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ORIGINAL ARTICLE ***Reproductive endocrinology***

Do we need to measure progesterone in oocyte donation cycles? A retrospective analysis evaluating cumulative live birth rates and embryo quality

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STUDY QUESTION: Does late follicular-phase elevated serum progesterone (LFEP) during ovarian stimulation for oocyte donation have an impact on embryo quality (EQ) and cumulative live birth rate (CLBR)?

SUMMARY ANSWER: LFEP does not have an influence on EQ nor CLBR in oocyte donation cycles.

WHAT IS KNOWN ALREADY: Ovarian stimulation promotes the production of progesterone (P) which, when elevated during the follicular phase, has been demonstrated to have a deleterious effect in autologous fresh IVF outcomes. While there is robust evidence that this elevation results in impaired endometrial receptivity, the impact on EQ remains a matter of debate. The oocyte donation model is an excellent tool to assess the effects of LFEP on EQ from those on endometrium receptivity separately. Previous studies in oocyte donation cycles investigating the influence of elevated P on pregnancy outcomes in oocyte recipients showed conflicting results.

STUDY DESIGN, SIZE, DURATION: This is a retrospective analysis including all GnRH antagonist down-regulated cycles for fresh oocyte donation taking place in a tertiary referral university hospital between 2010 and 2017. A total of 397 fresh donor-recipient cycles were included. Each donor was included only once in the analysis and could be associated to a single recipient.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The sample was stratified according to serum P levels of ≤ 1.5 and >1.5 ng/mL on the day of ovulation triggering. The primary endpoint of the study was the top-quality embryo rate on Day 3, and the secondary outcome measure was CLBR defined as a live-born delivery beyond 24 weeks.

MAIN RESULTS AND THE ROLE OF CHANCE: Three hundred ninety-seven fresh oocyte donation cycles were included in the analysis, of which 314 (79%) had a serum P ≤ 1.5 ng/mL and 83 (20.9%) had a serum P > 1.5 ng/mL. The average age of the oocyte donors was 31.4 ± 4.7 and 29.9 ± 4.5 years, respectively, for normal and elevated P ($P = 0.017$). The mean number of oocytes retrieved was significantly higher in the elevated P group with 16.6 ± 10.6 vs 11.5 ± 6.9 in the P ≤ 1.5 group ($P < 0.001$).
In parallel, the total number of embryos on Day 3, as well as the number of good-quality embryos at this stage, was significantly higher in the elevated P group (6.6 ± 5.6 vs 4.15 ± 3.5 and 8.7 ± 6.3 vs 6.1 ± 4.4; respectively, $P < 0.001$). However, maturation and fertilization rates did not vary significantly between the two study groups and neither did the top- and good-quality embryo rate and the embryo utilization rate, all evaluated on Day 3 ($P = 0.384$, $P = 0.405$ and $P = 0.645$, respectively). A multivariable regression analysis accounting for P groups, age of the donor, number of retrieved oocytes and top-quality embryo rate as potential confounders showed that LFEP negatively influenced neither the top-quality embryo rate nor the CLBR.

LIMITATIONS, REASONS FOR CAUTION: This is an observational study based on a retrospective data analysis. Better extrapolation of the results could be validated by performing a prospective trial. Furthermore, this study was focused on oocyte donation cycles and hence the results cannot be generalized to the entire infertile population.

WIDER IMPLICATIONS OF THE FINDINGS: This is the first study providing evidence that LFEP does not influence CLBR and is adding strong evidence to the existing literature that LFEP does not harm EQ in oocyte donation programs.

STUDY FUNDING/COMPETING INTERESTS: Not applicable.

Key words: progesterone / oocyte donor / cumulative live birth rate / embryo quality / endocrinology

Introduction

Late follicular-phase elevated progesterone (LFEP) is an unpreventable event with a prevalence of up to 38% of all stimulated cycles, independently from the protocol used for stimulation (Bosch *et al.*, 2010). LFEP has been clearly associated with endometrial abnormalities (Labarta *et al.*, 2011; Liu *et al.*, 2017), with impaired embryo quality (EQ) (Huang *et al.*, 2016; Vanni *et al.*, 2017) and a decrease in CLBR in autologous cycles (Bu *et al.*, 2014; Racca *et al.*, 2018).

