Narrative Review

Premature Progesterone Elevation in *in vitro* Fertilisation Cycles – Current Perspectives

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Abstract

The impact of premature elevation of progesterone (PPE) on the day of the trigger on pregnancy outcome in *in vitro* fertilisation (IVF) cycles has been a matter of contention and debate for decades. Research over the last 30 years has indicated that PPE >1.5 ng/ml is associated with declining live birth rates following fresh embryo transfer. Freeze-only approach has become a universal solution to overcome the issue of PPE. However, the topic is still mired with controversy. Few studies have not shown a negative impact on pregnancy rates. The impact of PPE on embryological parameters such as oocyte and embryo quality and ploidy is still very controversial. An important contentious issue is the choice of the threshold P value above which it is considered abnormal and a freeze-all strategy would be cost-effective. Currently, though a cutoff of >1.5 ng/ml is widely used, practices are not uniform and varying thresholds from 0.4 to 3 ng/ml are utilised. This review addresses the current understanding of PPE in IVF and the above controversies. The incidence, aetiology and source of progesterone rise, impact on endometrial receptivity, oocyte and embryo quality, impact on live birth and cumulative live birth and impact on frozen embryo transfer and donor oocyte cycles are discussed. Current controversies regarding the optimal threshold, assay performance and future directions are addressed.

Keywords: *High progesterone, in vitro fertilisation, pregnancy rate, premature progesterone elevation*

Introduction

The global utilisation of assisted reproductive techniques has been on the rise. Since its inception, there has been a constant endeavour to enhance pregnancy rates following embryo transfer. Premature elevation of progesterone (PPE) on the day of human chorionic gonadotropin (hCG) administration has been evaluated as a factor influencing the probability of pregnancy after fresh embryo transfer. Despite the universal use of gonadotropin-releasing hormone (GnRH) analogues for pituitary suppression for prevention of luteinising hormone (LH) surge, PPE in the late follicular phase is still observed in many *in vitro* fertilisation (IVF) cycles before the administration of hCG.[1] This early, inappropriate and prolonged exposure to progesterone (P) is expected to cause advancement of secretory endometrium, increased chance of embryo-endometrial asynchrony and implantation failure.[2] This hypothesis has been corroborated by data from basic research and supported by observations that embryos generated in such cycles implant better in subsequent frozen embryo transfer (FET) cycles and in donor recipients.[3] Freeze-only approach has emerged as a universal solution amongst most clinicians to overcome the issue of PPE on the day of trigger.[4,5] PPE might also have an impact on oocyte and embryo quality.[6]

Early publications in 1991 by Schoolcraft *et al*. were the first to demonstrate a lower pregnancy rate with

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elevated progesterone >0.5 ng/ml.[7] Subsequent research over the last 30 years has convincingly indicated that a negative effect does exist, with declining pregnancy rates as progesterone rises.[3,5] However, not all studies have shown a negative effect and some clinicians doubt the concept and do not measure P on trigger day.[8,9] The impact of PPE on oocyte number, number of mature oocytes, fertilisation and cleavage rates, blastocyst formation, embryo quality and ploidy are still very controversial with conflicting references. One of the most important contentious issues regarding PPE in IVF is the choice of the threshold P value above which is considered abnormal and a freeze-all strategy would be cost-effective.[10] Currently, though a cutoff of >1.5 ng/ml is widely used, literature and practices are not uniform and varying thresholds from 0.4 to 3.0 ng/ml have been seen.[3] However, is this cutoff of >1.5 ng/ml justified and does a uniform cutoff for all poor, normal and high responders hold valid? Should the freeze-all policy be universally applied to all patients with elevated P? Questions have also been raised about the timing of P measurement; whether a single measurement is a true reflection and the high variability in assay performance.

This review is an attempt to unravel the controversies and current understanding about PPE in IVF, its incidence, aetiology and source of *P* rise, predisposing factors, impact on endometrial receptivity, oocyte and embryo quality, impact on live birth and cumulative live birth and impact on FET and donor oocyte cycles. Current controversies regarding the optimal threshold, assay performance and future directions will also be addressed.

Methods

This narrative review involved a systematic search of electronic scientific databases PubMed, Medline, Google Scholar and Cochrane database and included published articles in English language from 2010 to 2022. The search involved keywords of search terms 'elevated progesterone', 'premature progesterone elevat*', 'high progesterone', 'progesterone', 'IVF', 'Assisted Reproductive Technology (ART)' and 'live birth'. Articles were screened, and their reference lists were checked for relevant publications [Figure 1].

