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Original Article

Obesity attenuates the beneficial effect of an intrauterine infusion of autologous platelet-rich plasma during in vitro fertilization

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Abstract: Objective: To assess how obesity, normal weight (NW) versus overweight/obese (OW/OB), impacts platelet-rich plasma's (PRP) effectiveness during *in vitro* fertilization and how obesity affects platelets during the menstrual cycle. Methods: Endometrial mean thickness (EMT), embryo implantation, and clinical pregnancy were assessed using a self-controlled retrospective study that enrolled 59 patients with two failed cycles and treated with autologous PRP (three-dose scheme). The NHANES dataset was used to assess platelet changes during the menstrual cycle, using the mean platelet volume to platelet count ratio (MPR) index. The COSINOR packages for R were used to determine rhythmicity. Results: PRP treatments significantly improved the EMT (2.5 ± 1.4 mm, P<0.001), unaffected by obesity. After the PRP treatment, one patient spontaneously became pregnant; therefore, 58 patients underwent embryo transfer (62 cycles), of which in 39 cycles the embryos implanted (63.9%). This was a significant improvement from their previous cycle (vs. 22.6%, P<0.001). Clinical pregnancy also improved with the PRP treatment over the previous cycle (57.4% vs. 16.1%, P<0.001). When stratified by obesity, there was an appreciable decrease in embryo implantation and clinical pregnancy rates for the OW/OB group; nevertheless, the PRP treatment significantly improved embryo implantation and clinical pregnancy (P<0.05). A rhythm was observed with the MPR index (P<0.05) only for the NW group, suggesting that the platelets normally fluctuate during the menstrual cycle. Conclusion: PRP improved embryo implantation and clinical pregnancy rates; however, these beneficial effects were attenuated by obesity. PRP presumptively promoted a change in the uterine environment to mimic the normal findings associated with normal-weight women.

Keywords: Endometrial receptivity, IVF, intrauterine platelet-rich plasma, menstrual cycle, recurrent implantation failure, refractory thin endometrium

Introduction

A healthy human endometrium is crucial during embryo implantation, as it is like the soil that promotes the embryo's growth [1]. It has been widely reported that embryo implantation depends on coordinated crosstalk between intrauterine factors and the embryo [2]. In addition, endometrial receptivity and thickness play an essential role in pregnancy outcomes, especially during assisted reproduction [3], in which platelet quality and quantity significantly affect the endometrium [4]. Under certain circumstances, the endometrium fails to reach an optimal condition [5], related to poor circulation and sub-optimal perfusion of the uterus [6]. This leads to low concentrations of cytokines and growth factors, which are secreted by endometrial epithelial and stromal cells during the window of implantation [7]. Interestingly, during the window of implantation, fluctuations concerning platelets have not been investigated;

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nevertheless, the administration of platelet-rich plasma (PRP) is a promising strategy for treating endometrium-associated infertility [8, 9].

PRP is a concentrated sample of human platelets obtained from the patient's blood (autologous) that is 5- to 10-fold higher than the physiologic concentration of thrombocytes in the whole blood [10]. PRP contains a variety of growth factors and bioactive molecules involved in clotting, inflammation, cell growth, cell adhesion, and host defense, among others [11]. The mechanism for PRP's effect on endometrial tissue is unknown; nevertheless, the induction of cell proliferation, chemotaxis, regeneration, extracellular matrix synthesis, remodeling, angiogenesis, and epithelialization are the main pathways for PRP to affect female reproductive organs [8]. The critical role cytokines and growth factors play during embryo implantation has been well-described [2]. PRP contains many growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-β, vascular endothelial growth factor (VEGF), and epidermal growth factor [11], that are present and are released by the blood during normal endometrial growth and embryo implantation [2].

The concept of an optimal endometrium is still under debate, and its characterization remains elusive; however, it is accepted that a minimum endometrial thickness (EMT) of 7 mm is required for embryo transfer, as it is the cutoff to define thin endometrium [12]. *In vitro* studies have shown that PRP exposure, as a single agent or a combination with growth factors, was associated with increased endometrial stromal and mesenchymal cell proliferation, increased expression of regenerative enzymes, and enhanced cell migration [13]. With *in vivo* animal studies, PRP treatments demonstrated a decrease in the expression of inflammatory and fibrotic markers. At the same time, there was an increase in the endometrial proliferation rate, expression of proliferative genes, and pregnancy rates [3, 8]. This suggests that PRP would benefit infertile women, especially those suffering from thin endometrium and recurrent implantation failure. Indeed, intrauterine infusions of autologous PRP have been used in infertile women with recurrent implantation failure and thin endometrial lining [3]. The first study to show how effective an intrauterine infusion of PRP as a therapy for infertile women with thin endometrium was published in 2015, in which the PRP injections promoted endometrial growth and improved pregnancy outcomes in five patients [14]. A systematic review demonstrated that following intrauterine PRP, EMT was increased as did the embryo implantation rate, chemical pregnancy rate, and clinical pregnancy rate [9].

Clinical studies using PRP in women undergoing assisted reproductive technologies have conflicting results [9], as PRP preparation and application protocols for different therapeutic purposes have yet to be standardized. Moreover, especially in females, obesity, which has been shown to affect fertility [15], has also been shown to affect platelet concentration and activation [16, 17]. Our study aimed to determine whether the intrauterine infusion of autologous PRP improved the EMT and enhanced embryo implantation and clinical pregnancy rates in patients with thin endometrium with a history of previously failed *in vitro* fertilization (IVF) cycles. Furthermore, we assessed whether the PRP effect is altered by obesity and how obesity affects the platelets during the menstrual cycle.

Methods

Study design and participants

A chart review for a retrospective self-controlled study was conducted from November 2019 to May 2023 following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [18]. The cohort pool consisted of patients who attended any of the seven centers of the Ingenes Institute and received a three-dose scheme intrauterine infusion of autologous PRP during an embryo transfer cycle. They had to fulfill the following inclusion criteria: 1) age >25 years, 2) have demonstrated a refractory thin endometrium (EMT<7 mm) at Day 10 during a gonadotropin-free estrogen-primed cycle of endometrial preparation for embryo transfer, 3) had a history of at least two failed IVF cycles with good quality embryos, 4) failed to achieve a pregnancy in the previous IVF cycle that used endometrial preparation with high-dose estradiol valerate, 5) as determined by hysteroscopy or hysterosonography, had a normal uterine cavity

or had an abnormal uterine cavity but was corrected, and 6) had good quality embryos available for embryo transfer, either from donated oocytes or the patient's ova. The patients were excluded if it was documented or suspected that: 1) the patient suffered from an autoimmune disease, thrombophilia, hematologic disorders, uncontrolled endocrine or other medical conditions, 2) congenital and untreated acquired uterine abnormalities, 3) couples with genetic or chromosomal abnormalities, or 4) only produced poor-quality embryos and were not willing to accept gamete donation. Once the potential records were identified, the patients were contacted, and of the patients who agreed, written informed consent was obtained according to the Declaration of Helsinki. The Ethics Committee of the Ingenes Institute approved this study (approval number: ISF201219).

IVF

All patients underwent controlled ovarian stimulation for 10 to 14 days with gonadotrophin-releasing hormone agonists and antagonists. On the second day of the menstrual cycle, recombinant human FSH (Corneumon, Corne, UPC: 7502242700449) was administered daily, in which the dose was adjusted by weight and antral follicle count according to an individualized protocol. Gonadotropin-releasing hormone antagonist, Cetrotide (Merk, UPC: 4054839325359, 0.25 mg/day), was administered from day 6 of ovarian stimulation until ovulation trigger was performed with 10,000 IU of human chorionic gonadotropin (hCG, Choriomon, Corne, UPC: 7680335240802). The ovarian response was assessed by measuring serum estradiol levels, and an ultrasound examination evaluated follicular development. Oocyte retrieval was conducted 36 hours after administering hCG. At the same time, the semen was prepared by density gradient centrifugation. The oocytes were inseminated by intracytoplasmic sperm injection, and fertilization was confirmed by the formation of two pronuclei 19 hours after insemination. Embryos were cultured in Global Total for Fertilization media (Cat # LGGT-30, Life Global) and incubated at 37°C, 8% CO_2, 5% O_2, and 87% N_2. An embryologist monitored and recorded all information about the antral follicle count, fertilization rates, embryo development, and embryo morphology for each oocyte.