In standard IVF, it is difficult to distinguish the effects of LFEP on oocytes from those on the endometrium, whereas oocyte donation and embryo cryopreservation are interesting models to assess separately the impact of LFEP on oocytes–embryos from that on the endometrium. However, some caution should be used when considering oocyte donors as a model, as they are significantly younger than the general IVF population, and not infertile; therefore, they may respond differently to ovarian stimulation. Indeed, detrimental effects have been found for LFEP neither in the hyper-responders (Wu *et al.*, 2019) nor in the oocyte donors (Melo *et al.*, 2006).

Previous studies on oocyte donation recipients, aiming to investigate the effects of LFEP on EQ and endometrial receptivity, reported controversial conclusions. While some researchers showed no influence of LFEP on EQ and on pregnancy outcomes in the recipients (Chetkowski 1997; Melo *et al.*, 2006), some others reported an association of elevated P with lower pregnancy outcomes (Schoolcraft 1991; Silverberg 1991; Dirnfeld 1993; Fanchin 1993; Yovel 1995). Nonetheless, the abovementioned studies had three fundamental limitations: first, the use of a progesterone (P) cutoff that has not been replicated by further studies (Bosch 2010; Hill *et al.*, 2018); second, the lack of data about EQ; and third, the use of pregnancy rate as the outcome of the fresh cycles. In fact, in our current clinical practice, it is well known that multiple embryo transfer procedures may be required to achieve a live birth (LB); hence, increasing efforts have to be made to report IVF success rates as cumulative live birth rates (CLBRs) in order to provide patients with better prognostic information (Viardot-Foucault *et al.*, 2015). Furthermore, CLBR can provide more complete information about the recipient's outcomes out of a whole stimulated cycle, considering that the fresh outcome of the recipient is influenced by neither the stimulation nor the serum hormone levels and considering the frequent need for multiple embryo transfers (ETs) to reach a LB.

Therefore, a well-established cutoff of P rise was used in the present study to investigate whether LFEP during ovarian stimulation in oocyte donation cycles has an impact on EQ and CLBR. The hypotheses were in open contrast with what is expected for the general IVF population. In particular, it was considered that, when comparing high vs normal P groups, EQ would not be hindered by LFEP, and CLBR would be comparable or higher in the high P group. Furthermore, when considering the whole sample, it was hypothesized that elevated P level would not be a significant predictor of CLBR.

Materials and Methods

Design and definition of LFEP

This was a retrospective analysis including all GnRH antagonist down-regulated ICSI cycles for fresh oocyte donation that took place in a tertiary university hospital between 2010 and 2017. Approval to retrieve and analyze the data was provided by the Ethics Committee of the Universitair Ziekenhuis Brussel (B.U.N. 143201939775).

Each donor was included only once in the analysis, and in order to simplify and have definitive results, only oocyte donors who donated their oocytes to a single recipient were included.

The study population was divided into two arms, according to serum P level; specifically, the donors included in the analysis with a P level $\leq$1.5 ng/mL were assigned to the normal P group, whereas those with a P value $>$1.5 ng/mL on the day of the ovulation trigger were included in the elevated P group. A cutoff of 1.5 ng/mL was used to subcategorize the included cycles according to the results of previous studies reporting poorer cycle outcomes with higher P levels on the day of ovulation trigger (Bosch *et al.*, 2010; Santos-Ribeiro *et al.*, 2014; Venetis *et al.*, 2015; Racca *et al.*, 2018). Both anonymous and known donations were included in the study, and only fresh ejaculated sperm was used. Cycles where mixed Day 3 and Day 5 embryos were obtained and cycles where IVF or ICSI vs IVF procedures were performed were excluded.

