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Progesterone peak influences embryonic developmental morphokinetics on trigger day? A retrospective study

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Abstract

Objective Premature Progesterone Rise (PPR) is characterized by elevated serum progesterone concentrations either towards the end of the follicular phase or on the trigger day, surpassing a pre-defined threshold value. Aim of the study is to evaluate the impact of PPR exceeding 1.5 ng/ml at the time of hCG-trigger on embryo morphokinetic parameters and to identify predictive biomarkers of in IntraCytoplasmic Sperm Injection (ICSI) cycles outcomes.

Methods It is a retrospective study including patients underwent ICSI cycles in the period 2020–2023. 58 patients were recruited in the study group showing P levels in the trigger day greater than or equal to 1.5 ng/ml. A matching control group of 58 patients with P levels below 1.5 ng/ml was after selected. The general characteristics of these patients, including age, Body Mass Index (BMI), antral follicle count (AFC), anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) levels, the type of infertility and smoking/non-smoking patients, were recorded on the day of their initial visit. Subsequently, data were collected regarding the number of eggs retrieved, mature eggs, successfully fertilized eggs, and embryos reaching the blastocyst stage. Additionally, the timing of embryonic development and the quality of obtained blastocysts, as assessed by the degree of expansion and the characteristics of the inner cell mass (ICM) and trophectoderm (TE), were evaluated using Time-Lapse technology.

Results Elevated P levels exceeding 1.5 ng/ml on the trigger day were directly associated with a significantly larger number of antral follicles, consequently leading to a higher count of retrieved eggs, mature eggs, successfully fertilized eggs and embryos reaching the blastocyst stage. Furthermore, the analysis of morphokinetic parameters indicated faster division times and a notably greater number of high-grade blastocysts in the study group compared to the control group.

Conclusions P levels ≥ 1.5 ng/ml on the trigger day did not negatively impact embryonic morphokinetic parameters, instead resulting in faster embryo development in the initial stages.

Keywords In vitro embryo culture, Progesterone peak, Time-Lapse monitoring, Embryo Morphokinetics

