Live birth rates after IVF are reduced by both low and high progesterone levels on the day of human chorionic gonadotrophin administration

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STUDY QUESTION: Are low serum progesterone levels on the day of human chorionic gonadotrophin (hCG) administration detrimental for live birth delivery rates during in vitro fertilization (IVF)?

SUMMARY ANSWER: Progesterone levels ≤0.5 ng/ml on the day of hCG administration hinder live birth rates.

WHAT IS KNOWN ALREADY: Fundamental research has shown that the presence of late follicular phase progesterone is essential for follicular development, ovulation and endometrial receptivity. However, previous studies in patients undergoing ovarian stimulation have only assessed if progesterone levels in the higher range are detrimental for pregnancy or not. That said, information on the effect of the full range of late follicular progesterone on IVF outcomes is still lacking.

STUDY DESIGN, SIZE, DURATION: This was a retrospective, single-centre cohort study with 2723 cycles performed in patients aged between 19 and 36 and undergoing controlled ovarian stimulation between January 2006 and March 2012 for their first or second attempt of IVF followed by a fresh embryo transfer (ET).

PARTICIPANTS/MATERIALS, SETTING, METHODS: All patients underwent ovarian stimulation using a gonadotrophin-releasing hormone (GnRH) antagonist for pituitary down-regulation. Final oocyte maturation was triggered with hCG 36 h before oocyte retrieval. On the day of hCG administration, serum progesterone evaluation was performed. Live birth delivery rates were compared amongst various ordinal and regular progesterone intervals (<0.50, 0.50–0.75, 0.75–1.00, 1.00–1.25, 1.25–1.50, >1.50 ng/ml) using logistic regression.

MAIN RESULTS AND THE ROLE OF CHANCE: The average age of our sample was 30.5 years. Almost 82% of all embryo transfers were of a single embryo and 51.8% were performed with a Day 5 embryo. The average value (± standard deviation) of progesterone on the day of hCG administration was 1.02 ± 0.50 ng/ml and the live birth rate was 23.4%. The live birth rates (according to the above-described ordinal serum progesterone intervals) were 17.1, 25.1, 26.7, 25.5, 21.9 and 16.6%, respectively. The live birth rates were significantly lower in patients with both low (<0.5 ng/ml) and high (>1.5 ng/ml) late follicular progesterone levels (P < 0.05).

LIMITATIONS, REASONS FOR CAUTION: The main limitation of our study was its retrospective nature. Furthermore, our study was restricted to patients under GnRH antagonist pituitary suppression and requires confirmation in a GnRH agonist setting.

WIDER IMPLICATIONS OF THE FINDINGS: This study comprehensively assessed the relationship between live birth delivery rates and progesterone levels on the day of hCG administration during ovarian stimulation for IVF. Clinically relevant lower (<0.5 ng/ml) and higher (>1.5 ng/ml) progesterone level limits were determined.

STUDY FUNDING/COMPETING INTEREST(S): No funding was received for this study and the authors have no conflicts of interest to declare.

Key words: ovarian stimulation / progesterone / in vitro fertilization / live birth rate
Introduction
Pituitary suppression with gonadotrophin-releasing hormone (GnRH) analogues has drastically reduced the incidence of premature luteinizing hormone (LH) surge during controlled ovarian stimulation (COS) to <2% per cycle (Smits et al., 1992; Felberbaum and Diedrich, 1999). Nonetheless, subtle late follicular progesterone (P) elevations unrelated to a premature LH surge still occur in up to 38% of all in vitro fertilization (IVF) cycles regardless of artificial pituitary suppression (Ubaldi et al., 1996; Bosch et al., 2003).

Many research groups have attempted to assess how late follicular high P levels affect IVF. The majority of available trials have demonstrated that high P levels may hinder IVF success (Feldberg et al., 2006 and March 2012 including all women between 18 and 36 years of age. We performed a retrospective, single-centre cohort study between January 2006 and March 2012 including all women between 18 and 36 years of age undergoing their first or second COS for IVF in our centre. As IVF success is influenced by cycle rank (Malizia et al., 2009; Luke et al., 2012), especially after three or more attempts, we excluded all cycles of patients who had more than one previous cycle performed in our centre. To avoid other potential confounders, only patients younger than the age of 36 years planned for fresh embryo transfer using autologous oocytes and under GnRH antagonist pituitary suppression were included. Furthermore, couples with planned blastocyst biopsy were excluded from the analysis.

