedure typically reserved for POR women., Ten days following the onset of menstruation, the patient underwent a PRP. The study Started from from May 2021 to August 2023. the study protocol was registered by the faculty of medicine ethical scientific committee. (**Ethical clearance letter number 218G/2017**).

Results: No substantial relation was noticed among pregnancy outcome with infertility duration ($P=0.792$), pre-AFC ($P=0.457$) and post AFC ($p=0.483$). There was no significant relation between pre and post (FSH, LH, AMH) ($P>0.05$). While, pre and post estradiol were significantly decreased among women with positive pregnancy outcome (34.93 ± 0.12 , 36.67 ± 0.58) than women with negative pregnancy outcome (48.60 ± 20.13 , 49.92 ± 19.74), ($P=0.003$). There was no significant relation between pre and post ovarian volume, ovarian doppler (PSV1) and (PSV2), ($P>0.05$).

Conclusion: There was no substantial increase in the pregnancy rates, embryo formation, oocyte, antral follicle count, and Anti-Mullerian Hormone associated with intraovarian PRP injection.

Keywords: Intraovarian injection of Platelet-Rich Plasma, ovarian rejuvenation, poor ovarian reserve.

INTRODUCTION

The pregnancy rate in this group of patients remains low, even though many different approaches have been studied to improve the success rate of ART in PORs. In re-

cent years, platelet-rich plasma (PRP) has seen widespread use for regenerative therapies across several medical fields, such as cosmetic surgery, orthopedics, dentistry, and dermatology [1]. PRP, or platelet-rich plasma, is a kind of blood plasma that has been supplemented with cytokines and growth factors [2] responsible for reversing cellular damage and rejuvenating tissues. Resuming menstruation in women showing symptoms of climacteric has been studied in situations of ovarian insufficiency [3]. One such example is the concept of using platelet-derived growth factors to activate germline stem cells (GSCs) in the ovaries, which are a source of renewable cells [4]. Cell migration, proliferation, differentiation, and division; angiogenesis; remodeling of the extracellular matrix; regeneration of tissues; and healing are all significantly aided by PRP [5].

PATIENTS AND METHODS

A prospective observational study involved 28 women with Primary Ovarian Insufficiency who were in attendance at the department of Gynecology and Obstetrics at University Hospital of Menoufia, Shebin Elkom, El-Menoufia Governorate, Egypt, from May 2021 to August 2023.

Ethics approval and consent to participate: After the study objective was explained in detail and clearly, the patient was asked to sign an informed consent form. In Menoufia, Egypt, The research design was authorized by the local ethical scientific committee of the Menoufia Faculty of Medicine. (Ethical clearance letter number 218G/2017)

Sample size: The outcomes of 28 infertile patients with poor ovarian reserve based on Bologna criteria.

Inclusion criteria: The patient has a history of underperforming ovaries (less than 3 eggs per in vitro fertilization cycle), ovarian reserve anomalies (such as an antimullerian hormone level below 1.1 ng/mL or antral follicle count below 5), and no underlying dis-

orders that might cause female infertility.

Exclusion criteria: All patients had male factor or tubal factor infertility; patients were not included in the study if they did not fulfill the inclusion criteria. These patients could have a history of myomectomy, other uterine anomalies, autoimmune diseases, thrombophilia disorders, endometriosis, thyroid dysfunction, ovarian insufficiency due to sex chromosome etiology, renal failure, cancer, or multiple fibroids larger than 5 cm.

Method: The study protocol (ID: 218G/2017) was accepted by the ethical scientific committee. Complete medical history, physical examination and investigations as prior to the operation, the following was collected from each patient in the study: hormonal profile: The following hormones are present: thyroid stimulating hormone (TSH), estradiol, luteinizing hormone (LH), anti-mullerian hormone (AMH), Ft3, and Ft4.