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Introduction

Progesterone (P) plays a pivotal role in facilitating embryo implantation in the endometrium, both in natural menstrual cycles and those induced by controlled ovarian stimulation (COS). During the early follicular phase, P levels are low, primarily originating from the adrenal glands, and are usually below 1.5 ng/ml [1]. P levels steadily rise as the follicular cycle advances, primarily due to accumulation from developing follicles and an elevated estradiol (E2) peak. In COS cycles, there is a risk of premature progesterone rise (PPR). It is characterized by an increase in serum P concentrations towards the end of the follicular phase or on the trigger day, surpassing a predefined threshold [2]. The exact causes of PPR in COS cycles are not fully elucidated, but they may involve factors such as the accumulation of P production by numerous developing follicles, excessive use of exogenous gonadotropins, premature luteinization of the dominant follicle and suboptimal ovarian response associated with early increased luteinizing hormone (LH) sensitivity related to the duration of COS [3–5], all more common in Polycystic Ovary Syndrome (PCOS). Elevated P levels exceeding 1.5 ng/ml at the time of hCG administration may lead to the over-expression of various genes in endometrial cells with receptive functions. These genes include GPX3 (Glutathione peroxidase 3), PAEP (Placental protein 14), DPP4 (Dipeptidylpeptidase 4), GADD45A (Growth arrest and DNA-damage-inducible, alpha), HABP2 (Hyaluronan-binding protein 2), EDNRB (Endothelin receptor type B), MPA2 (Inositol(myo)-1(or 4)-monophosphatase 2), CAPN6 (Calpain 6), TFPI2 (Tissue factor pathway inhibitor 2), MAP2K6 (Mitogen-activated protein kinase kinase 6), HLA-DOB (Major histocompatibility complex, class II, DO beta), NDRG2 (NDRG family member 2), and FOLR1 (Folate receptor 1) [6]. These genes are involved in various cellular functions, including cell adhesion, developmental processes, and the immune system, all of which are essential for normal endometrial function development [6]. Additionally, significantly higher concentrations of interleukins such as IL-1β, IL-5, IL-10, IL-12, IL-17, and TNF-α, as well as heparin-binding EGF, eotaxin, and Dkk-1 were observed in endometrial stromal and epithelial cells leading to alterations in endometrial secretory profiles and affecting endometrial receptivity [5–8]. These factors may contribute to a lack of synchronization between embryos and the endometrium, resulting in reduced clinical pregnancy rates (CPR) and live birth rates (LBR) [9–16]. *Hofmann et al.* [2] conducted the first investigation into the effects of high doses of P ($P>1.1$ ng/ml) on oocytes and embryo quality during an oocyte donation program. They found that embryos from patients with high P levels had comparable morphological grades to those without high P levels, with similar outcomes in terms of oocyte quality, fertilization rates, polyspermia rates, embryo quality, implantation rates, and delivery rates. Subsequent studies by *Hill et al.* [17] and L*efebvre et al.* [18] also supported these findings, showing that P levels on the trigger day did not significantly impact embryo quality, implantation rates, or developmental timelines [17, 18]. Moreover, *Baldini et al.* [19] demonstrated that the transfer of cryopreserved blastocysts obtained from cycles with PPR resulted in the same pregnancy outcomes as those blastocysts where no PPR had occurred. This suggested that PPR did not negatively impact embryonic development. Additionally, a retrospective study involving 1078 fresh ICSI cycles suggested that elevated serum P levels (ranging from 2.0 to 2.5 ng/ml) on the day of oocyte maturation were associated with a higher rate of top-quality embryo (TQE) formation. TQEs were defined as those with specific characteristics including the absence of multinucleated blastomeres, appropriate cell numbers on specific days, and minimal anucleated fragments [20]. However, the data in the literature are conflicting. Indeed, despite the above-mentioned studies showing positive effects of PPR in terms of the number and quality of obtained eggs and embryos, a few authors reported that PPR could harm embryo quality. *De Cesare et al.* [21] reported decreased CPR and LBR after day 3 embryo transfers with P levels > 1 ng/ml on the day of trigger. For day 5 embryo transfers, negative outcomes were recorded for *P* values > 1.75 ng/ml. However, blastocyst-stage embryos showed no difference in CPR or LBR regardless of P levels. *Çiftlik et al.* [22] found that PPR on the day of hCG administration had a significant negative effect on embryo development due to increased cumulus cell apoptosis, leading to lower pregnancy rates. *Kong et al.* [23] reported that serum P levels > 1.5 ng/ml on the day of hCG administration did not influence clinical pregnancy outcomes in cleavage-stage embryo transfers but negatively impacted CPR after blastocyst transfer. They suggested that opting for cleavage-stage embryo transfer might be more reasonable when progesterone levels exceed 1.15 ng/ml on the hCG day. Further evidence of the negative role of PPR on embryo quality came from *Villanacci et al.* [24], who observed an inverse relationship between P levels on the day of trigger and the rate of blastocyst formation on day 5. Higher P levels on the trigger day were associated with a higher percentage of blastocysts developing on day 6 or day 7 in COS cycles. Our study aimed to identify potential predictive and non-invasive biomarkers related to morphokinetics in cases where there was PPR, since, so far, no one had investigated the potential effects of PPR on embryonic morphokinetic parameters.

Methods

Study design

This single-center retrospective cohort study was performed in the Momò Fertilife Private Center for Reproductive Medicine Bisceglie, Italy, in the period between April 2020 and July 2023, in which there were performed 1217 oocyte retrievals. Couples with the following clinical characteristics were excluded from the study:

- Karyotype abnormalities.
- Male partner with severe oligoasthenospermia.
- Female partner over 40 years old.
- Female partner with BMI ≥ 30.
- Female partner with reduced ovarian reserve.
- Female partner with endocrinopathies.