Oocyte donors and ovarian stimulation

The donors were stimulated from Day 2 of the menstrual cycle with either recombinant FSH (rFSH: Gonal-F®, Merck Pharmaceuticals, Darmstadt, Germany; Puregon®, Merck Sharp & Dohme, Whitehouse Station, NJ, USA, or Elonva®, Merck Sharp & Dohme) or highly purified HMG (HPHMG; Menopur®, Ferring Pharmaceuticals, Saint-Prex, Switzerland). Pituitary down-regulation was performed using daily administration of a GnRH antagonist either cetrorelix 0.25 mg (Cetrotide®, Merck Pharmaceuticals) or ganirelix (Orgalutran®, Merck Sharp & Dohme) starting from Day 7 of the menstrual cycle onwards. Whenever necessary, dose adjustments of rFSH/HPHMG were performed according to ovarian response. As soon as three follicles $\geq$20 mm were observed during pelvic ultrasound, final oocyte maturation was triggered with either a single subcutaneous dose of GnRH agonist, 0.2 mg of triptorelin (Gonapeptyl®, Ferring Pharmaceuticals,

Saint-Prex, Switzerland, or Decapeptyl®, Ipsen Farma, Paris, France), or 5000 or 10 000 IU of highly purified urinary hCG, according to female body weight (Pregnyl®, MSD, Oss, Netherlands, or Ovitrelle®, Merck Serono Europe Ltd, London, UK). Cumulus–oocyte complexes (COCs) were collected by transvaginal aspiration 36 h following the ovulation trigger.

Endometrial preparation in the recipients

All the recipients underwent endometrial preparation in an artificial cycle (HRT) using 4 mg of estradiol valerate (Progynova®, Bayer-Schering Pharma AG, Berlin, Germany) daily for 12 from Day 1 to 3 of their menstrual cycle onwards, until the endometrial thickness reached ≥7 mm (monitored by ultrasound). Then, 200 mg t.i.d. of micronized vaginal P (Utrogestan®, Besins, Brussels, Belgium) was administered (van de Vijver *et al.*, 2014). The ET was performed on the fourth day of P supplementation for Day 3 embryos and on the sixth day of P supplementation for Day 5 embryos.

EQ assessment and ET policy

As described in the previous paper by our group (Racca *et al.*, 2018), fertilization was assessed 16–18 h after ICSI by the presence of two pronuclei, after which stage the embryo development was evaluated daily until the day of ET or the cryopreservation of the cleavage stage embryo or the blastocyst. Supernumerary embryos of good quality were cryopreserved by means of vitrification using a closed vitrification device with high-security straws (CBS-ViT HS®, Cryobiosystems, Normandy, France) in combination with dimethyl sulfoxide and ethylene glycol as cryoprotectants (Irvine Scientific Freeze Kit®, Irvine Scientific, Santa Ana, CA, USA) (Van Landuyt *et al.*, 2011). EQ was classified similarly to what is described in a previous study (De Munck *et al.*, 2015), with a minor update in the classification (good-quality embryos included up to <50% fragmentation). Day 3 embryos were evaluated on the basis of the number and symmetry of their blastomeres, percentage of fragmentation, vacuolization, granulation and multinucleation. Based on all these parameters, an EQ score was assigned to all normally fertilized embryos using a predefined algorithm, which is divided into four categories: excellent, good, moderate or poor. On Day 3, fresh embryos were considered eligible for transfer if at least four blastomeres were present with a maximum of 50% fragmentation.

The following embryos were considered eligible for cryopreservation: Day 3 embryos with ≥6 blastomeres and ≤50% fragmentation; Days 5 and 6 fully expanded or hatching blastocysts with a type A/B/C inner cell mass (ICM) and type A/B trophectoderm (TE.) After warming (Van Landuyt *et al.*, 2011) (Irvine Scientific Thaw Kit®, Irvine Scientific), Day 3 embryos were cultured overnight in a blastocyst medium until being transferred the following day (Day 4). Survival was scored based on the number of surviving blastomeres. If >2 cells degenerated after warming, a surplus embryo was warmed if available. An embryo score was given on Day 4 at the moment of transfer: excellent, good, moderate or poor as previously described (Racca *et al.*, 2018). EQ was evaluated on Day 3 in order to assess all the embryos in development; furthermore, embryos were transferred on Day 3 or Day 5 according to the decision of the treating physician and the center policy (preferable Day 3 transfer for donor-recipient programs).