Autologous PRP preparation and intrauterine infusion

For preparing each PRP infusion, 3 ml of venous blood was drawn from patients using a 4.5 ml sample collection tube with 3.8% sodium citrate (BD vacutainer). Afterward, the samples were centrifuged at 1200 rpm for 12 minutes. Then, the plasma was obtained, transferred to a 15 ml conical tube (Falcon), and centrifuged at 3300 rpm for 7 minutes. 0.5-1 ml of PRP was collected and infused into the uterine cavity within 10 minutes to avoid protein degradation. Intrauterine autologous PRP administration was performed during an estrogen-primed cycle. Endometrial preparation with 6 mg of estrogen valerate (Primogyn UPC: 00770333-115702) was started on Menstrual Cycle Day (MCD) 2 or 3. On MCD 10, EMT was measured by ultrasonography, and autologous PRP was infused into the uterine cavity using a Wallace Embryo Transfer Catheter (Sure View Soft 2, CE 123). The procedure was repeated on MCD 12 and 14 (Supplementary Figure 1).

Embryo transfer

For fresh or frozen-thawed embryos, 1 to 3 good-quality embryos were transferred during an estrogen-primed cycle, free of gonadotropin stimulation. Clinical decisions about the number of embryos to transfer were determined by the physician, with the patient's approval. Blastocysts were cryopreserved by vitrification. The uterine transfer occurred during a controlled endometrial development cycle with a daily dose of 6 mg of estrogen valerate (Primogyn UPC: 00770333115702) for ten days after menstruation. Embryo implantation was confirmed on Day 14 by serum β-hCG concentrations (>10 mUI/ml was considered positive) or by the presence of a fetal heartbeat using ultrasound at 6-8 weeks. Demographic data, IVF cycle information, embryo implantation rate, and IVF outcomes (pregnancies and miscarriages) were recorded by the physician. The primary outcomes were EMT, embryo implantation, and clinical pregnancy rates. The secondary outcome was the ongoing pregnancy/live birth rate.

Sample size

For this self-controlled study, the sample size was calculated using the SCCS package for R

[19, 20]. The inputs were based on the results from a similar study [21], in which the clinical pregnancy rate for the control and the treated group was 3.3% and 24.1%, respectively. With an alpha = 0.05 and power = 0.95, the calculated sample size was 15 subjects. However, using the inputs from another study [22], in which the control and treated group rates were 2.5% and 12.5%, respectively, the sample size was determined to be 25 subjects. Therefore, minimally, each group should contain between 15 and 25 participants.

National health and nutrition examination survey (NHANES) dataset

The National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention conducts the NHANES (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2017). This large cross-sectional survey systematically gathers data on medical examinations, laboratory testing, and interviews for studying a range of variables of medical importance. The survey includes dietary, laboratory, body measurement examination, and demographic information. Before collection of the data, informed consent was obtained from each participant. The Ethics Review Board approved data gathering for the NCHS (https://www.cdc.gov/nchs/nhanes/irba98.htm), and the files were posted online for public use. NHANES fully describes the data collection procedures and methods (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?Cycle=2017-2018). All procedures were performed under the ethical standards of the institutional and national research committee and in agreement with the Declaration of Helsinki. All data generated and analyzed during this study are available on the NHANES website (https://wwwn.cdc.gov/nchs/nhanes/default.aspx).

A set of eligibility criteria was constructed according to the patient population, intervention, comparison group, outcomes, and study design (PICOS) question scheme. The PICOS question was: in overweight/obese women, when compared to normal-weight women, does the platelet count, platelet volume, and the mean platelet volume to platelet count ratio (MPR) index, differ during the menstrual cycle, as determined using the NHANES cross-sectional dataset? The eligibility criteria reflected the PICOS components and the subsequent inclusion and exclusion criteria. In this study, the data came from the collection years between 1999 and 2006 (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2017). To be included, the participants had to be 1) non-pregnant women aged ≥16 years, 2) body-mass index (BMI) of ≥18.5 kg/m^2, and 3) had information about the number of days since their last period (Days 1 to 35). They were excluded for 1) having liver disease (Hepatitis B/C/D, autoimmune, or hepatocarcinoma), 2) having liver problems, 3) having thyroid problems, 4) having HIV, 5) taking insulin, 6) having/had cancer, 7) had a pregnancy/delivered within one year or were currently breastfeeding, 8) had a hysterectomy, ovariectomy, or endometrioses, or 9) taking hormones.

Key demographic variables collected were age, ethnicity, marital status, income, and poverty-to-income ratio. The age (years) was determined at the interview. NHANES categorizes ethnicity as Non-Hispanic White, Mexican American, Other Hispanics, Non-Hispanic Black, or Other Races (including multiracial). Anthropometric variables [weight (kg), height (m), BMI (kg/m^2), waist circumference (cm), systolic and diastolic blood pressures (mmHg)] were collected according to a standardized protocol (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?Cycle=2017-2018). BMI was categorized into normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), and obese (30-39.9 kg/m^2), according to the World Health Organization criteria (https://apps.who.int/iris/handle/10665/37003). For laboratory data, red and white blood cell count (n), platelet count (10^3/μL), mean platelet volume (fL), and luteinizing hormone (LH, IU/L) were analyzed according to a standardized protocol (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?Cycle=2017-2018). MPR was defined as mean platelet volume (fL)/platelet count (10^3/μL) × 100%.

Statistical analysis

All analyses were done with the Statistical Package for the Social Sciences software (SPSS, v. 22.0, Chicago, IL, USA) or with the R software [23]. The paired T-test was used for

the clinical study to compare the pre-PRP and post-PRP EMT. The two-way repeated measures ANOVA determined differences between pre and post-PRP EMT when stratified by IVF outcome and obesity. The embryo implantation, clinical pregnancy, and live birth rates were analyzed using McNemar's test. For the NHANES dataset, the normality of continuous variables was determined using the Kolmogorov-Smirnov test. Differences between categorical data were assessed with the Chi-Square test. Levene's test was used to examine differences in the variances of continuous variables. Differences between groups for continuous variables were determined using either Student's *t*-test for parametric data or the Mann-Whitney U test for non-parametric data. The COSINOR [24] and COSINOR2 [23] packages for R were used to determine rhythm. These packages fit a cosine curve with a free phase to data as well as calculate the MESOR (Midline Estimating Statistic Of Rhythm, a rhythm-adjusted mean), amplitude (half the predictable change within a cycle), and acrophase (time of highest value within a cycle) [24]. The cosinor models were adjusted by obesity [normal weight (BMI: 18.5-24.9 kg/m^2) and overweight/obese (BMI: 25.0-39.9 kg/m^2)] as a covariate. Including the obesity category in the cosinor model allows the MESOR, amplitude, and acrophase to change with respect to the binominal categories. The overall significance of the cosine model was established using the zero-amplitude test. Wald tests were conducted for differences in the amplitude and acrophase due to the covariates. Unless noted otherwise, data are represented as the frequency, percent, or mean ± standard deviation or standard error. *P*-values <0.05 (two-tailed) were considered significant.

Results

PRP treatment improved IVF

Over 300 patients who underwent intrauterine PRP infusion were identified during the study period. However, using the inclusion/exclusion criteria, 134 patient records were identified. After a thorough chart review and informed consent retrieval, 59 patients' data (62 IVF cycles) were included in this study (Supplementary Figure 2). The ages of the patients ranged between 30 and 49 years, with over 56.5% of the cohort being overweight to obese (BMI = 26.8 ± 5.0 kg/m^2, **Table 1**). The average duration of infertility was 4.9 ± 3.9 years, with an average of 3.0 ± 1.5 previous IVF attempts with embryo transfers at the Ingenes Institute. The most common reason for IVF was advanced age (49.2%), followed by polycystic ovary syndrome (PCOS) (15.3%), tubal factor (15.3%), and low ovarian response (10.2%). Male factor was suspected in 24 couples, and two patients had a female partner.