Once ICSI cycles were performed, patients showed serum P levels measured on the trigger day greater than or equal to 1.5 ng/ml were included in the study group. MedITEX – IVF software (CRITEX GmbH; Regensburg; Germany) was then used to identify a matching control group of patients with similar characteristics based on their epidemiologic characteristics and data resulting from anamnesis (age, body mass index (BMI), antral follicle count (AFC), Anti-Müllerian hormone (AMH) and Follicle-Stimulating Hormone (FSH) blood levels, the type of infertility and smoking/non-smoking). To obtain the evaluation of the sample to be analyzed, we applied the formula of the sample size calculator with G*Power software (Heinrich Heine Universitat, Dusserdolf, Germany). We set a dichotomous or binomial endpoint (only two possible results). The parameters used to calculate the sample size were: an expected incidence of 4.5% (this incidence was related to the possibility of having a premature P peak in stimulated cycles) and an expected study group of around 30% (with the prediction that a morphokinetic development anomaly could occur in at least 30% of cases), with a Type I/II error rate of alpha 0.05 and beta 0.05. The power of the study was set at 95%. All these parameters determined the need for a sample of at least 47 patients in the study group. We selected 58 patients to further increase the power of the study. The study design is summarized in Fig. 1.

Patients' selection

Patients were selected based on their serum P levels measured on the trigger day after underwent ICSI cycle. In detail, the study group consisted of 58 women with P levels greater than or equal to 1.5 ng/ml, while the matching control group comprised 58 women with P levels below 1.5 ng/ml selected using MediTEX software as described above. Epidemiological characteristics of the patients, including age, BMI, AFC, AMH and FSH blood levels, the type of infertility and smoking/non-smoking patients were recorded. Additionally, data on the outcomes of the assisted reproductive cycle, such as the number of retrieved eggs, mature (MII) eggs, fertilized eggs, and blastocysts formed were collected.

Progesterone (P) measurement

Serum P levels were assessed through analysis of a blood sample taken from patients on trigger day, which was immediately delivered to the laboratory to complete the measurement by MINDRAY PROG (CLIA) CL-1200i

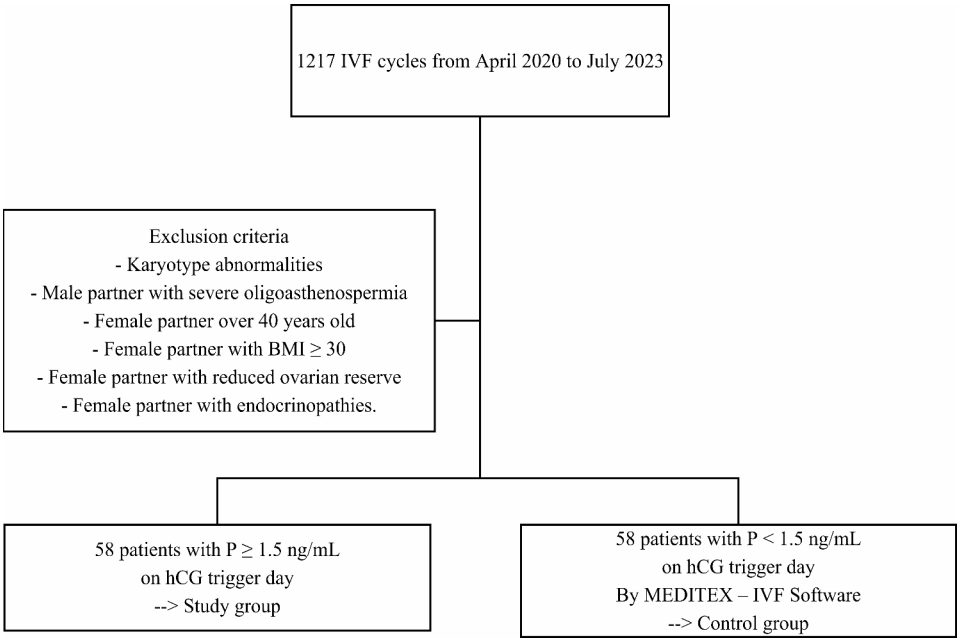

Fig. 1 Flowchart of study participation

(Mindray Medical International Limited, Shenzhen, China). It is a chemiluminescent immunoassay based on the principle of competitive binding for the quantitative determination of P in human serum. Each determination of P was performed in triplicate order to ensure the best accuracy and the mean was considered the final value. The assessment device kit showed a sensitivity ≤ 0.1 ng/ml. Precision: Intra-assay CV: 5.15%, Inter-assay CV: 7.4%.