Ovarian stimulation and pituitary suppression
The choice of stimulation protocol was made on a case-by-case basis according to clinician preference and patient characteristics. Women began ovarian stimulation on Day 2 of the menstrual cycle with either recombinant follicle-stimulating hormone (rFSH; Gonal-F®, Merck Serono Pharmaceuticals, Darmstadt, Germany; or Puregon®, Merck Sharp & Dohme, Whitehouse Station, NJ, USA; or Elonva®, Merck Sharp & Dohme) or highly purified human menopausal gonadotrophin (HP-HMG; Menopur®, Ferring Pharmaceuticals, St. Prex, Switzerland). Pituitary down-regulation, using a GnRH antagonist, was performed with daily administrations of either cetrotelix (Cetrotide®, Merck Serono Pharmaceuticals) or ganirelix (Orgalutran®, Merck Sharp & Dohme) starting from Day 7 of the menstrual cycle onwards.

Cycle monitoring and final oocyte maturation triggering
Cycles were monitored by means of serial vaginal ultrasound scans and serum determination of estradiol (E2) and P. Whenever necessary, dose adjustments of FSH/HP-HMG were performed according to ovarian response. As soon as three follicles of ≥ 17 mm were observed, final oocyte maturation was triggered with either highly purified urinary hCG (5000 IU or 10 000 IU, according to the physicians’ preference; Pregnyl®, Merck Sharp & Dohme) or 250 IU of recombinant hCG (Ovitrelle®, Merck Serono Pharmaceuticals). Cumulus–oocyte complexes (COCs) were collected by transvaginal aspiration ~36 h after hCG administration.

Progesterone assessment immunoassay
Serum P levels were assessed on the day of hCG administration using a validated electrochemiluminescence immunoassay (Cobas 6000®, Roche, Basel, Switzerland) with a measured sensitivity and total imprecision (% coefficient of variation (CV)) of 0.03 μg/l and <7%, respectively. The same assay was performed during the full duration of the study and was regularly calibrated to minimize variation of the results associated with time and reagent batch renewal.

Statistical analysis
To avoid bias of the results by assuming that any relationship between P and PR may be linear (Bosch et al., 2010), patients were divided into the following six distinct ordinal interval groups (ng/ml): ≤0.50, 0.51–0.75, 0.76–1.00, 1.01–1.25, 1.26–1.50 and >1.50. These thresholds were selected taking into account the cut-offs applied amongst previous studies (Bosch et al., 2010; Xu et al., 2012) and a balance between the use of clinically relevant intervals that would divide our sample homogenously so as not to hinder the inter-interval statistical analysis.

Live birth delivery rates per oocyte retrieval were calculated for each P interval, determining odds ratios (ORs) between each of the P level intervals and the highest P group (>1.5 ng/ml) using logistic regression and adjusting the standard errors to allow repeated cycles performed in the same women to be included. Using this approach, we would be able to further analyse the relationship between P and IVF outcomes to eventually determine the existence of a lower P threshold under which these end-points were hindered as much as they are already known to be in P levels >1.5 ng/ml.

Materials and Methods
Study population and design
We performed a retrospective, single-centre cohort study between January 2006 and March 2012 including all women between 18 and 36 years of age undergoing their first or second COS for IVF in our centre. As IVF success is influenced by cycle rank (Malizia et al., 2009; Luke et al., 2012), especially after three or more attempts, we excluded all cycles of patients who had more than one previous cycle performed in our centre. To avoid other potential confounders, only patients younger than the age of 36 years planned for fresh embryo transfer using autologous oocytes and under GnRH antagonist pituitary suppression were included. Furthermore, couples with planned blastocyst biopsy were excluded from the analysis.
Potential confounder adjustment for the number of embryos transferred (1 or ≥2), stage of embryo development (Day 3 cleavage-stage or Day 5 blastocyst) and the continuous variables age, serum E2 levels on the day of hCG administration, total dose of exogenous FSH and number of COCs retrieved was performed. Only one potential confounder amongst the groups were assessed using either logistic regression (for binomial variables) or Kruskal–Wallis (for continuous variables), followed by a post hoc pairwise analysis when significant. A P-value was considered significant if <0.05, adjusted for multiple comparisons (using the Bonferroni correction) when performed. The statistical analysis was performed using the Stata software version 12 (StataCorp, College Station, Texas, USA) and SPSS version 20 (IBM, New York, USA).