Ultrasound examination of AFC, Ovarian volume and Doppler study. On the second day of menstruation or withdrawal bleeding, women underwent a transvaginal ultrasonography examination while lying in the lithotomy position.

Prepared PRP was prepared by standard method at the lab of Menoufia university hospital. The PRP was activated with calcium gluconate (CG) in a 1:9 ratio after being kept for one hour at 4°C before injection [3]. Procedure for Intraovarian Injection In this case, PRP was administered ten days subsequent to the onset of menstruation. Each ovary was injected with 1.5 ml of activated PRP using a 17-gauge single lumen needle during a multifocal intramedullary injection while transvaginal ultrasonography was being monitored. The patient was under minimum anesthesia. After the treatment was finished, precise ultrasonography was taken to assess the pelvic region for vascular integrity and quantity of leaking. It was necessary to assess the serum AMH, FSH, LH, and E2 levels twice: once before the PRP injection and

again three months later. On the third day of menstruation, hormone levels were measured in PORs. We also monitored pregnant women all the way to birth and checked in with them after the PRP injection to see how their pregnancies were doing.

Outcome Measures:

Three months later, the serum levels of FSH, LH, estradiol, and AMH were examined. Ovarian volume, rate of pregnancy, number of retrieved oocytes, AFC, and Doppler ultrasonography.

RESULTS

The study population flowchart shown in Figure 1. Thirty women were diagnosed with primary ovarian insufficiency at Menoufia UH. Only two patients were not included in the study: one woman who did not want to participate withdrew, and another whose ovaries were not visible. The other twenty-eight patients were all eager to take part. The mean age was (34.60 ± 5.44) and body mass index was (27.40 ± 3.27), (Table 1). Also, the mean pre and post FSH were (13.53 ± 5.68 , 14.28 ± 5.43), pre and post LH were (11.60 ± 6.33 , 12.40 ± 5.86), pre and post AMH were (0.38 ± 0.29 , 0.35 ± 0.27) and pre and post E2 were (48.87 ± 19.96 , 50.16 ± 19.55). (Table 2). Additionally, the mean pre and post ovarian volume were (1.60 ± 0.48 , 1.50 ± 0.43), ovarian Doppler PSV1, PSV2 were (0.18 ± 0.08 , 0.18 ± 0.08) and pre and post AFC were (3.92 ± 2.00 , 3.48 ± 1.61). (Table 3). 10.71% of women were pregnant and 89.29% were not pregnant. (Table 4).

There was no significant relation between pre and post (FSH, LH, AMH) ($P > 0.05$). While, pre and post estradiol were significantly ($P = 0.003$). (Table 5) Distribution Hormonal data in relation to pregnancy outcome. (fig.2) there was no significant relation between pre and post ovarian volume, ovarian doppler (PSV1) and (PSV2), ($P > 0.05$). (Table 6). The mean change of FSH, LH, AMH, E2, AFC

and Ovarian volume were ($-.74000$, $-.72964$, $.02500$, -1.31071 , $.39286$, $.10714$) respectively with substantial difference among pre and post ($P \leq 0.05$). (Table 7). Mean change distribution of different parameters among the studied cases. (Fig.3).

DISCUSSION

One percent of reproductive-age women go through postmenopausal ovarian reserve (POI), the most severe kind of POR, which is marked by a substantial reduction in ovarian reserve before the age of 40.

PRP therapy is a relatively new approach to treating a wide range of medical conditions, including infertility and reproductive health [6]. Indicators of ovarian reserve such serum FSH, serum AHM, and count of antral follicle (AFC) were shown to improve after PRP injections in comprehensive review research included 663 infertile women [7]. Sills et al., [8] studied the impact of injecting PRP into the ovaries on women with a mean age of 42 ± 4 years. Patients' ovarian function was shown to be improved in every case two months after receiving trans-vaginal calcium gluconate-activated autologous PRP injection [9].