Controlled ovarian stimulation protocol and oocyte collection

Patients of both groups were down-regulated with a conventional GnRH antagonist (Cetrotide, Merck Serono, Germany) and stimulated with a recombinant FSH preparation (GONAL-f., Merck Serono, Darmstadt, Germany). Once at least 3 follicles with diameters ≥ 18 mm were reached, a dose of 10.000 IU of hCG was administered to induce ovulation (GONASI, IBSA, Lugano, Switzerland). On the same day, serum P concentration was also determined through a blood sample. The cumulus-oocyte complexes (COCs) were retrieved via transvaginal ovarian pick-up (OPU) under ultrasound guidance (VOLUSON S8, GE Healthcare; Chicago, IL, USA) [25]. This procedure was performed approximately 35 to 36 h after induced ovulation. Three hours after the retrieval of cumulus-oocyte complexes, they were denuded from the corona radiata by repeated pipetting in a solution containing 25 IU/ml hyaluronidase (LifeGlobal Group, Guildford, CT, USA) [19, 25–27]. The ICSI procedure was performed on a heated stage at 37 °C under an inverted microscope (Nikon Eclipse TE 200) at 400X magnification. ICSI was performed by an oil-hydraulic microinjection system (Nikon Eclipse TE 200) [19, 25–27].

Embryo culture

Injected oocytes were transferred to an EmbryoSlide culture dish (Geri, Genea Biomedx, Australia) prefilled with 80 µl of commercial IVF culture single-step medium (Gtl, Vitrolife, Sweden). The culture dish was then covered with 4 mL of paraffin oil. Embryos were cultured in a time-lapse incubator (Geri, Genea Biomedx, Australia) at 37 °C under a controlled atmosphere of 6% CO_2 and 5% O_2. The fertilization rate was assessed approximately 16 to 20 h after ICSI and was defined as the presence of two pronuclei and two polar bodies within the matured MII oocytes that were inseminated. Time-lapse images of each embryo were retrospectively analyzed using external image analysis software (Geri Connect & Geri Assess Software, Genea Biomedx, Australia). Embryonic events were annotated with corresponding timing in hours after ICSI. These annotations included key events after insemination such as the extrusion of the second polar body (*t2PB*), appearance of the two pronuclei in the cytoplasm (*t2PN*), cleavage to the two- and four-cell stage (*t2*, *t4* respectively), the developmental timelines of the morula (*tm* - when the blastomeres exhibited compaction forming a continuous outer covering composed of the plasma membranes of the outermost cells), and the time of early blastocyst formation (*tb* - when the blastocyst began to exhibit the blastocoelic cavity) [28, 29]. Blastocyst morphology was evaluated using conventional scoring criteria on day 5 blastocyst embryos according to Gardner's classification [30].

Statistical analysis

First, the two selected groups were statistically evaluated to demonstrate that the two samples were homogeneous. For this reason, epidemiological data were studied as mean ± standard deviation for continuous parametric variables or as a percentage for categorical variables. IVF outcomes, embryonic morphokinetic parameters, and blastocyst quality were assessed. Student's t-test was used to compare age, BMI, AFC, AMH, FSH, type of infertility, smoking and non-smoking patients, number of retrieved oocytes, mature oocytes (MII), fertilized oocytes, obtained blastocysts, and embryonic developmental timelines between the study and control groups. The chi-square test was employed to assess if there were statistically significant differences between the different stages of embryonic development reached in terms of the total number of embryos. Additionally, a t-test was used to compare blastocyst quality in terms of the degree of expansion and morphology of the Inner Cell Mass (ICM) and Trophectoderm (TE) between the two groups. All statistical evaluations of patient parameters were performed by comparing the study group and controls and conducted using GraphPad Prism software (GraphPad Software , 225 Frankli Street. Fl. 26 Boston MA 02110) .