Results

Patient demographics and general characteristics of the treatment protocol

The baseline and treatment characteristics of the 2723 cycles (performed in 2157 patients) included in the study are detailed in Table I (Supplementary data, Tables SI and SII compare these results according to IVF outcomes and P levels, respectively). The average age of our sample was 30.5 years (ranging from 19.0 to 36.0 years). Almost 82% of all embryo transfers (ET) were of a single embryo and 51.8% were performed with a Day 5 embryo. The average value (± standard deviation) of P on the day of hCG administration was 1.02 ± 0.50 ng/ml and the live birth rate was 23.4%.

Relationship between P levels on the day of hCG administration and live birth delivery rates

Figure 1 illustrates the live birth delivery rates for the various intervals of P levels on the day of hCG administration, while Fig. 2 shows the ORs (with a 95% confidence interval) for each interval when compared with the highest P group (>1.5 ng/ml). Adjusted live birth rates were significantly higher for all groups with a P level <1.5 ng/ml, except for the group with a P level ≤0.5 ng/ml. These results show that the live birth rates in the group with serum P levels ≤0.5 ng/ml on the day of hCG administration do not vary significantly from the hindered live birth rates in the group with P >1.5 ng/ml (P = 0.24).

To further assess for potential confounding factors and the effect of serum P levels on late follicular oocyte development, we applied the previously determined P lower and upper limits to divide our sample in three groups: patients with low P (≤0.5 ng/ml), normal P (0.5–1.5 ng/ml) and high P (>1.5 ng/ml) levels (Table II). As hypothesized before, patient age, the total dose of exogenous FSH administered, number of COCs retrieved, percentage of Day 3 embryo transfer’s and E2 levels on the day of hCG administration were different amongst these groups. Nonetheless, our analysis still showed that, when compared with the normal P group, patients with both low and high P had significantly lower live birth rates, even after adjusting for potential confounding variables (Table II). Conversely, maturation and fertilization rates did not vary significantly amongst the groups (Table II).

As this is a retrospective analysis, patients were not randomized between the available options of exogenous FSH. In our study, HP-HMG was the selected stimulation in 12.9% of all cases (Table II). Despite that, it was interesting to see that the trend of HP-HMG use decreased from the low to high P groups (P < 0.0001 for overall trend analysis using the Cochran–Mantel–Haenszel trend test). Having said that, while P values over 1.5 ng/ml occurred in 13.8% of the patients using rFSH and 6.3% of the HP-HMG users, P levels <0.5 ng/ml occurred in 8.5% of the rFSH users and 12.6% of the HP-HMG users.

Discussion

This study comprehensively assessed the relationship between live birth delivery rates and P levels on the day of hCG administration during COS in a GnRH antagonist protocol. Furthermore, clinically relevant lower (≤0.5 ng/ml) and higher (>1.5 ng/ml) P level limits between which live birth rates seem to be optimal, were determined.

High progesterone levels and live birth rates

A previous study performed with a similar number of patients using GnRH antagonist down-regulation had already determined that P levels >1.5 ng/ml were detrimental for IVF pregnancy outcomes [n = 2855 (Bosch et al., 2010)]. However, these results were obtained using an unselected population and ongoing PR as the study outcome. The strength of our results come from the fact that they are less prone to bias, as the inclusion and exclusion criteria that were applied limited the possibility for unverified confounding factors. Furthermore, they
are of more clinical relevance, since live birth delivery was the evaluated outcome.