Before treatment, the average EMT was 5.6 ± 1.4 mm (minimum = 2.1 mm; maximum = 10.9 mm) for the cohort; however, after the PRP treatment, the average EMT was 8.1 ± 1.5 mm (minimum = 5.5 mm; maximum = 12.3 mm). This significantly improved in the EMT (2.5 ± 1.9 mm, P<0.001, **Figure 1A**). Interestingly, only six cycles did not achieve the ideal EMT of 7.0 mm (**Table 2**). When stratified by obesity, there was no difference in the net increase in the EMT (P = 0.354, **Figure 1B**), nor when stratified by IVF outcome (P = 0.574, **Figure 1C**). There was also no significant difference when the cohort was separated into patients who did attain embryo implantation versus those who did not (P = 0.909, **Figure 1D**), as well as with successful (live birth/ongoing) pregnancy (P = 0.499, **Figure 1E**). Of the 59 patients, 58 underwent embryo transfer (62 IVF cycles), and one became pregnant naturally. Between 1 and 3 embryos were transferred per patient and implanted in 39 cycles (63.9%). The results of each pregnancy are presented in **Table 2**. When the PRP-treated cycle outcomes were compared to the previous cycle (control), the implantation, clinical pregnancy, and ongoing pregnancy/live birth rates all significantly increased (P<0.01, **Table 3**). When stratified by obesity, there was an appreciable difference between normal-weight patients and overweight/obese patients, in which obesity was associated with lower rates of implantation, clinical pregnancy, and ongoing pregnancy/live birth. Nevertheless, compared to the previous cycle, even for overweight/obese patients, there was significant improvement due to the PRP treatment.

Platelets significantly fluctuate during the menstrual cycle

Using the NHANES dataset, 41,474 participants were identified (Supplementary Figure 3).

Table 1. Characteristics of the study participants

Pt ID	Age (years)	BMI (kg/m^2)	Infertility (years)	# Failed IVF cycles with ET	Parity				Hysteroscopic findings	Other medical history
					T	P	A	L		
001	42	33.9	5	4	0	0	0	0	Uterine septum (removed)	None indicated
002[a]	41	21.9	1	4	0	0	0	0	Normal	None indicated
003	44	32.0	10	3	0	0	0	0	Cervix hypoplastic, endometrial polyps (dissected), and ostium in central orientation	None indicated
004	40	33.3	5	3	0	0	0	0	*Uterus arcuatus*, polyps (removed)	None indicated
005	44	23.6	5	4	0	0	0	0	Normal	None indicated
006	34	25.1	2	1	1	0	0	1	Normal	None indicated
007	41	28.7	1	5	3	0	0	3	Normal	None indicated
008[a]	48	35.4	12	3	0	0	0	0	No permeable left ostium	Laparoscopy for tubal factor
009	46	31.0	2	2	1	0	1	1	Normal	None indicated
010	32	21.2	5	4	0	0	0	0	Normal	None indicated
011	49	39.0	1	6	0	0	0	0	*Uterus arcuatus* (remodeled), vascularized uterine polyps (removed)	Complex endometrial hyperplasia
012	45	21.1	10	1	0	0	0	0	Stenosis was observed in the internal cervical orifice with access to a problematic cavity	None indicated
013	46	23.0	5	1	0	0	2	0	Normal	Myomectomy
014	45	26.8	7	4	0	0	0	0	Adhesions (removed)	None indicated
015	36	28.8	4	5	0	0	0	0	*Uterus arcuatus* and adhesions	None indicated
016	30	22.3	6	1	0	0	0	0	NR	Drug addictions, tobacco (G1, E1)
017	42	32.2	3	5	0	0	0	0	Normal	None indicated
018	44	39.0	4	3	0	0	0	0	Normal	None indicated
019	41	27.9	1	4	2	0	0	2	Normal	None indicated
020	38	24.1	12	2	0	0	2	0	Normal	None indicated
021	42	21.0	4	3	0	0	1	0	Normal	PCOS, endometriosis
022[a]	36	27.1	4	3	0	0	2	0	Myoma (dissected, normal by pathology)	None indicated
023	39	23.9	1	4	0	0	0	0	Hyperplasia (normal by pathology)	None indicated
024	45	22.6	4	3	0	0	0	0	Adhesions (removed)	None indicated
025	43	19.8	2	2	0	0	0	0	Normal	None indicated
026	37	25.4	6	4	4	0	1	4	Normal	None indicated
027	38	27.8	10	2	0	0	0	0	Normal	Moderate endometriosis
028	43	21.5	1	1	0	0	0	0	Normal	None indicated
029	39	21.7	2	2	0	0	0	0	Normal	None indicated
030	41	33.3	19	3	0	0	4	0	Endometrial and cervical polyps (normal by pathology)	Amenorrhea
031	44	38.1	1	1	1	0	0	1	Normal	None indicated
032	46	25.7	7	4	0	0	0	0	Normal	Polypus remotion
033	46	24.2	6	1	0	0	0	0	Normal	Polypus remotion
034	40	26.4	5	1	0	0	0	0	Polyps (removed)	None indicated
035	36	23.1	5	2	0	0	0	0	Unicorn uterus	Uterine adhesions, cyst salpingooforectomy, and adhesions treated by hysteroscopy

036	30	27.6	2	1	0	0	2	0	Endometrial hypotrophy (reactivation)	Salpingectomy
037	49	23.7	7	7	3	0	2	3	NR	Curettage
038	39	22.1	3	6	0	0	1	0	Normal	None indicated
039	43	20.1	<1	3	0	0	0	0	Normal	None indicated
040	40	23.9	2	4	0	0	0	0	Polyps (removed)	None indicated
041	30	28.3	1	2	0	0	1	0	Normal	Right oophorectomy
042	38	32.1	8	5	NR	NR	NR	NR	Normal	Hypertrophic uterus
043	35	29.9	3	2	0	0	3	0	Normal	None indicated
044	39	32.7	2	2	0	0	3	0	Polyps (removed)	None indicated
045	37	24.7	3	2	0	0	0	0	Asherman Syndrome	None indicated
046	35	25.1	7	1	0	0	2	0	Adhesions (removed)	PCOS
047	35	28.9	10	6	0	0	0	0	Normal	None indicated
048	39	22.6	6	2	1	0	2	1	Normal	None indicated
049	45	20.2	5	5	1	0	0	1	Normal	Refractory endometrium
050	49	28.8	5	2	0	0	1	0	No visible right ostium-suspected unicorn uterus	None indicated
051	36	25.4	3	4	0	0	1	0	Normal	None indicated
052	45	26.7	3	3	0	0	2	0	*Uterus arcuatus*	None indicated
053	42	22.6	3	2	0	0	0	0	Normal	Atrophic endometrium
054	39	20.8	1	2	0	0	0	0	The left ostium is not permeable; a small cavity with a partition in the bottom	Severe endometriosis left ovarian wedge
055	48	30.1	18	2	0	0	0	0	Normal	Polypus remotion
056	46	23.4	4	6	0	0	1	0	Irregular hypersecretory endometrium; coronary villosities; ischemic necrosis	Curettage
057	36	33.3	3	3	0	0	1	0	Polyps (removed)	Myomectomy
058	36	26.4	3	1	0	0	0	0	Normal	None indicated
059	38	23.8	4	3	0	0	0	0	Normal	None indicated

Abbreviations: BMI: body mass index; ET: embryo transfer; IVF: *in vitro* fertilization; NR: not recorded. Parity: T, Term; P, Preterm; A, Abortion; L, Live birth; PCOS, Polycystic Ovary Syndrome. [a]Patients underwent 2 IVF cycles of autologous platelet-rich plasma treatment and embryo transfer.