Incidence

The reported incidence of PPE in IVF cycles is highly variable, not infrequent and ranges from 12% to 46%.[3] This variability in the reported incidence is largely due to the diverse thresholds for 'high progesterone' used in various studies and methods of P assessment. Data presented in the systematic review showed that the observed incidence was 46.7% when thresholds as low as 0.4–0.6 ng/ml were used and 12.3% at 1.9–3.0 ng/ml. About 17.2% of patients had values above the popular 1.5–1.75 ng/ml cutoff.[3]

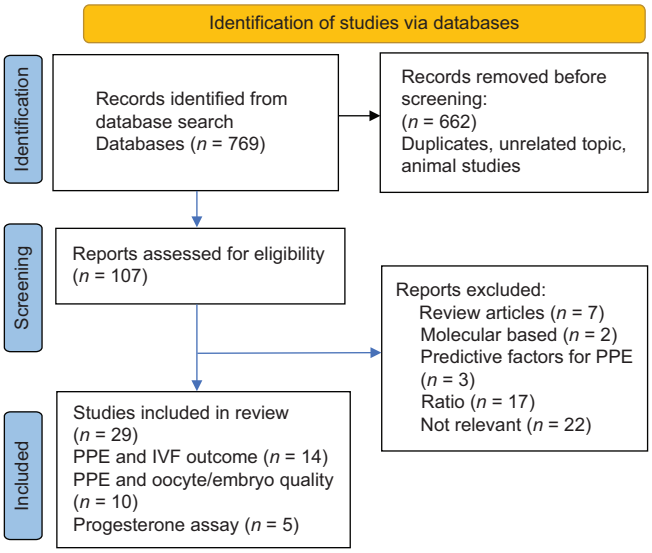

Figure 1: Flow diagram of search strategy

Pathophysiological Mechanisms of Premature Progesterone Elevation

In the late follicular phase, the main source of progesterone shifts to the ovary, and this *P* contributes to follicular development and timing of ovulation. After ovulation, the endocrine machinery of the corpus luteum is directed to enhance progesterone production; which reaches >15 ng/ml in the mid-luteal phase. Progesterone production is pulsatile, correlating closely with episodic LH release. This exposure of the endometrium to progesterone converts the secretory endometrium into a receptive state. Hence, the timing, concentration and duration of exposure to P are vital for normal implantation and receptivity.[11]

Till date, the exact aetiology of premature progesterone elevation on the day of hCG in ovarian stimulated cycles is unclear. There are multiple proposed mechanisms: (a) Excess production of P from the theca cells of the multiple growing follicles, (b) direct stimulatory effect by exogenous follicle-stimulating hormone (FSH) on the granulosa cells. FSH stimulates the 3 β– hydroxy steroid dehydrogenase and progesterone biosynthesis in granulosa cells.[12] Increase in the precursor steroids may exceed the conversion capacity to oestrogens and the excess P may leak into the systemic circulation and (c) delaying the hCG trigger and prolongation of the follicular phase can cause persistent FSH stimulation, increased granulosa cells and increased P production.[13]

Impact of Premature Elevation of Progesterone on Endometrial Receptivity–Molecular Mechanisms

The establishment of pregnancy in IVF requires a euploid blastocyst, a receptive endometrium and optimal embryo-endometrial synchrony. In view of premature and prolonged exposure to progesterone, the aetiology of poor outcomes in IVF cycles with PPE is most likely due to impairment of endometrial receptivity. The molecular mechanisms involved have been evaluated in many studies and they include (a) significant alterations in gene expression profiles of the endometrium, (b) histological advancement in endometrial development and increased uterine natural killer cell (uNK) count, (c) altered epigenetic modification status in three compartments of the endometrium, (d) disruptions in lipid homeostasis of the endometrium and (e) DNA hypermethylation and low expression of adhesion molecules on the endometrium.

In a pioneering study published in 2011, the authors used microarray technology to compare gene expression profiles at the window of implantation in six healthy oocyte donors serum P levels >1.5 ng/ml on hCG day. They found 140 genes essential for normal endometrial function related to cell adhesion, developmental processes and immune modulation significantly dysregulated.[2] A similar microarray and quantitative reverse transcription–polymerase chain reaction-based study in eight women undergoing IVF with P > 1.5 ng/ml found an alteration in the gene expression shift from pre-receptive to the receptive stage and hence accelerated endometrial maturation.[14] Endometrial samples from 106 women undergoing IVF were evaluated for histological staging and uNK cell count. They found advanced endometrial development and higher uNK cell count in women with high progesterone.[15]

Impact of high P on epigenetic modifications of endometrium was studied by comparing the endometria of 20 women with a P serum level of >1.7 ng/ml. Endometrial biopsies were taken on hCG +7 and they found high P levels associated with altered epigenetic modification in all three compartments of the endometrium, which in turn could disrupt endometrial receptivity.[16] The same group published further work in 2020 and found that high progesterone is associated with DNA hypermethylation and low expression of adhesion molecules in the implantation window.[17]

Predisposing Factors for Premature Elevation of Progesterone

Certain patient factors and stimulation protocols may have an influence on late follicular phase progesterone levels. These include– type of protocol (agonist or antagonist), type of gonadotropin (urinary or recombinant), dose of gonadotropin (standard or step down), duration of stimulation and type of responder (poor, normal or high responder).