The ETs were performed in most of the case on Day 3 of embryonic development, following the policy of the center. The trend towards Day 3 transfer in the last years is mainly based on avoidance of poor blastulation and also on the recently published manuscript of (De Vos *et al.*, 2016), stating similar CLBR between cleavage- and blastocyst-stage embryos. However, in some cases the transfers were performed on Day 5/6 of embryonic development following the treating physician choice.

P assessment immunoassay

Serum P was assessed on the day of trigger using a validated electrochemiluminescence immunoassay (Cobas 6000®, 6000®, Roche, Basel, Switzerland) with a measured sensitivity and within-assay coefficient of variation of 0.03 µg/L and <7%, respectively. The same assay was used during the entire course of the study and was regularly calibrated to minimize variation of the results associated with time and reagent batch renewal.

Main outcome measures

The primary outcome parameter was the top-quality embryo rate on Day 3, and the secondary outcome parameters were CLBR, defined as a live-born delivery after 24 weeks after either the fresh or one of the subsequent frozen ETs, and embryo utilization rate, defined as the total number of embryos transferred and cryopreserved per number of fertilized oocytes. CLBR was calculated per cycle where at least one ET was performed.

Statistical analysis

Continuous variables were analyzed using the independent *t* test or Mann–Whitney *U* test depending on the normality of the distribution. Continuous data were reported as mean ± standard deviation (SD), whereas dichotomous data were reported as number (*n*) and percentage. Normality was examined using the Shapiro–Wilk test. Categorical variables were analyzed by Pearson's chi-squared test or Fisher's exact test, as appropriate. To identify characteristics that may be related with the CLBR, multivariate logistic regression analysis was performed with CLBR as the dependent variable and the P categories, the number of COCs retrieved, the age of the donors and the top-quality embryo rate as the independent variables.

All selected variables were simultaneously entered into the logistic regression model. All statistical tests used a two-tailed *alpha* of 0.05. A *P* value of 0.05 was considered statistically significant and the analyses were performed using Stata 15.1 (StataCorp, College Station, TX, USA).

Sample size calculation

The sample size calculation was performed based on the fact that the control group (with low P levels) had a top EQ rate of 80% which represents the standard of care of oocyte donation in our center. We hypothesized that a sample size of 265 patients (212 in the normal P group and 53 patients in the elevated P group) with a 1:4 allocation was essential in order to have 80% power to detect a difference of 10% in top-quality embryo rate under the null hypothesis that in both groups the top-quality embryo rate was assumed to be 80% and the alternative hypothesis that the top quality embryo rate in the elevated P group would be 70%, using a two-sided *t* test, at significance level alpha of 0.05.

Table I Oocyte donor parameters depending on progesterone levels on the hCG day; Group I (P ≤ 1.5 ng/mL) and Group II (P > 1.5 ng/mL).