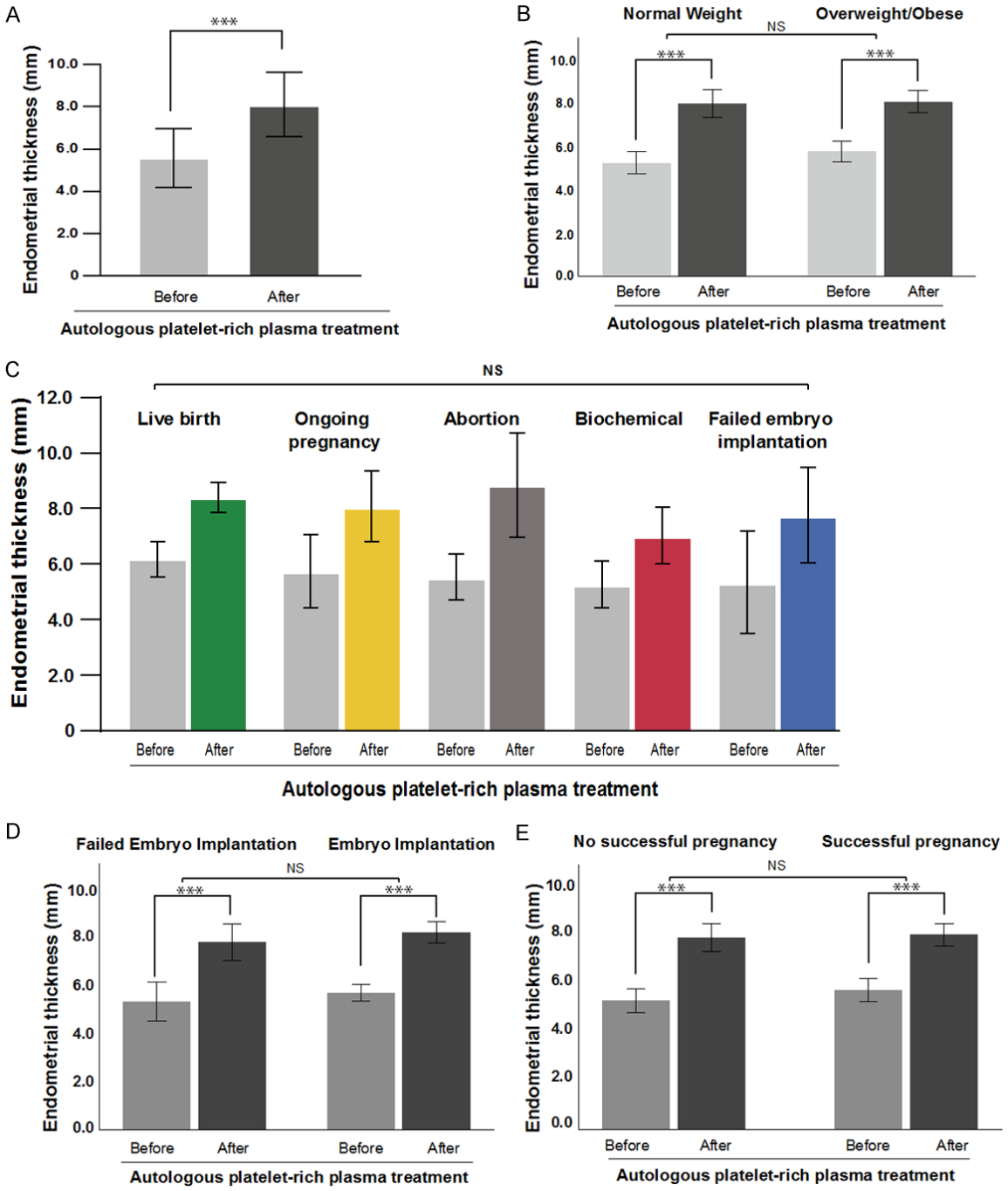

Figure 1. Endometrial mean thickness (EMT) improved after intrauterine infusion of platelet-rich plasma (PRP). (A) The EMT for each patient was graphed pre- and post-PRP treatment. EMT for pre- and post-PRP treatment was stratified by (B) normal weight and overweight/obese, (C) *in vitro* fertilization outcome, (D) embryo implantation result, and (E) successful pregnancy (live birth/ongoing). Column height represents the average, and bars represent the standard deviation. The results were compared using the two-way repeated measures ANOVA. NS: Not significant, *P<0.05, **P<0.01, ***P<0.001.

48.9% of the cohort were men, and 36.5% of the cohort did not have acceptable information on the number of days since the end of their period; therefore, these participants were excluded. Additionally, 3,132 participants were removed for having BMIs or ages outside the defined acceptable range, and 983 participants were removed for having conditions that

Table 2. Results of autologous platelet-rich plasma treatment

Obstetric result		EMT (mm)				Ova source	Sperm source	Frozen embryo	β-hCG (mUI/mL)	# Embryo transferred	Embryo Quality (stage/ ICM quality + trophectoderm quality)	# Gest. sacs
Result	Week	Pre	Post	Diff	>7							
Live Birth	36+6	6.4	9.2	2.8	+	Patient	Partner	Yes	229.4	3	BE/BB	1
	36+6	6.0	8.9	2.9	+	Donor	Partner	Yes	321.2	2	BE/AB	1[b]
	25+3	5.0	8.0	3.0	+	Donor	Partner	No	146.7	2	BE/BB	1
	38+6	6.9	7.8	0.9	+	Donor	Donor	Yes	179.8	2	BE/BB	1
	40+1	6.5	8.0	1.5	+	Donor	Partner	Yes	NR	2	BE/BB	1
	32+1	6.6	8.7	2.1	+	Donor	Partner	Yes	50.0	3	BE/BB	3
	38+3	5.9	8.2	2.3	+	N/A	N/A	N/A	QTPC	N/A	N/A	1[c]
Ongoing	30+0	5.6	9.0	3.4	+	Donor	Partner	Yes	1208.0	2	BE/AB, BE/BB	1[b]
	8+2	3.8	9.1	5.3	+	Donor	Partner	Yes	108.1	3	BE/BC	1
	26+3	4.0	7.0	3.0	+	Donor	Partner	Yes	131.1	2	BE/BB, BE/BC	1
	3+0	6.9	7.0	0.1	+	Donor	Donor	Yes	343.2	3	BE/BB	2
	6+4	4.3	7.2	2.9	+	Donor	Partner	Yes	243.7	2	BE/BB, BE/BC	2
	19+2	6.0	7.9	1.9	+	Patient	Partner	Yes	438.4	3	BC/BC	3
	9+1	4.0	7.5	3.5	+	Donor	Donor	No	766.9	3	BE/BB	3
	5+1	8.1	7.8	-0.3	+	Donor	Partner	Yes	246.9	2	BE/BC, BC/BC	1
	2+1	6.3	7.3	1.0	+	Donor	Partner	Yes	435.0	3	BE/BB	1
	15+3	6.6	7.8	1.2	+	Patient	Partner	Yes	319.0	2	BE/BC, BC/BC	2
	16+3	4.9	7.9	3.0	+	Donor	Partner	Yes	158.0	2	BE/BB	1
	7+4	6.1	7.3	1.2	+	Donor	Partner	No	306.0	3	BE/BB(2), BC/BB	2
	20+4	5.0	7.0	2.0	+	Patient	Partner	Yes	355.1	2	BE/BB	1
	19+1	8.0	12.0	4.0	+	Donor	Partner	Yes	53.0	2	BE/BB	1
	16+5	7.0	7.2	0.2	+	Donor	Partner	Yes	1905.3	2	BE/BB	1
	10+4	4.5	9.7	5.2	+	Donor	Partner	Yes	1768.0	3	BE/BB	1
	12+2	6.0	8.7	2.7	+	Patient	Partner	Yes	186.0	2	BE/BC	1
	9+5	6.0	8.0	2.0	+	Donor	Donor	Yes	162.5	2	BE/BB	2
Aborted	11+1	5.6	9.0	3.4	+	Donor	Partner	No	1208.0	2	BE/BB	1[a]
	13+5	6.9	10.6	3.7	+	Donor	Donor	Yes	201.5	3	BC/BB	1
	11+0	6.0	8.4	2.4	+	Donor	Partner	No	68.5	3	BE/BA, BE/AB, BE/BB	1[a]
	27+3	6.1	7.8	1.7	+	Donor	Donor	Yes	638.3	2	BE/BC	2
	10+2	5.5	9.4	3.9	+	Donor	Donor	Yes	299.6	2	BE/AA, BE/BB	1
	14+2	4.0	11.2	7.2	+	Donor	Partner	No	57.0	3	BE/BB(2), BE/BC(1)	1
	5+6	5.1	7.0	1.9	+	Patient	Partner	Yes	150.5	2	BE/BB	1
	NR	4.6	12.3	7.7	+	Donor	Partner	Yes	1231.5	2	BE/BB	0
	NR	6.4	8.2	1.8	+	Donor	Partner	Yes	88.0	2	BE/BB	NR
	5+2	5.0	6.2	1.2	-	Patient	Partner	Yes	293.0	3	BE/BB(1), BE/BC(2)	1
	10+0	5.8	7.3	1.5	+	Patient	Partner	Yes	262.0	3	BE/AA(1), BE/AB(2)	1
Biochemical	8+3	4.5	5.6	1.1	-	Donor	Partner	Yes	40.3	3	BE/BB	0
	6+1	6.0	7.4	1.4	+	Donor	Partner	Yes	255.5	3	BE/BB(1), BE/BC(2)	1
	2+3	4.6	7.1	2.5	+	Donor	Partner	Yes	65.0	3	BE/BB	1[b]
	3+2	6.0	8.0	2.0	+	Donor	Partner	Yes	167.0	3	BE/BB	NR
Not Pregnant	NA	5.2	8.0	2.8	+	Donor	Donor	Yes	0	3	BE/BB	0[a]
	NA	5.4	7.1	1.7	+	Donor	Partner	Yes	0	2	BE/AB	0
	NA	4.5	7.0	2.5	+	Donor	Donor	Yes	0.1	2	BE/BB	0
	NA	6.3	12.3	6.0	+	Donor	Partner	Yes	0	2	BE/BB, BE/BC	0
	NA	2.1	6.2	4.1	-	Patient	Partner	Yes	0.1	3	BC/BB, BE/BC, BE/BB	0
	NA	6.3	7.6	1.3	+	Patient	Partner	Yes	0	2	BC/BB	0[a]
	NA	4.0	7.0	3.0	+	Patient	Partner	Yes	1.7	1	BE/BB	0
	NA	6.6	9.0	2.4	+	Donor	Donor	Yes	0	3	BE/BB(1), BE/BC(2)	0
	NA	6.0	8.2	2.2	+	Donor	Partner	No	0	3	BE/BB(2), BE/BC(1)	0
	NA	5.5	8.7	3.2	+	Donor	Partner	Yes	0	2	BE/AA	0
	NA	5.0	7.0	2.0	+	Donor	Partner	Yes	0.1	3	BE/CC	0
	NA	10.9	7.3	-3.6	+	Donor	Donor	Yes	0	2	NR	0