In vitro fertilisation protocol

The type of GnRH analogue used for pituitary suppression may be a potential effect moderator and its influence has been evaluated in a limited manner. The recent meta-analysis showed marginally significant evidence that GnRH antagonist protocol was associated with a decreased incidence of PPE irrespective of the threshold used compared to agonist cycles.[3] However, few other publications have found a similar incidence of PPE in both agonist and antagonist protocols.[18,19]

Type of gonadotropin

Before the routine use of GnRH analogues in IVF stimulation, premature luteinisation and elevated progesterone were common and were attributed to the excessive effect of LH on the growing follicles causing luteinisation and progesterone production. Since routine use of GnRH agonist and antagonist prevent this LH surge, what could be the cause of PPE? It seemed intuitive that exogenous LH in gonadotropins for stimulation may cause luteinisation. This resulted in the introduction of pure FSH preparations, hoping that the risk of premature P rise could be mitigated by a lack of LH/hCG activity. However, contrary to the expectation, the incidence of PPE was lower in HMG-stimulated cycles.[20] Recent theories that propose that excessive FSH stimulation may result in increased production of P from granulosa cells concur with the above observation. In a large retrospective cohort study including 10,280 patients, the authors attempted to determine whether different ratios of LH/FSH gonadotropins have an influence of PPE on trigger day. They found that stimulations using no LH had the highest risk of P elevation. The lowest risk of PPE was found in the group that received a LH: FSH ratio of 0.3:0.6; irrespective of the type of responder. They defined this ratio as a sweet spot in ovarian stimulation to protect against PPE and suboptimal outcomes.[21]

Taking the same concept of incessant FSH stimulation as a cause for PPE, it was studied whether a step-down dose towards the late follicular phase could reduce the prevalence of PPE. *Post hoc* analysis of data from two randomised controlled trials (ENGAGE and PURSUE trials) showed that the incidence of PPE was significantly lower in cycles stimulated with corifollitropin alfa (CFA) 5.4% versus recombinant FSH 18.3%.[22] CFA has a long duration of action for a week but has the highest FSH activity during the first 2 days and declines later,

mimicking a step-down protocol. This strengthened the concept that excessive FSH stimulation contributes to elevated P in the late follicular phase and stepping down the FSH dose could be considered a mode of prevention.

Type of responder

Increased number of obtained oocytes and higher number of available embryos in high responders were postulated to overcome the negative impact of PPE on endometrial receptivity and exert a protective effect on IVF outcome.[23] However, the cumulative live birth rate (CLBR) per cycle started is significantly reduced in women with PPE regardless of the type of responder – poor, normal or high.[24-26] The threshold at which the negative impact on LBR begins is seen to increase with an increasing number of oocytes and ranges from 1.5 to 4 ng/ml.[25] Hence, high responders are not exempt from the detrimental effect of PPE but the discriminatory threshold seems to be higher – though there is no uniformity about the agreed level.

Premature Progesterone Elevation and Impact on IVF Outcome

Fresh embryo transfer

Ever since Schoolcraft *et al* reported a negative impact of elevated P on IVF outcomes in 1991, numerous studies with contradictory results have been published. Initial systematic reviews published a decade ago either showed a lower but non-significant pregnancy rate with PPE[27] or a significantly lower probability of pregnancy in GnRH antagonist IVF cycles.[28] A subsequent systematic review was published in 2013 to evaluate the association of PPE with the probability of pregnancy in fresh, frozen and donor–recipient cycles including 63 studies evaluating 55,199 cycles. This comprehensive meta-analysis confirmed that there was a decreased probability of pregnancy in fresh IVF cycles in women undergoing stimulation with gonadotropins and GnRH analogues. This decline was observed over a range of P thresholds, from values above >0.8 ng/ml. The impact increased at P values of 1.2 ng/ml and remained stable after >1.2.[3]

After the publication of this meta-analysis, 14 retrospective and few prospective cohort studies have been published. Two studies[6,24] have reported CLBR as an outcome measure. Seven studies[29-35] have evaluated the impact of PPE on the live birth rate in fresh IVF cycles. Five studies have reported the impact on clinical pregnancy rate (CPR)[36-40] [Table 1].