Thus, this study aimed to examine how injecting autologous PRP into a woman's ovary might affect her ovarian reserve and her ability to conceive in the event that she has a polycystic ovary syndrome (POR).

The current study showed that the mean age was (34.60 ± 5.44) and body mass index was (27.40 ± 3.27). Also, the mean duration of infertility was (5.96 ± 3.30).

Our study was close to with a study obtained by Rezk et al., [10] who found that Participants' ages ranged from 24 to 38 years old, with an average of $31.1 (\pm 4.48 \text{ SD})$. Of the patients, 39 (78%) had primary infertility and 11 (22%) had secondary infertility; the average infertility time was 3 years ($\pm 1.33 \text{ SD}$), ranging from 1 to 5 years. The mean BMI

was 31.11 kg/m^2 with a SD of 3.48., ranging from 25 to 37.6 kg/m^2 . Also, Stojkovska et al., [11] found that group A patients had a mean age of 37.47 ± 3.87 years and a body mass index (BMI) of $22.63 \pm 3.81 \text{ kg/m}^2$, and the duration of infertility was 4.0 ± 2.1 years. Additionally, Aflatoonian et al., [2] found that in the PORs group, women mean age was 35.47 ± 4.34 years, whereas in the group of POI, it was 33.66 ± 4.84 years. In women who experienced postpartum hemorrhage, the average time after menstruation stopped was 8.11 ± 3.29 years.

Also, Stojkovska et al., [11] examined the live birth rate (LBR) of two groups: twenty healthy controls and twenty low responders who were tracked using transvaginal ultrasonography and administered three to five milliliters (mL) of autologous PRP. When all known confounders were included, multivariate analysis did not show any substantial distinctions among the groups, and additionally, both groups had similar values for age, body mass index, partner's age, and baseline follicle-stimulating hormone. After 61 ± 18 days, when PRP was given, both groups followed the same protocol of low-dose activation with GnRH antagonist. Results showed no substantial relevance among clinical pregnancy and LBR.

In the current study, 1 of women were positive ICSI Live birth and 2 were positive spontaneous chemical pregnancy.

In this concern, in a study by Aflatoonian et al. [2] examined the impact of intraovarian injection of autologous PRP on ovarian reserve measures and pregnancy outcome in a study including women diagnosed with POI and poor ovarian responses (PORs). An equal number of PORs (17) and women (17) were each administered intraovarian PRP injections. Eight women (47%) in the POR group conceived naturally, with a 50% live birth rate; in contrast, twenty-two percent of the POI women did not conceive but did experience menstrual recovery.

Also, Sills et al., [8] discovered that four women who experienced amenorrhea and an absence of responsiveness to ovarian stimulation had intraovarian PRP injections. Following PRP injection, all women showed considerable improvements in their hormonal profiles, oocyte retrieval, and blastocyst development. Afterwards, Sfakianoudis et al. [12] described the first case of a biochemical pregnancy resulting from in vitro fertilization using autologous PRP which was injected into an egg. But this pregnancy ended in a spontaneous abortion midway through the fifth week of gestation.

Additionally, in a clinical trial, Farimani et al. [13] Documented a rise in the quantity of oocytes and embryos acquired following PRP administration and contrasted the number of oocytes recovered before to and subsequent to PRP injection. Out of the 19 women, three became pregnant, including two spontaneous chemical pregnancies and one in vitro fertilization clinical pregnancy that culminated in a successful live delivery. An additional case series demonstrated three women who were treated with autologous PRP ovarian infusion and were classified as poor responders. In addition to a live delivery that went well, they mentioned a pregnancy that was both easy and natural, lasting 17 weeks, and 24 weeks. Thanks to in vitro fertilization, the remaining two patients were able to conceive [12]. Moreover, Stojkovska et al. [11] tested the effects of injecting PRP intraovarianly into the eggs of 40 women who did not respond well to in vitro fertilization. Patients who received PRP therapy had a slightly greater rate of successful implantation and live births. While, in a study by Parvanov et al. [14] did not demonstrate a statistically substantial rise in embryo and oocyte counts.