	Progesterone level		*P*
	≤1.5 ng/mL (*n* = 314)	>1.5 ng/mL (*n* = 83)	
Age (years)	31.4 ± 4.7	29.9 ± 4.5	**0.017**
AMH (mcg/L)	3.3 ± 2.6	3.9 ± 2.7	0.14
Progesterone (ng/mL)	0.87 ± 0.3	2.9 ± 2.7	<**0.001**
BMI (kg/m^2)	24 ± 4.7	23.8 ± 3.9	0.65
Smoking habit *N* (%)			
No	196 (75.4)	56 (76.7)	
Yes	52 (20.0)	15 (20.6)	0.87**
Stopped	12 (4.6)	2 (2.74)	
Total dose of rFSH/HPHMG (IU)	2053.7 ± 831.2	2035 ± 603.1	0.847
Stimulations (days)	10.7 ± 1.9	10.8 ± 1.7	0.522
E_2 levels on the day ovulation trigger (pg/mL)	1925.5 ± 1104.2	2965.4 ± 1924.6	<**0.001**
P to E2 ratio (P * 1000/E2)	0.7 ± 0.04	0.5 ± 0.05	**0.002**
Number of oocytes retrieved	11.5 ± 6.9	16.6 ± 10.6	<**0.001**
Number of mature oocytes	9.3 ± 5.7	13.4 ± 8.6	<**0.001**
Number of oocytes assigned	11.4 ± 6.9	16.5 ± 10.6	<**0.001**
Maturation rate (%)	83.1 ± 17.9	82.1 ± 15.1	0.602

AMH, (anti-Mullerian hormone); rFSH, (recombinant FSH); HPHMG, (human purified human menopausal gonadotropin); P, (progesterone); E2, (estradiol).
Data are expressed as mean ± standard deviation (SD)
All the significant *p* values are reported in bold
P values for independent *t* test (two-tailed)
**Fisher exact test

Results

A total of 397 fresh oocyte donation cycles were included in the analysis, of which 314 (79%) had a serum P ≤ 1.5 ng/mL and 83 (20.9%) had a serum P > 1.5 ng/mL.

The baseline characteristics of the oocyte donors are reported in Table I, while the characteristics of the recipients are reported in Table II. With regard to the donors, the average age was 31.4 ± 4.7 and 29.9 ± 4.5 years, respectively, for normal and elevated P ($P = 0.017$); moreover, there were no differences in anti-Mullerian hormone (AMH), BMI and smoking habits. Furthermore, the total dose and duration of stimulation were comparable. The between-group comparisons showed that late-follicular estradiol (E2) levels (1926, 2965 pg/mL, respectively, in normal and high P) were significantly higher in the elevated P arm. The P to estradiol ratio (P:E2 ratio) (Lai *et al.*, 2009; Wu *et al.*, 2012; Hakan *et al.*, 2018) on the day of ovulation trigger was significantly lower in the elevated P group compared with the normal P group (0.5 vs 0.7, respectively, $P = 0.002$). However, the influence of the P:E2 ratio on the regression model was negligible. Moreover, the number of oocytes retrieved was higher in the elevated P group (11.5 and 16.6, respectively); nonetheless, even though the absolute number of mature oocytes was higher in the LFEP group (9.3 vs 13.4, respectively), the maturation rates were comparable among the two arms ($P = 0.602$).

Concerning the recipients, the age was comparable between the two groups as well as the fertilization rate; on the contrary, the number of oocytes assigned per recipient was significantly higher in the LFEP arm with 11.4 vs 16.5, respectively, for the normal P group and the LFEP group. As for the embryo development, the number of embryos cryopreserved on Day 3 was slightly higher in the LFEP arm compared to the normal P group ($P = 0.037$); in absolute numbers, there were significantly more top-quality embryos on Day 3 in the elevated P group (6.1 ± 4.4 vs 8.7 ± 6.3, $P < 0.001$), represented in Fig. 1; however, the good-quality embryo rate was similar between the two arms ($P = 0.405$). The EQ is reported in detail in Table II.

For patients with ET on Day 3, the total number of embryos was significantly higher in the LFEP group (6.62 vs 4.15, respectively, $P < 0.001$), but this was not the case for the utilization rate of patients undergoing extended culture to Day 5/6 (3.83 vs 3.33, $P = 0.368$). As described in a previous paper, the embryo utilization rate appeared not to be significantly higher in the elevated P group (60.9 vs 62.9%, respectively, $P = 0.645$), and the same was found for Day 5 embryos ($P = 0.415$). CLBR was evaluated according to embryo stage where an ET was performed and for the cycles where an outcome was present. As reported in Table II, for cleavage-stage embryos, the CLBR was slightly higher in the LFEP group, though not significant (76.2 vs 60.5%, respectively, $P = 0.058$), while for blastocyst-stage embryos there was no statistical difference found (82.7 vs 70%, respectively, for normal and high P groups, $P = 0.235$), represented in Fig. 2.