Results

As reported in Fig. 1, of 1217 IVF cycles available during the study period, 58 had progesterone greater than or equal to 1.5 ng/mL and were included in the "study group" of this research. We also included 58 matched controls (Control group) for comparison. In Table 1, the general characteristics at enrollment including age, BMI, AFC, AMH, FSH, the type of infertility, and smoking/non-smoking parameters of the women with P levels greater than or equal to 1.5 ng/ml (study group), and their matched controls were compared. The data indicate that the groups are well-matched concerning the analyzed parameters, except for AFC. Specifically, the AFC of the study group was 17.38 ± 4.80, whereas that of the controls was 14.38 ± 5.18. This difference in AFC between the study group and controls was statistically significant ($p < 0.05$; Table 1).

Table 1 General characteristics at enrolment into the cohort of study group and controls

	Study group (*n* = 58)	Control group (*n* = 58)	*P*-value
Age (years)	35.46 ± 4.16	35.15 ± 4.40	0.717
BMI (kg/m^2)	22.94 ± 3.88	24.82 ± 4.57	0.6618
AFC (n)	17.38 ± 4.80	14.38 ± 5.18	0.0073
AMH (ng/ml)	5.461 ± 4.55	2.70 ± 2.35	0.6988
FSH (IU/ml)	6.37 ± 1.57	7.53 ± 2.50	0.4555
Idiopathic infertility	16/58 (27.59%)	24/58 (41.38%)	0.1711
Male factor	26/58 (44.83%)	19/58 (32.76%)	0.2528
Female factor	16/58 (27.59%)	15/58 (25.86%)	1.0000
Smokers	19/58 (32.76%)	17/58 (29.31%)	0.8411
Non-Smokers	39/58 (67.24%)	41/58 (70.69%)	0.8411

Note: Data are presented as mean ± standard deviation or percentage

Table 2 IVF outcomes of the analyzed two groups

	Study group	Control group	*P*-value
Picked-up eggs	15.26 ± 5.93	11.51 ± 5.32	0.0005
MII eggs	12.26 ± 5.54 (80.34%)	8.12 ± 4.10 (70.58%)	0.0001
Fertilized eggs	7.93 ± 3.40 (64.66%)	5.81 ± 3.67 (71.49%)	0.0018
Blastocysts	4.84 ± 2.76 (61.06%)	3.57 ± 2.55 (57.10%)	0.0133

Note: Data are presented as mean ± standard deviation or percentage

IVF outcomes (picked-up eggs, MII eggs, fertilized eggs, and obtained blastocysts) of the two groups were reported in Table 2. As for the study group, 870 oocytes were retrieved during pick-up procedure (mean and standard deviation: 15.26±5.93); 80.34% (699/870) of these eggs were evaluated as MII eggs (12.26±5.54); 64.66% (452/699) of these MII eggs correctly fertilized (7.93±3.40) developing into embryos and 61.06% (276/452) became blastocysts (4.84±2.76). In the control group 656 oocytes were picked-up (11.51±5.32) with 70.58% (463/656) of MII eggs (8.12±4.10); 71.49% (331/463) of fertilized eggs (5.81±3.67) and 57.10% (189/331) of developed into blastocysts (3.57±2.55). All the four parameters assessed showed statistically significant differences between the two groups ($p<0.05$). In detail, the study group displayed a higher number of oocytes retrieved, MII, and fertilized eggs and obtained blastocysts rather than the control group.