The present study showed that there was no significant relation between pregnancy outcome with duration of infertility ($P=0.962$), pre-AFC ($P=0.451$) and post AFC ($p=0.436$).

In disagreement with our study Adiga et al.,

[15] noted statistically significant improvement in the AFC. In a study by Otsuka et al., [16] discovered that atresia and cell death can happen in any step of folliculogenesis, from the primordial follicle all the way to the antral follicle, in POI.

Additionally, Hosseini et al., [9] observed that Low viability and implantation following PRP injection in POI may occur when the aberrant growth gene mutations are expressed more often, or when PRP does not revive the primordial follicles that are already atretic. Furthermore, Adiga et al., [15] determined that there is insufficient data to support the claim that PRP increases the number of antral follicles. There is no clinically relevant utility in the mean difference of just 1.78 following PRP.

In our study, there was no significant relation between pregnancy outcome with pre and post (FSH, LH, AMH). While pre and post estradiol were significantly decreased among women with positive pregnancy outcome (34.93 ± 0.12 , 36.67 ± 0.58) than women with negative pregnancy outcome (48.60 ± 20.13 , 49.92 ± 19.74).

In line with our study, Farimani et al. [13] found the same outcomes. Serum FSH, LH, and AMH levels remained unchanged in this case. Also, Cakiroglu et al. [5] found that women suffering from primary ovarian insufficiency showed modest improvement in AMH levels and no significant effect on FSH levels after receiving intra-ovarian injections of autologous PRP. Although, Aflatoonian et al. [2] found no substantial change in the LH and FSH profiles of women injected with PRP who had primary ovarian insufficiency or polycystic ovary syndrome. Prior research indicated that intra-ovarian PRP therapy increased estradiol levels. By week six following autologous PRP treatment, FSH levels had dropped substantially [12].

In contrast, the other studies by Sfakianoudis et al. [12]; Sfakianoudis et al. [12]; Sfakianoudis et al. [12] identified a considerable

decrease in FSH and LH levels as well as a rise in AMH and E2 levels following PRP injection.

CONCLUSION

Our results demonstrated that patients with POR did not benefit from a single intra-ovarian injection of autologous PRP. Further investigation is required in upcoming clinical studies about this. The results shown here do not support the idea that autologous intraovarian PRP injection may revive the ovaries or get the folliculogenesis process going again. Hormone testing revealed no substantial rise in follicle-stimulating hormone (FSH) or luteinizing hormone (LH) levels following PRP intraovarian injection for ovarian rejuvenation across all age groups studied.

LIST OF ABBREVIATIONS

primary ovarian insufficiency (POI), Anti-Mullerian hormone (AMH), assisted reproductive technology (ART), Body mass index (BMI), platelet-rich plasma (PRP), polycystic ovary syndrome, antral follicle counts (AFC), Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Chi-Squared (χ^2), Standard student-t test (t) and The Paired- t test (t), live birth rate (LBR), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF).

DATA SHARING STATEMENT

All data and materials included in this work are available.

CONSENT FOR PUBLICATION

There were no conflicts of interest, all authors have read the manuscript, then revised well and agree to publish.

AUTHORS' CONTRIBUTIONS

All authors contributed to the drafting, revi-

sion, or critical review of the article. They also provided final approval for the published version, concurred on the journal to which the article was submitted, and agreed to be accountable for all aspects of the work. Furthermore, all authors played a substantial role in the reported work, whether through the conception, study design, execution, data acquisition, analysis, and interpretation, or in all of these instances.

DISCLOSURE

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Fig (1): Flowchart of women with primary ovarian insufficiency.

Fig(2): Distribution Hormonal data in relation to pregnancy outcome.

Fig 3. Mean change distribution of different parameters among the studied cases.