Furthermore, multivariate logistic regression analysis allowing adjustment for relevant confounders (P groups, donor age, number of oocytes retrieved and top-quality embryo rate) showed that CLBR was positively associated with the number of COCs retrieved and with the age of the donors, while the P groups and the EQ rate did not show any association with CLBR (Table III).

Table II Recipients/embryo quality/embryo utilization rate/CLBR.

	Progesterone level		*p*-value
	≤1.5 ng/mL (*n* = 314)	>1.5 ng/mL (*n* = 83)	
Age (years)	35.7 ± 5.8	35.7 ± 6.1	0.893
Fertilization rate (%)	80.5 ± 20.3	80.8 ± 17	0.896
Embryo quality A Day 3 (*n*)	4.1 ± 3.5	6.1 ± 5.6	**0.003**
Embryo quality A + B Day 3 (*n*)	6.1 ± 4.4	8.7 ± 6.3	**<0.001**
Rate embryo quality A Day 3 (%)	55.9 ± 29.9	52.9 ± 27.5	0.384
Rate embryo quality A + B Day 3 (%)*	83.3 ± 23.5	80.8 ± 23.7	0.405
Total *n* of embryos			
on Day 3	4.3 ± 3.2	6.7 ± 4.7	**0.01**
on Day 5/6	3.1 ± 2.5	4.9 ± 2.7	0.06
Total cleavage stage embryos (for fresh ET and cryopreserved) (*n*)	4.15 ± 3.5	6.62 ± 5.4	**<0.001**
Total blastocyst stage embryos (for fresh ET and cryopreserved) (*n*)	3.33 ± 2.6	3.83 ± 2.9	0.368
Embryo utilization rate for Day 3 embryos (%)	62.9 ± 28.9	60.9 ± 25.3	0.645
Embryo utilization rate for Day 5/6 embryos (%)	35.6 ± 20.6	32.0 ± 20.7	0.415
CLBR for Day 3 embryos (per ET) *n* (%)	101/167 (60.5)	32/42 (76.2)	0.0581
CLBR for Day 5/6 embryos (per ET) *n* (%)	43/52 (82.7)	14/20 (70.0)	0.2351

CLBR (cumulative live birth rate)
*The rate embryo quality A + B includes all the embryos analyzed (the ones transferred or cryopreserved on Day 3 and the ones transferred or cryopreserved on Day 5/6).
Data are expressed as mean ± standard deviation (SD)
P values for independent *t* test (two-tailed)
All the significant *p* values are reported in bold
[1] Pearson chi-square

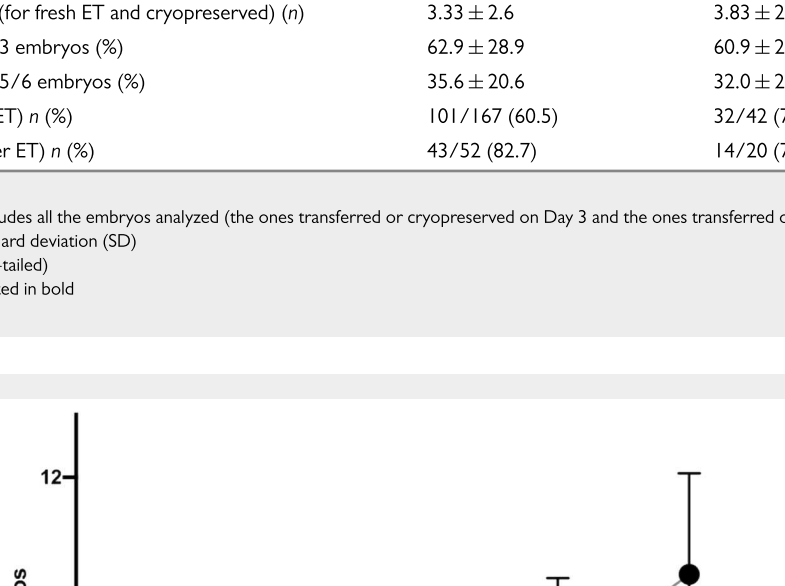

Figure 1 Relationship between serum progesterone (P) levels and top-quality cleavage-stage embryos (crude numbers).