Table 3 presents the data on correctly fertilized embryo morphokinetic parameters for both the study and control groups. A total of 381 embryos were analyzed for the study group, while 183 embryos were analyzed for the control group. The observed embryos were classified into five categories based on the developmental stage reached. As depicted in Table 3, embryos in both groups progressed to the blastocyst stage, with more blastocysts observed in the study group. Specifically, the blastocyst rate in the study group was 241/699 embryos (34.5% per injected oocyte), which was significantly higher ($p<0.05$) than that of the control group, where 154/463 embryos (33.3% per injected oocyte) developed into blastocysts. On TLM, the analyzed morphokinetic parameters were faster in all categories of embryos for the study group, except for the 4-cell arrested stage and the t2 timing of the 2-cell arrested stage, which were reached more quickly in the control group. Notably, the study group's blastocysts developed significantly earlier than those in the control group at the *t2PB* (2.55±1.24 vs. 3.32±1.09 h, $p=0.0001$), *t2PN* (8.70±2.85 vs. 9.79±1.89 h, $p=0.0001$) and *t2* (24.42±5.03 vs. 27.51±20.82 h, $p=0.03$). Instead, *t4* (37.38±6.96 vs. 37.89±4.20 h, $p=0.42$), *tm* (87.97±10.50 vs. 91.14±8.65 h, $p=0.17$) and *tb* (106.98±11.10 vs. 107.67±8.76 h, $p=0.99$) were comparable between the two groups.

Table 4 demonstrates that blastocysts of higher quality were observed in the study group, characterized by a higher degree of expansion and a type A quality for both

Table 3 Time-lapse profile of embryo development after ICSI

	n	Developmental stage	t2PB (h)	t2PN (h)	t2 (h)	t4 (h)	tm (h)	tb (h)
Study group	241	Blastocyst	2.55 ± 1.24	8.70 ± 2.85	24.42 ± 5.03	37.38 ± 6.96	87.97 ± 10.50	106.98 ± 11.10
	17	Morula arrested	2.35 ± 1.17	8.47 ± 2.31	26.71 ± 8.08	40.88 ± 12.74	91.06 ± 9.42	-
	39	4-cell arrested	3.29 ± 1.72	9.74 ± 2.70	34.17 ± 37.99	42.10 ± 8.98	-	-
	17	2-cell arrested	2.56 ± 1.40	7.6 ± 2.03	34.45 ± 23.50	-	-	-
	67	No cleavage	2.56 ± 1.05	9.63 ± 2.41	-	-	-	-
Control group	154	Blastocyst	3.32 ± 1.09	9.79 ± 1.89	27.51 ± 20.82	37.89 ± 4.20	91.14 ± 8.65	107.67 ± 8.76
	3	Morula arrested	3.67 ± 1.15	9.33 ± 2.08	31.67 ± 9.81	47 ± 13	115 ± 12.17	-
	10	4-cell arrested	3 ± 1.56	9.2 ± 1.55	28.2 ± 3.36	40.2 ± 4.24	-	-
	3	2-cell arrested	2.67 ± 1.53	12 ± 1.73	33.33 ± 2.08	-	-	-
	13	No cleavage	3 ± 1.15	10.54 ± 2.93	-	-	-	-
P-value		Blastocyst	0.0001	0.0001	0.0330	0.4245	0.1712	0.9949
		Morula arrested	0.2480	0.5431	0.3393	0.4422	0.0008	-
		4-cell arrested	0.5939	0.5461	0.6251	0.5204	-	-
		2-cell arrested	0.8617	0.0030	0.9365	-	-	-
		No cleavage	0.2220	0.2355	-	-	-	-

Note: Data are presented as mean ± standard deviation or percentage

Table 4 Assessment of blastocyst quality in terms of degree of expansion, ICM and TE

		Study group	Control group	*P*-value
Degree of expansion	1	-	-	
	2	14 (5.81%)	52 (33.77%)	0.0001
	3	66 (27.39%)	92 (59.74%)	0.0001
	4	145 (60.17%)	7 (4.54%)	0.0001
	5	16 (6.64%)	3 (1.95%)	0.03
	6	-	-	
ICM	A	133 (55.19%)	92 (59.74%)	0.37
	B	79 (32.78%)	56 (36.36%)	0.46
	C	28 (11.62%)	6 (3.90%)	0.01
	D	1 (0.41%)	-	0.42
Trophectoderm	A	115 (47.72%)	87 (56.49%)	0.08
	B	100 (41.49%)	50 (32.47%)	0.07
	C	23 (9.54%)	17 (11.04%)	0.63
	D	3 (1.24%)	-	0.16

Note: Data are presented as mean ± standard deviation or percentage

ICM and TE cells, according to Gardner's classification [30].