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Table 1: Demographic data, Past history of pregnancy or IVF failure and Duration of infertility among the studied cases (n=28).

Variable	The studied cases (n=28)	
	Mean± SD	Range
Age/year	34.60±5.44	20.00-39.00
BMI (kg/m ²)	27.40±3.27	24.00-35.00
Duration of infertility	5.96±3.30	2.00-15.00
	N	%
Previous Pregnancy		
Positive	17	60.71
Negative	11	39.29
Previous IVF Failure		
Positive		
1 ICSI	3	10.71
2 ICSI	2	7.14
3 ICSI	1	3.57
4 ICSI	1	3.57
Negative (No ICSI)	21	75.00

BMI: body mass index

Table 2: Hormonal data among the studied cases (n=28).

Variable	Mean± SD		P value
	Preinjection	Postinjection	
FSH	13.53±5.68	14.28±5.43	0.076
LH	11.60±6.33	12.40±5.86	0.092
AMH	0.38±0.29	0.35±0.27	0.041
E2	48.87±19.96	50.16±19.55	0.053

FSH: follicle-stimulating hormone, LH: luteinizing hormone, AMH: Anti-Mullerian hormone, E2: estradiol

Table 3: Ultrasound examination among the studied cases (n=28).

Variable	Mean± SD		P value
	Preinjection	Postinjection	
Ovarian volume (cc)	1.60±0.48	1.50±0.43	0.248
Ovarian Doppler (PSV)	0.18±0.08	0.18±0.08	1.00
AFC	3.92±2.00	3.48±1.61	0.125

PSV: peak systolic velocity **AFC:** antral follicle count

Table 4: Pregnancy outcome among the studied cases (n=28).

Variable	The studied cases (n=28)	
	N	%
Pregnancy outcome		
Negative	25	89.29
Positive	3	10.71

Table 5: Hormonal data in relation to pregnancy outcome (n=28).

Variable	Pregnancy outcome		t	P value
	Negative (n=25)	Positive (n=3)		
FSH-pre Mean± SD Range	13.64±5.67 6.56-32.00	13.07±0.90 12.20-14.00	0.461	0.649
FSH-post Mean± SD Range	14.31±5.42 8.00-31.00	14.33±1.15 13.00-15.00	0.011	0.991
LH-pre Mean± SD Range	11.40±6.40 3.10-33.00	11.33±2.08 9.00-13.00	0.040	0.969
LH-post Mean± SD Range	12.16±5.97 6.50-33.00	11.83±1.26 10.50-13.00	0.234	0.818
AMH-pre Mean± SD Range	0.36±0.28 0.01-0.90	0.42±0.20 0.20-0.58	0.500	0.650
AMH-post Mean± SD Range	0.33±0.26 0.01-0.85	0.37±0.19 0.15-0.50	0.235	0.829
E2-pre Mean± SD Range	48.60±20.13 20.00-85.00	34.93±0.12 34.80-35.00	3.40	0.002*
E2-post Mean± SD Range	49.92±19.74 22.00-85.00	36.67±0.58 36.00-37.00	3.34	0.003*

Table 6: Ultrasound examination in relation to pregnancy outcome (n=28).

Variable	Pregnancy outcome		t	P value
	Negative (n=25)	Positive (n=3)		
Ovarian				
Volume- pre Mean± SD Range	1.62±0.46 1.00-2.00	1.67±0.58 1.00-2.00	0.135	0.904
Volume-post Mean± SD Range	1.54±0.43 1.00-2.00	1.33±0.29 1.00-1.50	1.10	0.347
Doppler (PSV1) Mean± SD Range	0.18±0.07 0.10-0.30	0.20±0.10 0.10-0.30	0.370	0.743
Doppler (PSV2) Mean± SD Range	0.18±0.07 0.10-0.30	0.20±0.10 0.10-0.30	0.369	0.744