Discussion

To the best of our knowledge, this is the first study evaluating simultaneously the influence of LFEP on both EQ and CLBR in an oocyte donation program. The retrospective analysis provides important evidence that, in oocyte donation cycles, elevated P does not hinder EQ or CLBR. On the contrary, elevated P is associated with a superior number of COCs retrieved and consequently a higher number of usable embryos per patient. Despite recent evidence showing that elevated P has a negative effect on autologous EQ (Huang *et al.* 2016; Vanni *et al.*, 2017) and on CLBR (Bu *et al.*, 2014; Racca *et al.*, 2018), the present study showed that in oocyte donation

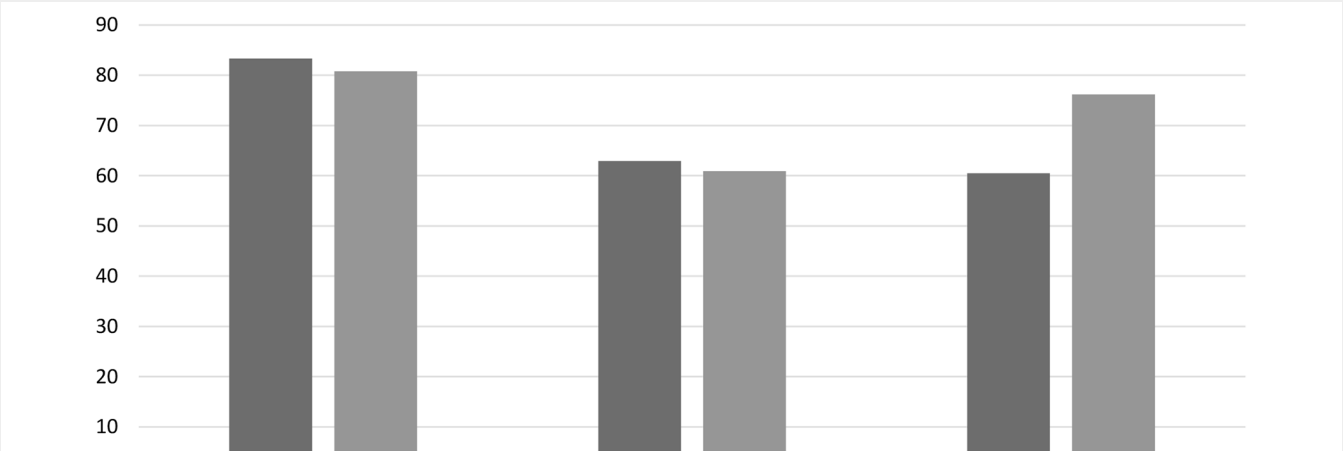

Figure 2 Measure of outcomes of the study (embryo quality A + B, embryo utilization rate (Day 3 embryos) and cumulative live birth rate (CLBR) for Day 3 embryos), crude analyses. Orange shows the late follicular-phase elevated serum-progesterone (LFEP) group while blue shows the normal P group. *Y*-axis shows the cycle outcomes (%).

Table III Multivariate regression.

CLBR	OR	*p*	95% C.I.
P (dichotomous)	1.19	0.592	0.62–2.28
Age donor	0.94	**0.029**	**0.89–0.99**
N oocytes retrieved	1.1	**<0.001**	**1.06–1.15**
Top-quality embryo rate	2.1	0.067	0.95–4.63

Odds ratios (ORs) for CLBR (adjusted for potential confounders, specifically P group, age of the donor, *n* oocytes retrieved and top-quality embryo rate).
All the significant *p* values are reported in bold

cycles, elevated P does not impair the reproductive outcome of recipients.