The discriminatory threshold of elevated P used in the studies is not uniform and varies from 1.0 to 2.1 ng/ml. Some studies have used statistical tools to identify their own thresholds and few studies have used different levels for patients based on the type of responder.

Clinical pregnancy rate

Three studies[38-40] concluded that elevated serum P on trigger day was associated with lower CPRs. In a large retrospective cohort of 11,146 patients, it was found that a P level of >1.5 ng/ml was detrimental in cleavage stage transfer and >1.75 ng/ml in blastocyst transfer.[40] However, Lepage *et al*. found a similar CPR, higher miscarriage rate and lower ongoing pregnancy rate with high P.[37]

Live birth rate

Two studies[31,33] did not find a significant difference in LBR in women with PPE. However, all the remaining five studies concluded that there was a significant reduction in LBR following fresh transfer in women with PPE on trigger day.[29,30,32,34,35]

Cumulative live birth rate–fresh and frozen ET included

Majority of the published literature seems to concur that there indeed does exist a detrimental impact of elevated P on trigger day on live birth outcome following fresh embryo transfer. However, questions have been raised about whether PPE has an additional impact on oocyte and embryo quality.[41] Any effect on embryo quality would reduce the embryo utilisation rate, and in turn, the CLBR– an outcome measure that provides patients with better prognostic information. In a retrospective analysis of 3400 GnRH antagonist cycles, the impact of PPE on embryo quality and CLBRs was assessed. They found that increasing P levels were associated with an increasing number of oocytes retrieved, lower embryo utilisation rates on day 3 and day 5 and decreased fresh and CLBRs.[6] Another retrospective cohort study explored the relationship between elevated P and CLBR in women with different ovarian responses. They included 4651 patients and found that serum P level adversely affected CLBR in patients with different ovarian responses, even after controlling for all confounding factors. There was no significant difference in high-quality embryo rate in groups with normal or elevated P.[24] The effect of PPE on subsequent FET cycle outcomes was compared in a paired analysis of women. They observed that PPE was associated negatively with the live birth rate in fresh transfer cycles but not on FET outcome.[4] All the above fairly large retrospective studies concluded that PPE was associated with significantly lower cumulative live birth after adjusting for multiple confounders and independent of ovarian response. Both the studies included fresh and subsequent FETs in their analysis and emphasised that a freeze-all strategy ameliorates the negative association.

Table 1: Pre-mature progesterone elevation and live birth outcomes

Author	Publication details	Type of study	Number of subjects	Inclusion criteria	PE threshold	Outcome measure	Results
CLBR							
Bu *et al.*[24]	PLoS One, 2014	Retrospective	4651	Poor ovarian responder (≤5 oocytes, 785 patients), intermediate ovarian responder (6-19 oocytes, 3065 patients) and high ovarian responder (≥20 oocytes, 482 patients)	Thresholds for serum progesterone elevation were 1.60 ng/mL, 2.24 ng/mL, and 2.50 ng/mL for poor, intermediate, and high ovarian responders, respectively (cumulative 95% of progesterone level in each ovarian response group)	CLBR	For all responders, patients with elevated progesterone level had significantly higher number of oocytes retrieved, but lower high quality embryo rate, and lower cumulative live birth rate compared with patients with normal serum progesterone level. In addition, serum progesterone level adversely affected cumulative live birth rate by both univariate and multivariate logistic regression analysis, independent of ovarian response
Racca *et al.*[6]	Hum Reprod, 2018	Retrospective	3400	All responders	≤0.50, 0.51-1.49 and ≥1.50 ng/mL	Embryo utilisation CLBR	Utilization rates decreased linearly as progesterone increased for Day 3 embryos (72.3, 63.0 and 45.4%, respectively), while for Day 5 embryos only the EP group was associated with a significant decrease (48.8, 47.8 and 38.8%, respectively). EP was also associated with decreased fresh and cumulative LBRs
LBR							
Zhang *et al.*[30]	Front Endocrinol, 2022	Retrospective	867 < 1 362 > 1	Normoresponders	1 ng/mL	LBR	For live birth, the rate for the P<1.0 ng/mL group was 35.3%, which was significantly higher than the 29.0% in the P≥1.0 ng/mL group (P=0.03)
Yadav *et al.*[29]	JBRA Assisted reproductive, 2022	Retrospective	2149	All responders	1.5 ng/mL	LBR	Live birth rates were 14.4%, 21.6%, and 21%
Roque Fernandez *et al.*[31]	J Obstet Gynaecol, 2022	Prospective cohort	400		Progesterone levels on day of OPU >90th centile	LBR	PE is not associated with the IVF outcome, but there is a trend to lower ongoing pregnancy rate and LBR and more miscarriages
Robati *et al.*[32]	J Reprod Infertil, 2020	Retrospective cohort	170		2.1	LBR CPR	As serum progesterone increased, a decrease in LBR was observed. Following multivariate logistical analyses, LBR significantly decreased with P4 thresholds of 4.0 ng/mL (OR 0.42, 95% CI: 0.17-1.0) and 4.5 ng/mL (OR 0.35, 95% CI: 0.12-0.96)
Huang *et al.*[33]	Taiwan J Obstet Gynecol, 2015	Retrospective	599		1.5	LBR CPR	No difference in CPR or LBR D5 ET was significantly higher LBRs
Hill *et al.*[34]	Fertil Steril, 2015	Retrospective	1620			LBR	An elevated serum progesterone level on the day of hCG administration was negatively associated with live birth, even in ETs with a good prognosis