The exact mechanisms that cause this P increase (still often misleadingly referred to as ‘premature luteinization’) remain a largely debated topic amongst researchers (Venetis et al., 2007; Bosch et al., 2010; Al-Azemi et al., 2012). Some believe that the excessive ovarian steroidogenic activity resulting from the stimulation of multiple follicles with exogenous FSH is the main source of late follicular P (Bosch et al., 2010; Elnashar, 2010). Nonetheless, alternative causes, namely increased LH production or an altered LH receptor sensitivity have been postulated for specific populations such as low responders (Fanchin et al., 1997a,b; Younis et al., 1998, 2001; Elnashar, 2010; kilcig et al., 2010; Bosch, 2011; Younis, 2011). In our study, patients with high P levels were younger (0.8 years), had a higher consumption of exogenous FSH, had more COC recovered and had higher E2 levels on the day of hCG administration (Table II). However, even after controlling for these factors, the live birth rates of the patients with high P levels remained significantly lower compared with the normal P group (Table II).

We found no significant difference with regard to oocyte maturation or fertilization rates amongst the high and normal P level groups. Previous
studies have also shown that oocyte and embryo quality seem to be comparable between high and normal P patients, while the endometrium significantly changes its gene expression profile (Labarta et al., 2011) and may have decreased receptivity (Fanchin et al., 1996; Chetkowski et al., 1997; Fanchin et al., 1997a,b). Furthermore, studies performed in acceptors of donated oocytes found no difference in PR between oocytes deriving from stimulated cycles with P levels above or below 1.0–1.2 ng/ml (Check et al., 1994; Melo et al., 2006), indicating that the high P levels may affect PR by altering endometrial receptivity.

**Low progesterone levels and live birth rates**

Until now, no previous trial has attempted to evaluate whether low P levels on the day of hCG administration may impair pregnancy following IVF or not. By comparing the live birth rates of patients with high P levels (>1.5 ng/ml) with the various ordinal regular groups of patients with decreasing values of P, we were able to conclude that P levels ≤0.5 ng/ml were as detrimental to live birth as high P. To the best of our knowledge, the only trial with similar results (lower PR in the low P range) was performed by Levy et al. in 254 patients undergoing COS with GnRH agonist pituitary suppression (Levy et al., 1995). In this small study, clinical PRs were significantly lower in patients with P levels <0.7 ng/ml and over 0.8 ng/ml.

Patients in our low P group (<0.5 ng/ml) were older (0.7 years), had less COC retrieved and lower E2 levels on the day of hCG triggering. Nevertheless, low P levels were still associated with lower live birth rates even after adjusting for these and other confounding variables (Table II). More interestingly, patients were administered similar amounts of exogenous FSH units as the normal P group, were triggered with hCG using the same criteria and had unaffected maturation and fertilization rates (Table II). Thus, the lower live birth rates observed do not appear to be related to inadequate stimulation nor to poorer oocyte maturation or fertilization competence. These results differ from previous animal experiments using rhesus monkeys in which late follicular P seems to be essential for adequate oocyte maturation and fertilization (Zelinski-Wooten et al., 1994; Hibbert et al., 1996; Borman et al., 2004).

As oocyte quality in patients with low P values seems to be unaltered, one can postulate that the reduced live birth rate may be due to either decreased luteinization, altered endometrial receptivity or both. Basic science research on the menstrual cycle has shown that small amounts of P are extremely important during the late follicular phase (Hibbert et al., 1996; De Geyter et al., 2002; Borman et al., 2004). Prior to ovulation, the main source of P shifts from the adrenal cortex to the ovary (De Geyter et al., 2002) and, at that same time, P receptor mRNA and proteins become more abundant in the nuclei of the granulosa cells (Natraj and Richards, 1993) and that only granulosa cells with P receptors in the pre-ovulatory phase were capable of luteinizing (Hild-Petito et al., 1988). Furthermore, endometrial P receptors reach their maximal concentrations during the mid- to late proliferative phase of the menstrual cycle (Barile et al., 1979;...
Lessey et al., 1988; Ravn et al., 1994) and P directly affects the action of E2 on the endometrium (Ozckar et al., 2004a,b). Studies that administered the anti-progestin RU-486 during final oocyte maturation in mice showed that it affected endometrial receptivity and not embryo development (Batten et al., 1988). Furthermore, a prospective trial on women undergoing IVF showed that an insufficient endogenous P production can be an early predictor of poor pregnancy outcome which cannot be rescued by exogenous supplementation (Ioannidis et al., 2005). Trials designed to specifically assess the endometrial development could shed further light on the mechanisms behind the decreased PR in patients with low serum P concentrations on the day of hCG administration.