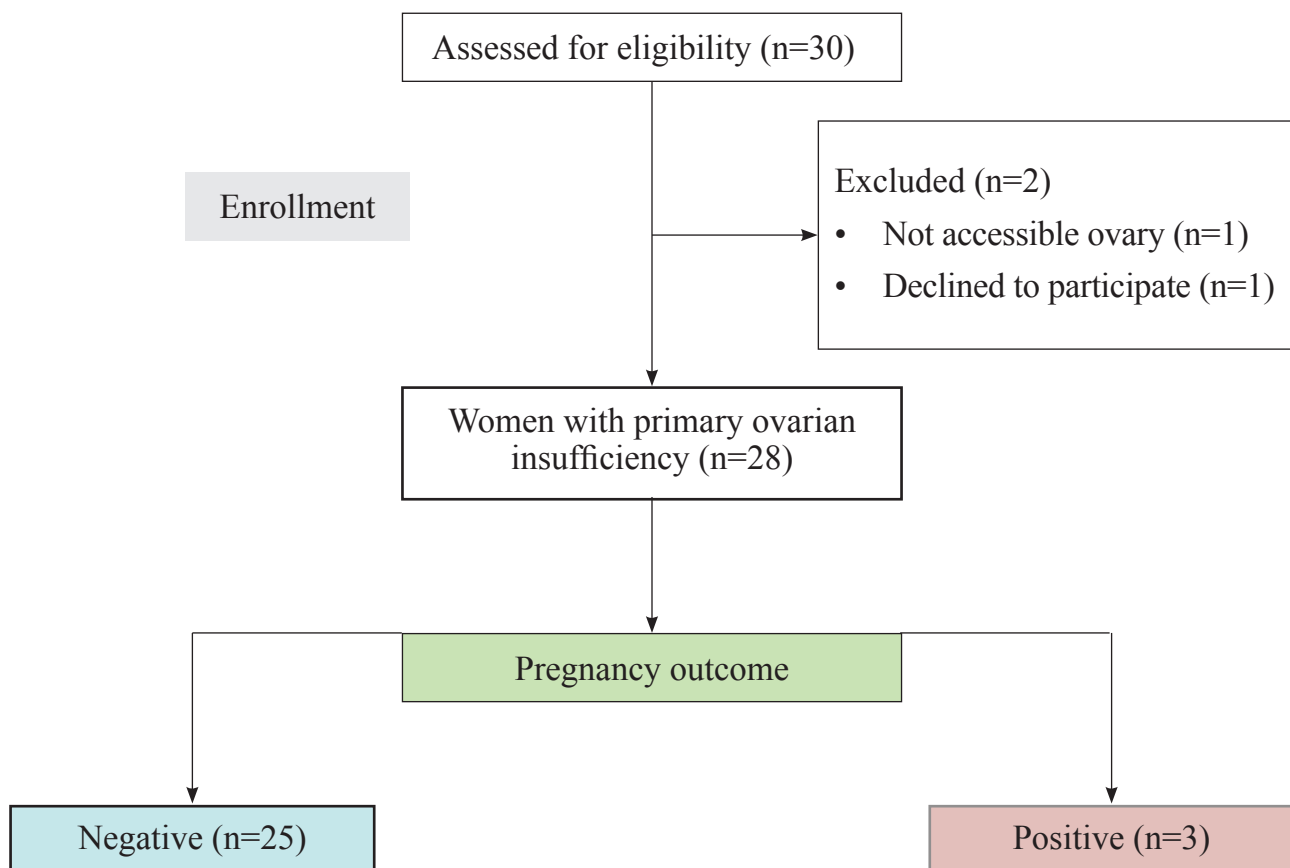
t = independent test, PSV: peak systolic velocity

Table 7: Mean changes of different parameters among the studied cases (n=28).