Contd...

Table 1: Contd...

Author	Publication details	Type of study	Number of subjects	Inclusion criteria	PE threshold	Outcome measure	Results
Venetis *et al.*[35]	Hum Reprod, 2015	Retrospective	3296		1.5	LBR	When a multivariable analysis was performed, controlling for the effect of the aforementioned confounders, live birth rates (OR: 0.68, 95% CI: 0.48-0.97) were significantly decreased in the group with PE on the day of hCG
CPR							
Nagaraja *et al.*[36]	J Hum Reprod Sci, 2019	Prospective observational study	380	All responders	1.5	CPR	No difference in *P* value in pregnancy and non-pregnancy groups
Lepage *et al.*[37]	J Gynecol Obstet Hum Reprod, 2019	Retrospective	1022	All responders	1.57	Ongoing PR	CPR similar Miscarriage rate higher >1.57 LBR lower
Ashmita *et al.*[39]	J Hum Reprod Sci, 2017		235	All responders	1.5	CPR	The clinical pregnancy rate in the patients with P4 <1.5 ng/mL was significantly higher than those with elevated levels, P4 ≥1.5 ng/mL (33.3% versus 12.9%; *P*=0.037)
Cui *et al.*[38]	Horm Metab Res, 2017		825	Good responders	1.04 ROC analysis	CPR	IR and CPR lower in women >1.04 *P*
Huang *et al.*[40]	Reprod Biol Endocrinol, 2015	Retrospective	11,146	All responders	Identified threshold	CPR	Serum PE was inversely associated with CPRs in both cleavage-and blastocyst-Stage ET cycles D3>1.5 D5>1.75

LBR=Live birth rate, CLBR=Cumulative LBR, CPR=Clinical pregnancy rate, ROC=Receiver operating characteristic, PE=Progesterone elevation, IVF=*In vitro* fertilisation, CI=Confidence interval, OR=Odds ratio, ET=Embryo transfers, hCG=Human chorionic gonadotropin, IR=Implantation rate, OPU=Oocyte pick Up, PR=Pregnancy Rate, EP=Elevated Progesterone

Cumulative live birth rate–using freeze-all strategy

The above studies calculated CLBR including fresh and subsequent FETs. It is now evident that PPE causes lower LBR following fresh transfer. Including the first fresh transfer would mean the loss of the best embryos in a lower receptive endometrium and hence reduction in CLBR. Some researchers tried to understand whether the same detrimental effect on CLBR would persist if all embryos were cryopreserved and embryos transferred in only FET cycles–using the freeze-only strategy.

Two studies[42,43] found that PPE in the fresh cycle did not hamper CLBR in subsequent FET cycles using a freeze-all approach. It was also demonstrated that in oocyte donation cycles, PPE had greater number of oocytes obtained and good quality cleavage stage embryos. Embryo utilisation rate and CLBR in the oocyte donor recipients were similar in both groups substantiating the lack of impact on oocyte and embryo quality.[44] In few other studies, euploidy rate after preimplantation genetic testing (PGT) was similar in women with elevated P and LBR following transfer of such euploid embryos in FET cycles were not different.[45-48]

Is freeze-only strategy advisable in women with PPE to achieve better pregnancy rates? A secondary analysis of data from three randomised trials comparing fresh versus frozen embryo[49-51] transfer in normal-or high responders was done and the effect of P concentration on trigger day on live birth rate was analysed. It was seen that in women with P level >1.14 ng/ml, live birth rates were higher following frozen versus fresh transfer. They recommended that a freeze-only strategy was superior in women with a P concentration of >1.14 ng/ml.[52]

Summary

Majority of existing data till date confirms a negative impact on live birth rate following fresh transfer in women with PPE. This robust evidence reiterates the deleterious effect of PPE on endometrial receptivity. However, preliminary data from two studies show a negative impact of PPE on embryo utilisation and CLBR after fresh and following FETs.