Discussion

Several studies reported how PPR could have a detrimental impact on endometrial secretory profiles, affecting endometrial receptivity and leading to a lack of synchronization between embryos and the endometrium, resulting in reduced CPR and LBR. Regarding embryo quality, the scientific literature shows conflicting opinions. This is the first study that focuses on the effects of PPR on embryonic morphokinetic parameters, highlighting whether there was a direct correlation between high serum levels of P on trigger day with embryonic development timelines and the quality of obtained blastocysts.

There were no significant differences observed among age, BMI, AMH and FSH levels, the type of infertility, and smokers/non-smokers among the patients evaluated in both groups. However, the incidence of PPR was found to be higher among patients with a greater number of antral follicles. Several studies have supported this observation, indicating an increased incidence of PPR in patients with a larger number of antral follicles, suggesting the impact of a larger follicle cohort on elevated progesterone levels. *Kyrou et al.* [31] investigated the relationship between PPR and follicle count in GnRH antagonist/rec-FSH stimulated cycles, revealing that patients with progesterone levels greater than or equal to 1.5 ng/ml on the trigger day exhibited a significantly higher number of follicles compared to those with levels below 1.5 ng/ml. This finding was consistent with the results reported by *Fatemi et al.* [32], who suggested that in stimulated cycles characterized by multiple follicular growths, the primary factors contributing to the extent of P secretion from the ovaries include the number of follicles, in addition to FSH stimulation of granulosa cells and LH stimulation of theca cells. Furthermore, *Ashmita et al.* [33] proposed that PPR is a consequence of FSH dose and ovarian response. They suggested that high FSH-only stimulation triggers the recruitment of a significant number of growing follicles, leading to increased ovarian steroidogenic activity and P production.

Our retrospective study has unveiled an association between elevated serum P levels on the day of oocyte maturation and an increased number of eggs retrieved during the pick-up procedure. This finding aligns with the study by *Lefebvre et al.* [18], which also demonstrated a similar trend, reporting that for *P* values exceeding 1.3 ng/ml, there was a significantly higher number of follicles measuring over 11 mm in diameter, resulting in a correspondingly higher number of retrieved oocytes. Furthermore, our data revealed a statistically significant difference in the maturation rate, as it was higher in the study group. These findings contrast with previous observations made by *Kofinas et al.* [16] and *Baldini et al.* [19], who reported no reduction in the number of eggs available for fertilization. However, it should be noted that in the study by *Baldini et al.* [19], the P back was set at a lower value of 1.2 ng/ml. This observation is consistent with the findings of *Hoffman et al.* [2] and *Alnasser et al.* [20], where P levels ranging from 0.9 ng/ml to 1.1 ng/ml resulted in a comparable number of mature oocytes. Our results regarding the maturation rate translated into a greater number of fertilized eggs and subsequently a higher number of blastocysts obtained from patients with P levels exceeding or equal to 1.5 ng/ml on the trigger day. This finding was consistent with the study by *Alnasser et al.* [20], which emphasized that in the presence of high P levels ranging between 2.0 and 2.5 ng/ml on the day of oocyte maturation trigger, there was an increase in the rate of top-quality embryo (TQE) formation. Therefore, there was a higher likelihood of obtaining

more correctly fertilized eggs and, consequently, a greater number of high-grade blastocysts.