In the current work, surprisingly, despite having more oocytes retrieved, more mature oocytes and a higher level of E2 in the LFEP group, we found no differences in CLBR between the two groups. On the contrary, we found, in crude numbers, a higher number of quality A and A + B embryos in the LFEP group, probably due to the higher numbers of oocytes obtained. Indeed, when we looked at the EQ rate, we found no difference between the two groups. Our results are in contrast with Melo *et al.* (2006) who reported no difference in number of oocytes retrieved, embryo development and pregnancy outcome between the normal and elevated P groups. On the other hand, against a superior absolute number of total embryos on Day 3 for the LFEP group, the embryo utilization rate was not significantly different between the two study groups for cleavage-stage embryos. This indicates that the inherent quality of the oocytes is not affected by the elevation in P and that every mature oocyte has the same capacity to develop into an embryo, irrespective of the P value at the moment of triggering. Although, previous studies showed hindered EQ in the autologous embryos obtained from LFEP cycles (i.e. Huang *et al.*, 2016; Vanni *et al.*, 2017), the evidence supporting the biological reason why the oocytes/EQ should be hindered by the elevated P is very poor; thus, further research is necessary to investigate this possible association.

In line with the results from the between-group analysis, the multivariable regression analysis for CLBR showed that the only significant predictors were the number of oocytes retrieved and the age of the donors, whereas high P level and top-quality embryo rate were not significantly associated.

Our study results are equivalent with previous findings where LFEP was not associated with lower EQ and resulted in similar pregnancy rates in recipients obtaining embryos from oocyte donors with or without LFEP (Hofmann *et al.*, 1993; Chetkowski *et al.*, 1997; Fanchin *et al.*, 1997). Nonetheless, these publications of two decades ago used a P threshold of 0.9 ng/mL that has not been confirmed as a valuable cutoff to detect differences in cycle outcomes (Bosch *et al.*, 2010; Hill *et al.*, 2018). On the other hand, the results of our retrospective analysis are in contrast with the finding of Yovel *et al.* (1995) where exposure to LFEP (threshold 1.9 ng/mL) in OD cycles was significantly associated with detrimental effect on oocyte/EQ. The results of the latter paper, nonetheless, have to be considered with caution as there was no consensus on a P threshold that could be considered detrimental for the IVF outcomes. In the present work, the 1.5-ng/mL value has been chosen following the evidence of more recent papers (Bosch *et al.*, 2010; Hill *et al.*, 2018).

The major strengths of our study rely on the confounder-adjusted analysis that offers a more accurate inference, the consideration of EQ and CLBR as main outcomes and the decision to use 1.5 ng/mL as P threshold to stratify patients. Furthermore, only fresh oocyte donations were included in the analysis, in order to avoid the possible bias of the vitrification procedure. Nonetheless, the present study has several limitations: first is its retrospective design; second, only GnRH antagonist cycles were considered for the analysis, hence our

results could not be extrapolated to all ovarian stimulation–suppression protocols. Finally, this research was conducted on oocyte donors who are relatively young compared to the infertile population and mostly without infertility problems. Our findings are not suitable to explore the underlying mechanism by which P levels are rising in the late follicular phase of infertile patients.

In summary, this study provides useful evidence to reduce the endocrinological monitoring in oocyte donors; specifically, we propose to stop measuring P during the donor stimulation, since we have excluded a potential negative impact of P value on the cycle outcome.

Authors' roles

A.R.: responsible for the concept and draft of the article. N.D.M.: participated in the writing, interpretation and editing of the article. S.S.-R.: participated in the writing, interpretation and editing of the article. P.D., J.E., A.G., B.P., S.M., G.V. and M. DV.: contribution in the interpretation and editing of the article. H.T.: contribution in the interpretation and editing of the article. C.B.: responsible for the concept and the final revision of the article.

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Conflict of interest

The authors have no conflicts of interest.

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