This negative effect is negated by following a freeze-all approach. By excluding fresh embryo transfer, PPE in the fresh cycle does not appear to hinder CLBR in subsequent FET cycles. Therefore, the freeze-all strategy seems to be an appropriate approach to counter PPE in fresh cycles.

Elevated Progesterone and Impact on Oocyte and Embryo Quality

Impact of elevated P on trigger day on oocyte and embryo quality remains contentious even today. Ten studies have been published evaluating the impact of PPE on oocyte and embryo quality since the systematic review of Venetis *et al*.[6,42-44,47,53-57] [Table 2]. They are all retrospective cohort studies using a P threshold of 1.5–2.0 ng/ml. Increased *P* is seen to be associated with a greater number of retrieved oocytes and the number of cleavage-stage embryos on day 3 in most studies. Four studies found PPE to be associated with a lower number of top-quality blastocysts.[6,53,55,56] They proposed that delaying the trigger with an intention to retrieve greater oocyte numbers may cause elevated P and a lower number of utilisable blastocysts; which in turn would reduce CLBR. However, the remaining six studies indicated a similar number of top-quality embryos in both groups with no obvious detrimental effect of PPE on oocyte and embryo quality.[42-44,47,54,57] The euploidy rate after PGT-A was also similar in most studies.[45-48]

In summary, existing evidence on the influence of PPE on oocyte and embryo quality is conflicting though largely reassuring. Nevertheless, though cycles with PPE are associated with higher number of obtained oocytes, embryological outcomes such as total number of available good quality embryos/embryo utilisation rates are similar in both groups. This is suggestive of a probable negative impact on embryo quality and is a matter that needs further thought and research.

Progesterone Assay

The universal use of a discrete P threshold for clinical decision-making in IVF practice requires sensitive, precise and reliable immunoassay systems that are accurate over a range of P levels. Reliability and accuracy of estimated values are also vital for comparisons between centres, countries and accurate reporting of meta-analysis data and formation of practice guidelines.

A study compared the precision of P measurements using four automated immunoassays and the standard liquid chromatography–tandem mass spectrometry (LC-MS). Two of the assays had inter-assay coefficients of variation of <10%. P levels as determined by LC-MS/MS were at times significantly different from P levels in three of the four analysers. Their work indicated that serum P level estimation should be interpreted cautiously and is influenced by laboratory and method-specific data.[58] In another study, *P* was measured in 28 serum samples from women undergoing IVF using the Siemens ADVIA Centaur Immunoassay System and the Abbott Architect i1000SR analyser. The values were compared with LC–tandem MS to define the accuracy of each immunoassay. They found that Siemens ADVIA Centaur Immunoassay System overestimated progesterone