	Paired Differences			t	P value
	Mean diff.	95% CI			
		Lower	Upper		
FSH (pre) – FSH (post)	-.74000	-.97736-	.50264	-.397	.000
LH (pre) – LH (post)	-.72964	-1.10951-	.34977	-3.94-	.001
AMH (pre) – AMH (post)	.02500	.01313	.03687	4.322	.000
E2 (pre) - E2 (post)	-1.31071	-1.64098-	.98045	-8.143	.000
AFC (pre) – AFC (post)	.39286	.20001	.58571	4.180	.000
Ovarian volume (cc) - Ovarian volume-post	.10714	.02613	.18816	2.714	.011

FSH: follicle-stimulating hormone, **LH:** luteinizing hormone, **AMH:** Anti-Mullerian hormone, **E2:** estradiol, **AFC:** antral follicle count, **t:** Paired- t test, *****: Significant.

This table showed that, the mean change of FSH, LH, AMH, E2, AFC and Ovarian volume were (-.74000, -.72964, .02500, -1.31071, .39286, .10714) respectively with significant difference between pre and post ($P \leq 0.05$).

**Fig 1.** Flowchart of women with primary

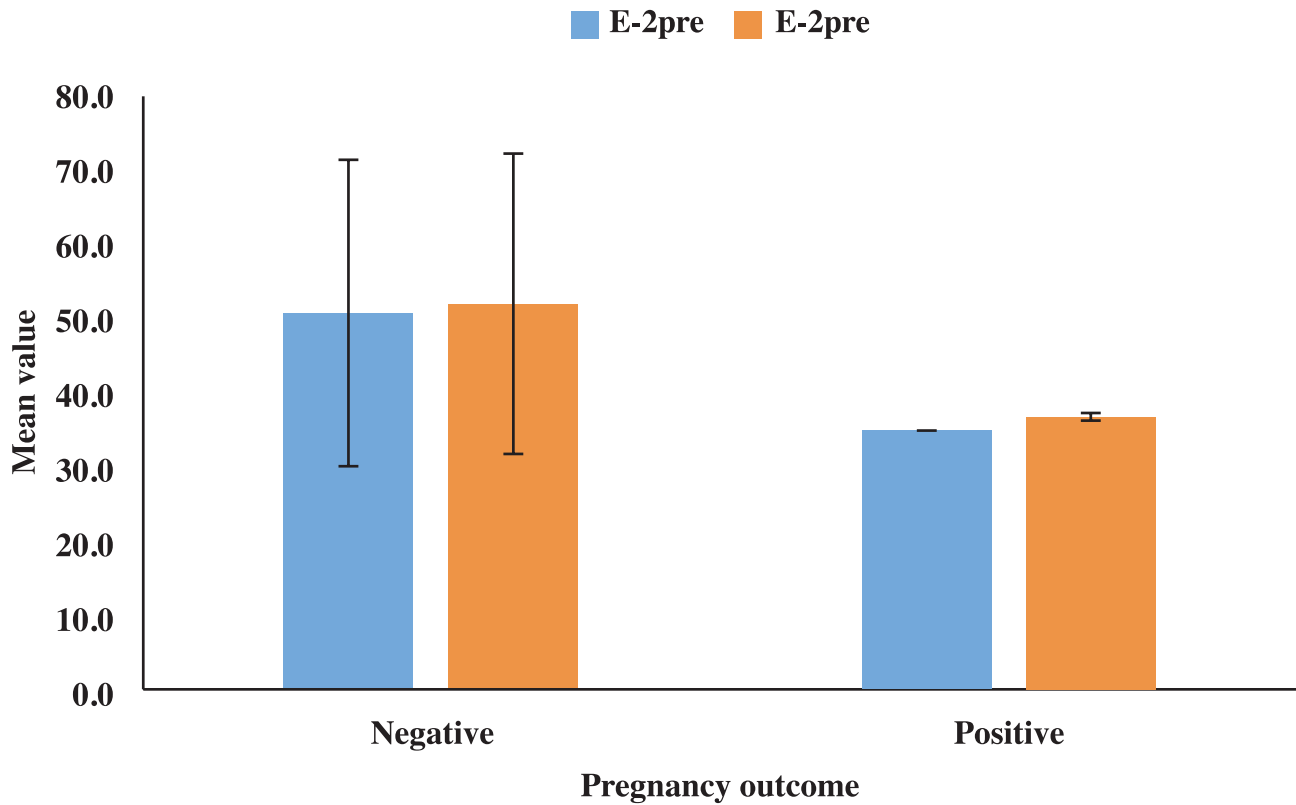


Fig 2. Distribution Hormonal data in relation to pregnancy outcome.

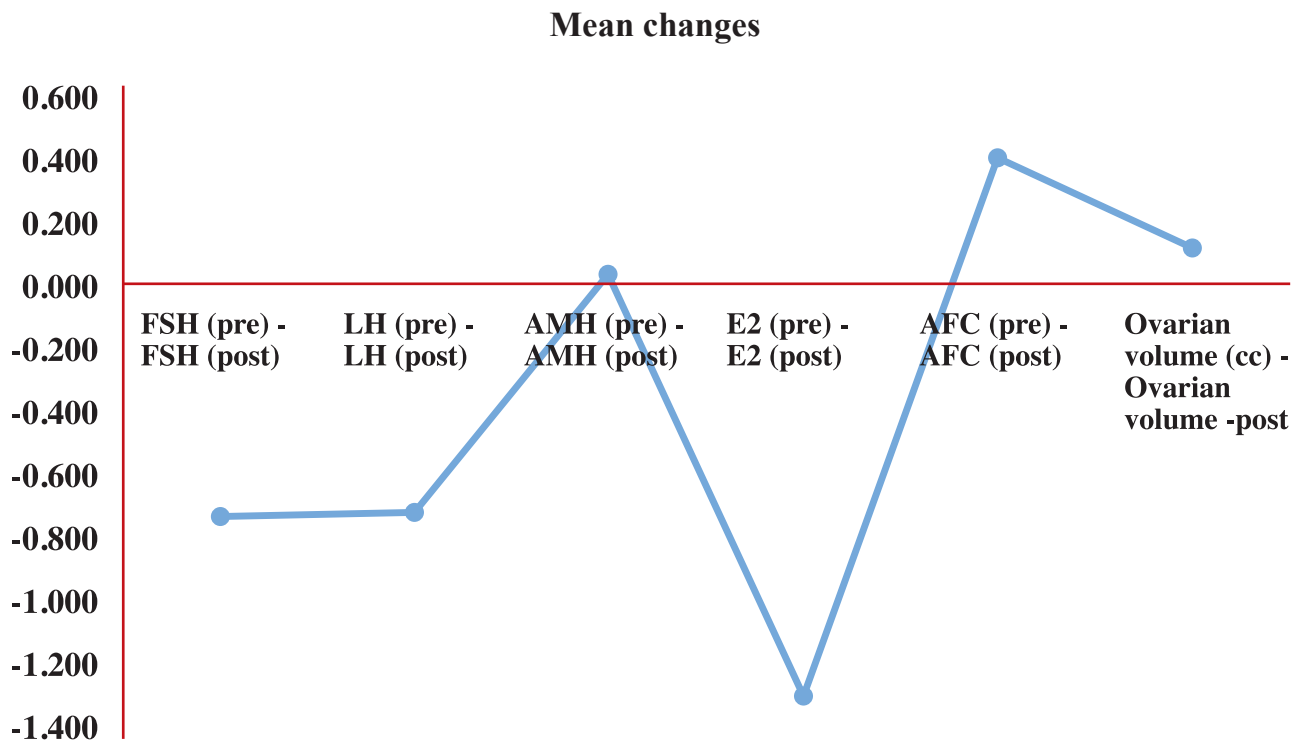


Fig 3. Mean change distribution of different parameters among the studied cases.