NA	4.1	5.6	1.5	-	Patient	Partner	Yes	0	3	BE/BC	0
NA	3.2	11.6	8.4	+	Donor	Partner	Yes	7.7	2	BE/BB	0
NA	5.3	7.2	1.9	+	Patient	Partner	Yes	0	1	BE/BC	0
NA	4.5	7.5	3.0	+	Patient	Partner	Yes	0	3	BC/BB(1), BC/BB(2)	0
NA	4.5	7.2	2.7	+	Donor	Partner	No	0	3	BC/BB(1), BE/BB(2)	0
NA	8.1	10.0	1.9	+	Donor	Donor	Yes	8.1	3	NR	0
NA	6.0	6.2	0.2	-	Donor	Donor	Yes	0	3	BE/BB	0
NA	6.7	8.6	1.9	+	Donor	Donor	Yes	0	2	BE/BB	0
NA	4.5	5.5	1.0	-	Donor	Partner	Yes	0	3	BE/BE	0
NA	3.0	7.3	4.3	+	Donor	Donor	Yes	0.16	2	BE/BB, BE/BC	0

Abbreviations: EMT: endometrial mean thickness; Diff: difference in mm between pre and post-EMT; ICM: Inner Cell Mass; NA: not applicable; NR: not recorded; QTPC: Qualitative test for pregnancy confirmation; β-hCG: beta-fraction of human chorionic gonadotropin. [a]The patient had multiple IVF cycles. These are the data for the first cycle. [b]The patient has multiple IVF cycles. These are the data for the second cycle. [c]After PRP and before assisted reproduction, the patient was able to get pregnant spontaneously.

Table 3. Comparison of outcomes between the treatment and the previous cycles

Category	Previous Cycle	Treatment Cycle	*p*-value[a]
Cycle implantation rate (%)	22.6 (14/62)	63.9 (39/62)	<0.001***
Normal weight	30.8 (8/26)	71.1 (19/26)	0.001***
Overweight/obese	17.1 (6/35)	57.1 (20/35)	0.001***
Clinical pregnancy rate (%)	16.1 (10/62)	57.4 (35/62)	<0.001***
Normal weight	19.2 (5/26)	69.2 (18/26)	0.001***
Overweight/obese	14.3 (5/35)	48.6 (17/35)	0.002*
Ongoing pregnancy/live birth rate (%)	0.0 (0/62)	40.3 (25/62)	<0.001***
Normal weight	0.0 (0/27)	51.9 (14/27)	<0.001***
Overweight/obese	0.0 (0/35)	31.4 (11/35)	0.001*

[a]*p*-value was calculated using the McNemar test. Significant results (two-tailed) are indicated as follows: *P<0.05, ***P<0.001.

could affect their menstrual cycle or their platelet concentration. Lastly, 88 participants were removed for lacking complete CBCs, resulting in 1,854 participants being included in this analysis (**Table 4**). The total cohort presented an average BMI of 26.5 ± 0.2 kg/m^2; however, most of the cohort were overweight or obese (51.7%). When the cohort was divided into two groups ("normal weight" and "overweight/Obese"), there were no clinical differences regarding age, systolic or diastolic blood pressure or LH. The platelet count was significantly higher in the normal weight group, which resulted in the MPR index scores being significantly higher in this group as well (P<0.05).

The COSINOR analysis was used to assess obesity's effect on platelet fluctuations during the menstrual cycle. For this cohort, to verify that the model was predictive, the well-characterized hormone LH, that oscillates during the menstrual cycle was examined (**Figure 2A**). As expected, LH presented a significant rhythm, as confirmed with the zero-amplitude test (P<0.05). A significant MESOR and amplitude (MESOR = 8.7 mg/dL, amplitude = 3.5 mg/dL) was observed, and the cosine fit conformed to the expected cycle, suggesting that the cohort represents a typical menstrual cycle. For the platelet count and the MPR index, in the overall cohort, a rhythm was observed (P<0.05, **Figure 2B-D** and **Table 5**), with platelet volume almost achieving significance (P = 0.056). However, when the cohort was stratified into normal weight and overweight/obese, the model presented with a significant rhythm (P< 0.05). Upon further investigation, only the normal weight group presented with a significant amplitude and acrophase (P<0.05). Even though the MESORS were significant for platelet count, platelet volume, and the MPR index, the MESORs for platelet count were significantly higher in the overweight/obese group than in the normal weight group (P<0.001). However, the MESORs for the MPR index, the normal weight group, presented a higher score (P< 0.001). Overall, this suggests that obesity affects platelet fluctuations during the menstrual cycle.