Table 2: Pre-mature progesterone elevation and oocyte and embryo quality

Author	Publication	Type of study	Number of subjects	Research question	*P* threshold	Results
Woo *et al*.[53]	J Clin Med, 2022	Retrospective	982	P4 and oocyte and embryo quality	1.25 1.5 2.25	>2.25 low oocyte maturation>1.25 low fertilisation rate>1.5 low good quality embryos
Racca *et al*.[42]	Hum Reprod, 2021	Retrospective	942	Is LFEP in the fresh cycle hindering CLBRs when a freeze only strategy is applied?	1.5	LFEP in the fresh cycle does not hinder CLBR of the subsequent frozen cycles in a FA approach
Boynukalin *et al*.[43]	Gynecol Endocrinol, 2021	Retrospective	1034	To evaluate the effect of trigger day progesterone levels on live birth in freeze-all cycles	1.5 0.8-1.49 <0.8	LBRs were similar in the three subgroups The proposal that trigger day PE exerts a detrimental effect on oocyte and embryo competence has no clinical validity
Racca *et al*.[44]	Hum Reprod, 2020	Retrospective	397	Does LFEP during ovarian stimulation for oocyte donation have an impact on EQ and CLBR	1.5	PPE had greater no of oocytes retrieved, total number of embryos D3, D3 good quality embryos. Fert rate, embryo utilisation rate, CLBR similar No impact on embryo quality
Hernandez-Nieto *et al*.[47]	Hum Reprod, 2020	Retrospective cohort	5806 euploid single FET	A LFPE on embryonic competence and reproductive potential in thaw cycles of PGT-A screened embryos?	2	Utilisable blastocysts Euploidy rate IR CPR, OPR LBR Similar in FET PGTA euploid No impaired embryo dvpt, aneuploidy No impact on FET outcome
Baldini *et al*.[54]	Clin Ter, 2018	Retrospective	131	Impact of PPE on outcome of FET cycles after D3 transfer	1.2	No difference in number of oocytes, fert rate, implantation rate, CPR, ongoing pregnancy rate after FET
Racca *et al*.[6]	Hum Reprod, 2018	Retrospective	3400	elevated late-follicular phase progesterone (EP) associated with a deleterious impact on EQ and cumulative LBRs after fresh and FET	1.5	Number of oocytes retrieved increased significantly with increasing serum *P* values. Utilisation rates decreased linearly as progesterone increased for Day 3 embryos while for Day 5 embryos only the EP group was associated with a significant decrease. EP was also associated with decreased fresh and cumulative LBRs
Vanni *et al*.[55]	Plos One, 2017	Two-center retrospective study	986	Impact of PPE on top quality blastocyst formation rate	>1.49 ROC	PPE is associated with lower rate of top quality blastocyst formation 1.49 is the best cut off to identify risk of absence of TQ D5 embryos
Huang *et al*.[56]	Plos One, 2016	Retrospective	4236	PPE and top quality embryo rate	>2	Serum progesterone levels >2 were associated with lower TQE
Zhu *et al*.[57]	J Assist Reprod Genet, 2014	Retrospective	2978	Between serum progesterone (P4) response after hCG administration and the number of oocytes retrieved and the embryo quality in fresh IVF cycles		PPE had higher oocytes retrieved, but similar oocyte and embryo quality and pregnancy rates

FET=Frozen embryo transfers, LFEP=Late follicular elevated progesterone, LBRs=Live birth rates, CLBRs=Cumulative LBRs, EQ=Embryo quality, PGT-A=Pre-implantation genetic testing for aneuploidy, PE=Progesterone elevation, PPE=Pre-mature PE, EQ=Embryo quality, hCG=Human chorionic gonadotropin, IVF=*In vitro* fertilisation, ROC=Receiver operating characteristic, CPR=Clinical pregnancy rate, FA=Freeze All, IR=Implantation rate, OPR=Ongoing Pregnancy Rate, PGTA=Pre-implantation genetic testing – Aneuploidy, TQE=Top quality embryo, EP=Elevated Progesterone

concentrations by 19% and the Abbott Architect overestimated progesterone concentrations by 5%.[59] Three assay systems – ELECSYS generation II by Roche (gen II), ELECSYS generation III by Roche (gen III) and Architect' by Abbott (Architect) were compared and it was seen that different P assays have limited reproducibility and that the results depend on the assay used and the range of P level.[60]

These studies highlight the variability and lack of reproducibility and agreement between current existing immunoassay systems at P thresholds that are clinically relevant in the follicular phase. This renders questionable combined data from different centres using dissimilar assays and the resultant implications for daily clinical practice. This also calls for globally uniform and accurate assay methods with good reproducibility. Consequently, the results of meta-analysis data must be interpreted with caution.

Is a Single Progesterone Estimation Sufficient and Reliable?

Traditionally, blood samples to estimate serum P are drawn on the morning of the trigger day and this single value determines further management. Considering the pulsatile nature of P secretion in natural cycles, it remains doubtful whether a single estimation of P reflects the P true picture. Most studies do not specify the time of sample collection and whether it was uniform across all patients.

Few studies have been published to understand the daily variability of P levels on trigger day in women undergoing IVF stimulation. In a prospective cohort study in 22 oocyte donors P levels at four different times on trigger day–8:00, 12:00, 16:00 and 20:00 were estimated. The mean P levels at these times were 1.75 ng/ml, 1.4 ng/ml, 1.06 ng/ml and 0.97 ng/ml, respectively. They observed a mean difference of 0.77 between the first determination at 8:00 and the last at 20:00; which was equivalent to a 44% reduction in mean level. In patients with P levels above the threshold of >1.5 ng/ml at 8 am, 70% had values below this level at the last measurement.[61] Another similar study evaluated four samples on trigger day drawn at 8:00, 11:00, 14:00 and 17:00. This study also observed a 37.8% decline between the first and last drawn sample and a highly significant decline in levels between 8 am and 11 am.[62]

These studies highlight the diurnal variation of P on trigger day and the remarkable decline in levels observed during the day. Although this finding does not deny a possible negative effect of PPE on fresh embryo transfer pregnancy rates, it raises several important and pertinent questions. Should the time of progesterone determination be standardised in future research? How relevant are the existing proposed threshold levels to decide fresh transfer or freeze-all policy without regard to the time of sample collection?