Furthermore, our study observed that embryos developed at a faster pace, except for the 4-cell arrested stage, which was reached more quickly in the control group, and the t2 timing of 2-cell arrested embryos. Blastocysts in the study group showed significantly earlier development compared to those in the control group at the early stages of *t2PB*, *t2PN*, and *t2*, while timings for *t4*, *tm*, and *tb* stages were comparable. Obtaining embryos that developed very rapidly in the initial stages could lead to the development of high-quality embryos, positively influencing the outcome of an ART cycle. This observation was supported by previous studies by *Sakkas et al.* [34] and *Li et al.* [35], which demonstrated that patients with early-cleaving embryos exhibited significantly higher PR and implantation rates.

It was expected that delayed embryo development would occur in line with the findings of *Villanacci et al.* [24], who noted an inverse relationship between P levels higher than 1.15 ng/ml on the day of hCG administration and the time required to reach the blastocyst stage. In the presence of high P concentrations, blastocyst formation was anticipated around day 6/7. Additionally, *Coticchio et al.* [36] demonstrated that delayed blastocysts could have higher percentages of anomalies during the cleavage phase, confirming what previously emerged from the findings of *Friedenthal et al.* [37], showing a positive effect of P on embryo development. They reported, indeed, that embryos undergoing rapid division exhibited elevated rates of euploidy and clinical potential, thus positively influencing the outcome of ART cycles.

The quality of the obtained blastocysts was notably higher in the study group, characterized by a superior degree of expansion and better quality of ICM and TE. These findings contrasted with those of *Lefebvre et al.* [20], who reported similar blastocyst quality between groups with P levels above and below 1.3 ng/ml on the day of hCG administration. However, it's worth noting that embryos developing at a faster pace might exhibit greater competence and higher quality, which could ultimately enhance the outcomes of ART cycles [36, 37].

Limitations

However, some limitations of the study should be taken into consideration in the interpretation of the results. One of the limitations of the study is that it is a retrospective study and not prospective. Furthermore, the number of patients examined is limited. Therefore, the chosen P cut-off of 1.5 ng/ml is not consistent across all studies in the scientific literature, so it would be necessary to identify a universal one.

Conclusions

In conclusion, P levels exceeding or equal to 1.5 ng/ml on the day of the hCG trigger may be correlated with the higher number of antral follicles observed and consequently with the higher number of retrieved, matured, properly fertilized oocytes and those reaching the blastocyst stage. Contrary to hypotheses in other studies, PPR not only fails to alter morphokinetic development but appears to enhance the trajectory, with faster developmental timelines observed during the initial phases of embryo development using time-lapse systems that may positively influence embryonic competence. Moreover, PPR resulted in better-quality blastocysts in terms of expansion degree and morphology of ICM and TE cells. These embryos might contribute to a greater likelihood of achieving pregnancy and successfully carrying it to term. Certainly, further investigation and additional studies will be necessary to provide more insights into this aspect since contradictory opinions exist in the literature. However, based on the large number of evaluated patients and the obtained data, it could confidently be stated that PPR on the day of the hCG trigger did not negatively impact the competence of retrieved oocytes or embryo development to the blastocyst stage. However, elevated P levels on the trigger day could lead to premature endometrial maturation and, consequently, asynchronization of the endometrium and embryos on the day of transfer. This should be prevented by choosing a frozen embryo transfer.

Author contributions

Each author have made substantial contributions to the manuscript. In detail: DB, VMB, AM conception; DB, VMB, AM design of the work; DB, VMB, AM, DF, GMB acquisition; VMB, AM analysis; DB, VMB, AM interpretation of data; DB, VMB, AM, DF, MD, AS, SH, GMB, AM, AV, GT have drafted the work, revised it and approved the submitted version. DB, VMB, AM, DF, MD, AS, SH, GMB, AM, AV, GT have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Written informed consents were obtained, at the first clinical evaluation, from all patients enrolled voluntarily in the study after being well informed about the hypothetical risks of the procedure. This study was regularly approved by the Local Ethical Board of the Momò Fertilife Institute (approval number 07/2018) according to the Declaration of Helsinki ethical principles.

Clinical trial number

Not applicable.

Conflict of interest
The authors declare no conflict of interest.

Competing interests
The authors declare no competing interests.

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