Table 4. Characteristics of the study participants from the NHANES dataset

Category	Total[a]	NW[a]	OW/OB[a]	*p*-value[b]
Sample (n)	1854	831	1023	-
Age (years)	32.7 ± 0.3	31.7 ± 0.4	33.5 ± 0.4	0.001**
Weight (kg)	70.5 ± 0.7	59.0 ± 0.3	81.2 ± 0.8	<0.001***
Height (m)	163.1 ± 0.3	163.6 ± 0.4	162.7 ± 0.3	0.075
Body-mass Index (kg/m^2)	26.5 ± 0.2	22.0 ± 0.1	30.6 ± 0.2	<0.001***
Normal weight (%)	48.2 ± 2.1	100	-	-
Overweight (%)	26.0 ± 1.6	-	50.3 ± 2.6	
Obese (%)	25.7 ± 1.7	-	49.7 ± 2.6	
Waist circumference (cm)	88.29 ± 0.55	78.6 ± 0.3	97.3 ± 0.7	<0.001***
Systolic blood pressure (mmHg)	111 ± 1	108 ± 1	114 ± 1	<0.001***
Diastolic blood pressure (mmHg)	68 ± 1	67 ± 1	69 ± 1	0.001**
Blood counts				
Red blood cell (n)	4.49 ± 0.19	4.45 ± 0.02	4.52 ± 0.02	0.002**
White blood cell (n)	7.32 ± 0.07	7.07 ± 0.08	7.57 ± 0.11	0.001*
Platelet count (10^3/mL)	293.7 ± 2.3	284.6 ± 4.7	302.2 ± 3.1	0.008*
Mean platelet volume (fL)	8.16 ± 0.02	8.14 ± 0.05	8.18 ± 0.04	0.553
MPR	2.98 ± 0.03	3.08 ± 0.06	2.89 ± 0.04	0.025
Laboratory results				
Follicle stimulating hormone (mIU/mL)	7.7 ± 0.5	8.1 ± 0.6	7.3 ± 0.6	0.262
Luteinizing hormone (mIU/mL)	9.7 ± 0.9	10.6 ± 1.1	9.0 ± 1.2	0.260
Days since the last period	15.1 ± 0.3	15.3 ± 0.4	14.9 ± 0.4	0.480

Abbreviations: NW: normal weight group; MPR: the mean platelet volume to platelet count ratio; OW/OB: overweight/obese group. [a]Data are presented as mean or frequency ± standard error. [b]*p*-value corresponds to the difference between the NW and OW/OB groups determined by either the Chi^2 test, Student's *t*-Test, or Mann-Whitney U test. Significant results (two-tailed) are indicated as follows: *P<0.05, **P<0.01, ***P<0.001.

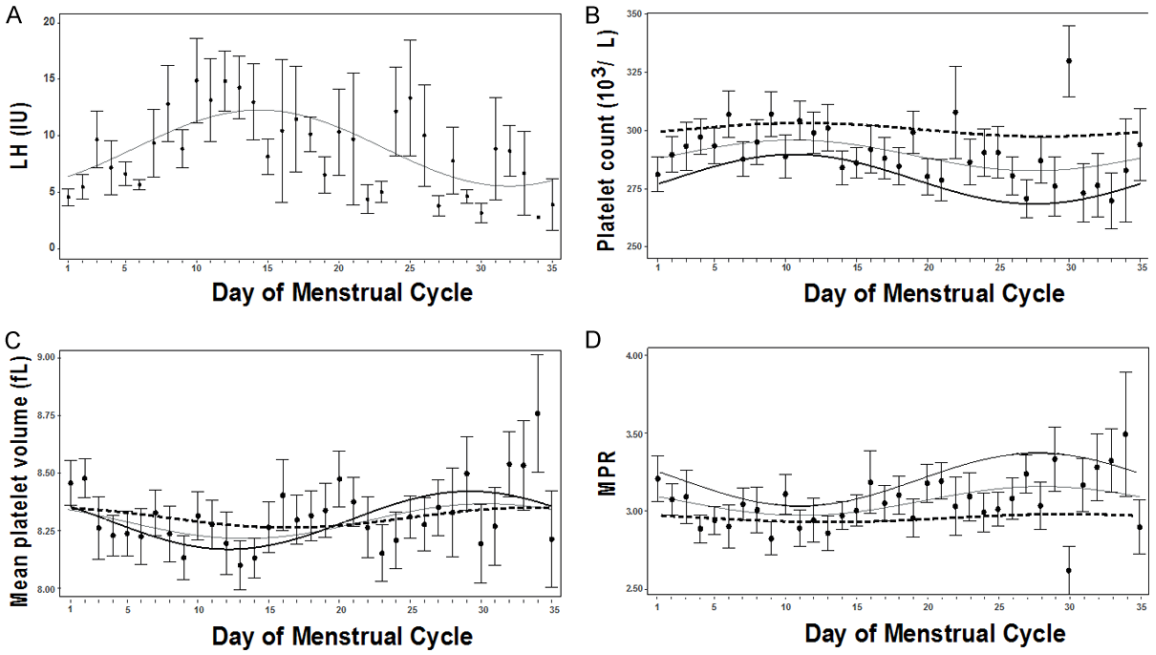

Figure 2. Platelets fluctuate during the menstrual cycle, which is affected by obesity. Means (dot) and standard error (bars) plots with cosine waves were constructed to demonstrate the changes for (A) luteinizing hormone (LH, IU/L), (B) platelet count (10^3/μL), (C) mean platelet volume (fL), and (D) the mean platelet volume to platelet count ratio index (MPR) during the menstrual cycle. Cosinor model fits are shown for all participants (grey line), normal-weight participants (black line), and overweight/obese participants (dashed line).

Table 5. Fluctuations in platelet count, platelet volume, and the mean platelet volume to platelet count ratio index during the menstrual cycle, stratified into normal weight and overweight/obese participants

Group	N[a]	MESOR[b]	Amplitude	Acrophase[b]	$p_{Zero\text{-}amp}$[c]
Platelet count					
Overall	1854	291 (288-294), <0.001*	7 (2-11), 0.006*	-1.4 (-2.1–0.7), <0.001*	0.022*
Normal weight	831	280 (275-285), <0.001*	11 (4-18), 0.003*	-1.4 (-2.0–0.7), <0.001*	<0.001***
Overweight/obese	1023	301 (290-313), <0.001*	3 (-4-9), 0.377	-1.3 (-3.3-0.8), 0.238	
		p_{MESOR}[d]<0.001*	p_{AMP}[e] = 0.108	p_{ACR}[f] = 0.907	
Platelet volume					
Overall	1854	8.30 (8.27-8.35), <0.001*	0.07 (0.01-0.14), 0.016*	-0.8 (-1.6–0.1), 0.022*	0.056
Normal weight	831	8.30 (8.24-8.36), <0.001*	0.12 (0.03-0.21), 0.007*	-1.1 (-1.7–0.4), 0.002*	0.012*
Overweight/obese	1023	8.31 (8.17-8.46), <0.001*	0.04 (-0.03-0.12), 0.302	-0.3 (-2.1-1.5), 0.739	
		p_{MESOR}[d] = 0.792	p_{AMP}[e] = 0.184	p_{ACR}[f] = 0.419	
MPR index					
Overall	1854	3.06 (3.01-3.10), <0.001*	0.09 (0.03-0.16), <0.001***	-1.9 (-2.0–0.6), <0.001*	0.022*
Normal weight	831	3.19 (3.12-3.26), <0.001*	0.17 (0.07-0.27), <0.001***	-1.3 (-1.8–0.7), <0.001*	<0.001***
Overweight/obese	1023	2.94 (2.78-3.10), <0.001*	0.03 (-0.07-0.12), 0.580	-0.9 (-4.1-2.2), 0.569	
		p_{MESOR}[d]<0.001*	p_{AMP}[e] = 0.034*	p_{ACR}[f] = 0.818	

Abbreviations: MESOR: Midline Estimating Statistic of Rhythm; MPR: the mean platelet volume to platelet count ratio. [a]Number of participants. [b]MESOR, amplitude, and acrophase were determined using the COSINOR and COSINOR2 packages for R. Values are estimated, as well as a 95% confidence interval and *p*-values. [c]*p*-value was calculated using the cosinor. Detect function of the COSINOR2 package, which detects rhythm (also called the zero-amplitude test) and tests the overall significance of the cosinor model. [d]*p*-value is for the difference in MESORS and was calculated using the summary function of the COSINOR package. The result assessed the difference between the second and primary categories. [e]*p*-value is for the difference in amplitude and was calculated using the test_cosinor function of the COSINOR package. [f]*p*-value is for the difference in acrophase and was calculated using the test_cosinor function of the COSINOR package. Significant results (two-tailed) are indicated as follows: *P<0.05, ***P<0.001.