Optimal Progesterone Threshold

One of the most contentious issues on this topic of PPE has been the selection of an optimal progesterone threshold above which outcomes are poor and a freeze-all strategy was reasonable and cost-effective. Currently, most studies use a popular cutoff of 1.5 ng/ml. The first published study by Schoolcraft *et al*. in 1991 used a cutoff of 0.5 ng/ml.[7] The systematic review by Venetis *et al*. in 2013 called attention to the immense variability in utilised thresholds ranging from 0.4 to 3.0 ng/ml.[3] While few studies choose their cut-off arbitrarily, some have used receiver operating characteristic (ROC) curves to determine their own values or based on their 90th or 95th percentile levels of P. Subsequent literature has also demonstrated variability in the use of cutoff from 1.0 to 2.1 ng/ml. Venetis *et al*. in their meta-analysis have also shown that the decline in pregnancy rates following fresh embryo transfer is observed much earlier with P values above 0.8 ng/ml.

Choosing a threshold value cannot be arbitrary, but goes beyond the point of differentiating women into good and poor outcomes. In PPE, the cutoff must accurately predict the absence of a live birth and at the same time, justify the cost-effectiveness of a freeze-only approach. P levels may not affect pregnancy outcomes linearly and reduction in live birth following fresh embryo transfer has also been observed in women with low P levels.[1,63,64] The threshold also appears to be different for women with different types of responses.

A retrospective study sought to critically assess various methodologies to determine the threshold value of P and makes for a very thought-provoking read on the topic.[65] Using threshold analysis and cost-effectiveness analysis, they studied 14 different statistical methodologies to generate P thresholds and applied them to 7608 fresh ART cycles. The 14 methods (95th centile, ROC analysis specificity 80%–95%, ROC analysis sensitivity 80%–95% and absolute reduction 5%–20% in live birth from baseline) generated P thresholds from 0.4 to 3.0 ng/ml. The lowest P level at which a reduction in a live birth was observed was 0.7 ng/ml (this was similar to the earlier results of the meta-analysis). However, they noted that a clinical and cost-effective benefit to a freeze-only approach was seen at values above 1.5–2 ng/ml. They opined that above these thresholds, a smaller percentage

of patients would be at risk, the number needed to treat would be a clinically meaningful 4–13 and the freeze-only strategy would be cost-effective.[65]

In their intriguing and stimulating editorial, Venetis and Tarlatzis introduce a new concept away from the current tendency to dichotomise patients into two groups based on a given threshold value.[10] They theorise that progesterone being a continuous variable, it would be wrong to divide a patient population into two groups based on a single threshold value. The decline in the live birth rate with increasing P levels is likely to be a gradual reduction and not an absolute all-or-nothing. A woman with a P level of 1.45 is likely to have a live birth rate not very different from 1.55 ng/ml. With the existing controversies around the time of P estimation, the method of assay used and their coefficients of variation, are such differences of 0.1 ng/ml clinically relevant? They suggest that the concept of progesterone elevation should migrate from a threshold concept to a continuous covariate in prediction models of live birth.

Drawbacks of Published Literature

The limitations in the existing literature on PPE preclude our endeavour to generate uniform practice guidelines. The data are largely retrospective in nature and there are no randomised trials to justify a freeze-only approach. There is huge heterogeneity in the thresholds used, the P assay utilised and no mention of the timing of measurement P levels are not the only determinant of live birth and other confounding factors are not adjusted for using multivariate analysis in some studies.

Conclusions

Interpretation and management of elevated P on the day of the trigger is still a matter of controversy and debate. Evidence till date confirms a negative effect of PPE on live birth outcomes following fresh embryo transfer. The likely impact of this early and prolonged exposure of P on the achievement of pregnancy seems to be on the endometrium causing altered endometrial receptivity. However, questions remain unanswered whether this effect extends to the oocyte, embryo and consequently on CLBR; with conflicting evidence. Greater clarity is needed to achieve agreement about the optimal threshold P value. A reduction in live birth rate is seen as early as 0.7–0.8 ng/ml, but for values above 1.5–2 ng/ml, the freeze-only strategy seems cost-effective or whether IVF practice should progress from single threshold levels to using P as a continuous variable in prediction models? Current assays lack reproducibility and agreement with a paucity of standardised tests. Research has also highlighted the diurnal variations in P levels on a single day and doubts whether a single measurement is a true reflection of the problem.

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Conflicts of interest

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