Finally, the low P levels may also be a confounding factor of another mechanism that hinders simultaneously late follicular P production and pregnancy after IVF. Therefore, a randomized controlled trial in patients with low P levels (≤ 0.5 ng/ml) when at least three follicles of 17 mm are present comparing immediate hCG administration with delayed triggering and serial P assessment might further explain the clinical importance of our findings.

Strengths, limitations and clinical impact of the study

To our knowledge, this is the first trial to comprehensively assess the effect of the full spectrum of serum P levels on the most important outcome of IVF, i.e. live birth delivery. Furthermore, this was performed in a large and homogeneous sample.

The main limitation of our study is its retrospective nature, an issue also encountered in previously mentioned high P studies (Fanchin et al., 1997a,b; Bosch et al., 2010; Kilicdag et al., 2010; Wu et al., 2012; Xu et al., 2012). We attempted to diminish the importance of this weakness by adjusting for many potential confounders, but there could still be important variables which we might have failed to consider. Furthermore, our results are limited to patients under GnRH-antagonist pituitary suppression and require confirmation in a GnRH agonist setting.

Regardless of its limitations, our research offers robust evidence that low late follicular P levels may be as detrimental as high P to live birth following IVF. Hence, the most important conclusion that can be extrapolated is that serum evaluation of late follicular P should not be ignored. Since 22% of all cycles included in our trial had either low or high P levels, a considerable number of cycles seemed hampered even before embryo transfer.

The knowledge that a patient has abnormal late follicular P could lead one to try to intervene to optimize IVF outcomes (Elbashar, 2010). To that extent, limiting the total dose of FSH administered could be a beneficial way to address the issue of high trigger-day P levels (Bosch et al., 2010; Xu et al., 2012). For example, in two small previous randomized trials, late follicular replacement of daily FSH with low-dose hCG showed comparable PR (Filicori et al., 2005; Blockeel et al., 2009) without the detrimental late follicular P elevation (Filicori et al., 2005). Alternatively, close P monitoring and hCG triggering before reaching excessively high P levels (i.e. P levels > 1.0 ng/ml) has also resulted in higher implantation rates in a small retrospective study (Harada et al., 1996). Although other authors have suggested the use of HP-HMG to reduce the incidence of high late follicular P (Bosch et al., 2010), we would use this advice with caution, as the results of our study lead us to conclude that the exclusive use of HP-HMG could cause a higher incidence of similarly detrimental late follicular low P levels.

On the other hand, low ‘hCG-trigger-day’ P values also require close monitoring and, eventually, an FSH step-up or hCG trigger postponement to give the necessary stimulus to reach the minimal level of steroidogenesis. Furthermore, as a luteinization defect could be the cause for lower live birth rates in this population, a more intense or earlier luteal phase support could counter-balance the negative influence of low P. Finally, a randomized controlled trial comparing fresh embryo transfer and cryopreservation-thawing embryo transfer protocols could definitively obviate the need for further attempts to rescue what seem to be suboptimal fresh cycles (with both low- and high late follicular P levels), especially in an era where vitrification cryopreservation systems are becoming increasingly widespread (Bosch et al., 2010; Xu et al., 2012).

In conclusion, our study demonstrated, for the first time, that low P levels on the day of hCG administration also significantly impair live birth rates in women undergoing IVF with GnRH antagonist co-treatment followed by fresh embryo transfer. This should be carefully considered when treating infertile patients in order to aim towards P levels between 0.5 and 1.5 ng/ml in an attempt to maximize the reproductive outcomes in this population.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors’ roles

S.S., N.P.P. and C.B. were responsible for the study conception, article drafting, statistical analysis and critical review. P.H. contributed during the study conception and aided with the statistical analysis. J.S., M.C. and H.T. participated in the study conception and review of the paper. C.B. was the research organizer.

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Conflict of interest

The authors have no conflict of interest to declare.

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Low and high P levels reduce live birth after IVF

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