Discussion

In this study, the implantation and clinical pregnancy rates improved in women who underwent intrauterine infusion of PRP. Patients who achieved a live birth or had an ongoing pregnancy showed an increase in their Endometrial thickness (EMT); however, patients in whom the IVF cycle resulted in a failed implantation, biochemical pregnancy, or an abortion also had similar increases. Thus, this suggests that, even though PRP did improve EMT, other factors should still be considered, such as obesity. Indeed, obesity was associated with low rates of embryo implantation and clinical pregnancy. Here, we also show that obesity is associated with loss of platelet fluctuations during the menstrual cycle.

EMT is postulated to be critical in determining an augmented probability of achieving successful embryo implantation [25]. Indeed, in 90% of the cycles included in our cohort, the patients' EMT was >7 mm after PRP treatment, and a higher portion had their embryos implanted (63.9%). Furthermore, it has been demonstrated that both clinical pregnancy and live birth rates decreased significantly when EMT was <7 mm, independent of whether the embryo transfer was fresh or frozen-thawed [5]. The mechanisms for thin endometrium-impaired receptivity have not yet been elucidated; nevertheless, the balance between the pro- and anti-inflammatory cytokines and their associated pathways are speculated to be key for endometrium receptivity [7, 26, 27]. Three proposed mechanisms involved in PRP's contribution to endometrial adequateness are angiogenesis, metabolic health, and the Th1/Th2 cell ratio.

Endometrial angiogenesis is crucial for endometrial regeneration. Insufficient vascularization may be a plausible cause of thin or inadequate endometrium, as uterine blood flow is essential for endometrial growth and vascular development [25]. PRP application has been demonstrated to improve reproductive outcomes due to increased tissue vascularization in mice [28]. Recently, it has been proposed that the therapeutical feature of PRP in endometrial repair for infertile patients is mediated by growth factors contained in platelet gran-

ules released in response to activating stimuli. PDGFs facilitate angiogenesis, cytoskeletal plasticity, and cell migration by exerting mitogenic action on cells of mesenchymal origin. At the same time, VEGF promotes angiogenesis by supporting tissue homeostasis and promoting accelerated repair in the case of damage. Even though highly variable in concentration, VEGF is a critical factor associated with endometrial receptivity [29]. Immunochemical characterization of PRP injected into patients with endometrial atrophy, who presented a significant increase in EMT, revealed that higher levels of PDGF-BB (2.8 times) and VEGF (2.4 times) are present in PRP when compared to ordinary plasma [30].

Within our cohort, many of the patients also suffered from other forms of infertility, affecting the endometrium's receptivity. Metabolic disorders, such as insulin resistance, PCOS, or Type 2 Diabetes Mellitus, are more frequent in overweight/obese patients. Recently, our group demonstrated that correcting insulin resistance in PCOS patients, which was also associated with weight loss, improved EMT and endometrial receptivity for successful ongoing pregnancies [31]. Recently, in PCOS patients, a significant increase in EMT was observed after PRP infusions; however, none of the patients were able to conceive. Although not significant, the increase in EMT for the PCOS group was less when compared to patients with other non-metabolic factors of infertility (tubal or diminished ovarian reserve) [32]. This may explain the observed differences between the normal-weight and the overweight/obese patients observed here, in which obesity was associated with lower rates of implantation, clinical pregnancy, and ongoing pregnancy/live birth. Therefore, future work should evaluate how metabolic disorders influence the effect PRP has on endometrial health.

Successful embryo implantation requires the immune system to tolerate the semi-allogeneic embryo by altering the balance of pro- and anti-inflammatory cytokines secreted by Th1 and Th2 cells, respectively [33]. Th1 dominance is associated with implantation failure after embryo transfer, potentially due to the toll-like receptor 4 (TLR4) [34], whereas anti-inflammatory cytokines from the Th2 cells favor implantation [33]. It has been demonstrated that repeated implantation failure and recurrent pregnancy loss have been linked to a Th1/Th2 cell ratio, favoring Th1 cells [35]. Therefore, a shift favoring Th2 cytokines would be beneficial for IVF. PRP downregulates the expression of TLR4, a receptor that protects against non-autologous antigens [36]. Using PRP alters the endometrium's inflammatory cytokine profile. Thus, it is postulated that PRP may act by promoting Th2 cytokines and diminishing Th1 cytokines. This would improve endometrial immune tolerance of endometrial decidualization and improve embryo implantation. Future studies should consider the concentration of cytokines within the PRP isolate and the patient's cytokine profile at the endometrium level.

In vitro studies have shown that prolonged exposure to PRP (three to seven days) leads to endometrial stromal and mesenchymal cell migration and increased proliferation in a time-dependent manner [13]. Therefore, our study proposed a three-dose scheme to mimic this effect. This is because multiple intra-articular injections of PRP in damaged tissues are more effective than a single application, as seen for knee osteoarthritis [37, 38]. This is supported by other studies where the clinical pregnancy rate was higher when at least two doses were applied over a single dose [21, 39, 40]. Therefore, it is posited that a multi-dose treatment should be considered for future studies.

The embryo's successful implantation depends on platelet activation [41, 42]. Platelet activation, which consists of the adhesion and aggregation of platelets, is part of primary hemostasis [43]. This activity not only occurs in epithelial lesions but is also activated by inflammatory processes or blood flow alterations, some of which thin the thickness of the endometrium. If platelet activation is inhibited, the body will suffer from hypofunction of its tissues [44]. Platelet count assesses platelet activation, and mean platelet volume is a marker of platelet size and function [45]. These last two hematologic parameters must be maintained at adequate levels to achieve excellent physiological reproduction [44, 46, 47]. Platelet activation causes changes in the female reproductive tract, such as increasing the motility of the cilia in the uterine tubes and improving the zygote's transport to the uterine cavity. If platelet activation fails or is diminished, early implantations

that are not viable for the embryo can occur in the uterine tubes. Decreased platelets in mice affected the rate of uterine ectopic pregnancies [42]. In the same way, platelet activation causes vasodilation and angiogenesis at the endometrial level to favor embryo implantation [42, 48]. Here, it was observed that the platelet count, the platelet volume, and the MPR index cycled for the normal weight group but were planar for the obese group. Adiposity has been associated with thrombotic changes by promoting platelet activation [49]. However, other studies have not established whether elevated platelet counts in obese individuals are associated with platelet activation [16]. Obesity directly affects the menstrual cycle, as shown by studies where obesity decreases the transport and production of female sex hormones due to altering the hypothalamus-pituitary-ovarian axis. In obese women, gonadotropin-releasing hormone levels are affected, altering the temporospatial production of FSH and LH, which are vital for the menstrual cycle [50, 51]. Due to the presence of estrogen receptors on the platelets, platelet function is affected by the concentration of LH and FSH [52]; therefore, it was expected that obesity would affect platelet function. In normal-weight patients, platelet function should be increased more during the luteal phase than in the follicular phase [53, 54]. Here, we observed that platelet function increased during the luteal phase in patients in the normal weight group and decreased during the follicular phase. It was found that the MPR index in overweight/obese women was arhythmic, which is why platelet activity is expected to be decreased in these patients. Therefore, the effect observed in the clinical study presented here is most likely due to the PRP injections mimicking the normal increase seen for normal-weight participants.

Our study has a few limitations. First, the quality of the PRP was not measured. The lack of a consensus among researchers about the optimal procedure for PRP preparation may affect the clinical outcomes, as the quality and contents of the isolate can vary significantly between each study, between the patient's IVF cycles, and each subsequent PRP preparation for the same patient with an IVF cycle [55]. However, the PRP was obtained and applied three times, and the sample was minimally degraded. Second, the self-control study design uses a repeated measure methodology instead of including a control group. With IVF, the probability of an embryo implanting decreases with each additional cycle. Therefore, the likelihood of an embryo implanting is low after two failed IVF cycles. Concerning the NHANES study, first, only women who presented information about days since their last period were utilized. This assessment did not consider the average number of days for each participant's cycle or whether there were any abnormalities associated with their cycle. Second, diseases affecting platelet count and quality, such as congenital pathologies, especially hematological diseases (hemophilia, hemoglobinopathies, von Willebrand disease, Wiskott-Aldrich syndrome), were not assessed. Moreover, patients taking certain medications, such as heparins, NSAIDs, or antiepileptics, that could directly affect the normal physiology of platelets were not excluded. Therefore, the potential effects of these conditions should be considered and assessed in future studies. Third, the results shown here were not adjusted. Platelet counts and volumes are affected by endometriosis, diet, and lifestyle. These factors should be considered in future studies.

In conclusion, we show that an intrauterine infusion of autologous PRP improved the embryo implantation rate and the clinical pregnancy rate of women suffering from refractory thin endometrium, most likely promoting a change in the uterine environment. In addition, we demonstrate that the platelets fluctuate during the menstrual cycle, which can be lost when subjects become obese. Therefore, intrauterine infusions of PRP are suspected to mimic the normal changes associated with normal-weight women, who are typically more fertile.

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Disclosure of conflict of interest

None.

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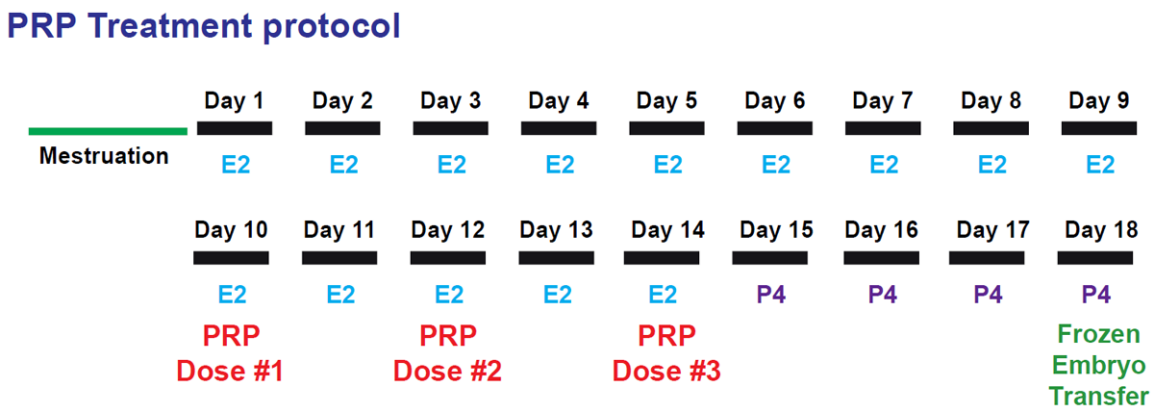
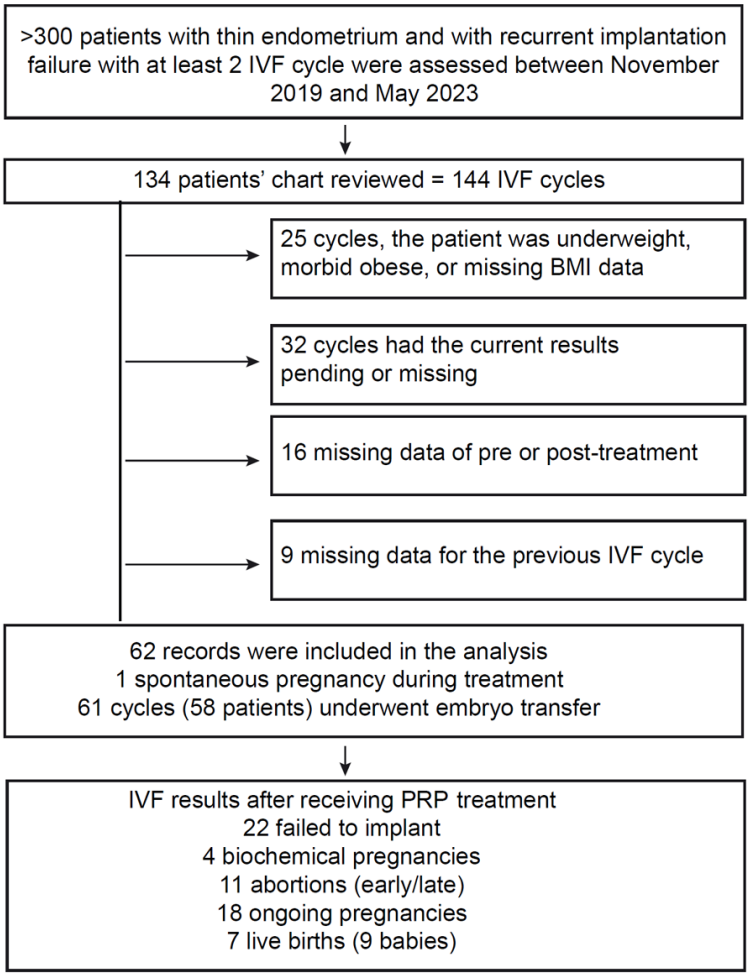
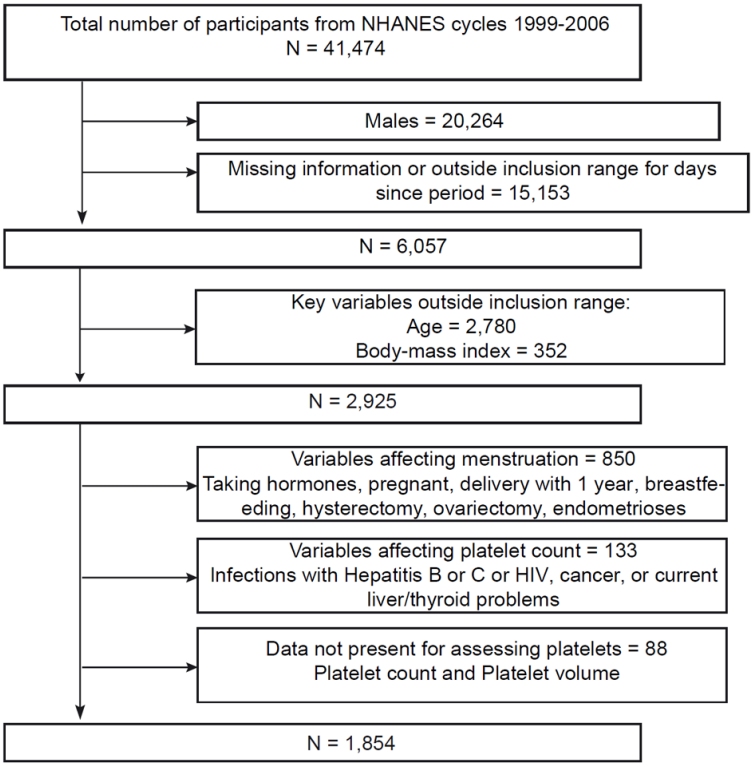


Supplementary Figure 1. Platelet Rich Plasma (PRP) treatment protocol for patients with atrophic endometrium under-going estrogenprimed cycle. Endometrial preparation with 6 mg of estrogen valerate was started on the menstrual cycle Day 2 or 3. On the menstrual cycle Day 10, PRP was infused into the uterine cavity. The procedure was repeated on the menstrual cycle Day 12 and 14 until a 7 mm endometrial thickness was reached. Then, progesterone support for the luteal phase was started, and embryos were thawed and transferred on their corresponding development day. Abbreviations: E2: estradiol, PRP: platelet rich plasma, P4: progesterone.



Supplementary Figure 2. The study flow chart demonstrating the selection of patients to examine the effect platelet-rich plasma has on endometrium thickness and in vitro fertilization outcomes, following the Strengthe-ning the Reporting of Observational Studies in Epidemiology (STROBE) statement.



Supplementary Figure 3. Flow diagram of the literature search and filtering results for the systematic review of the effectiveness of platelet-rich plasma on implantation and clinical pregnancy rates patients undergoing in vitro fertilization, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.