Daytime Variation in Serum Progesterone During the Mid-Luteal Phase in Women Undergoing In Vitro Fertilization Treatment

Lise Haaber Thomsen1,2*, Ulrik Schiøler Kesmodel3,4, Claus Yding Andersen5 and Peter Humaidan1,2

1The Fertility Clinic, Skive Regional Hospital, Skive, Denmark, 2Department of Clinical Medicine, Aarhus University, Aarhus, Denmark, 3The Fertility Clinic, Herlev Hospital, Herlev, Denmark, 4Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark, 5Laboratory of Reproductive Biology, The Juliane Marie Centre for Women, Children and Reproduction, University Hospital of Copenhagen, University of Copenhagen, Copenhagen, Denmark

Objective: To investigate whether mid-luteal serum progesterone (P4) exhibits significant fluctuations during a 12-h daytime period in women undergoing in vitro fertilization (IVF) and to explore whether the extent of these fluctuations could impact the interpretation of luteal progesterone levels in a clinical setting.

Design: Explorative pilot study.

Setting: Public hospital-based fertility unit.

Patients: Ten women undergoing IVF treatment.

Intervention: Seven days after oocyte pick-up, patients underwent frequent repeated blood sampling (every 60 min for 12 h and during two of these hours, every 15 min). Serum samples were analyzed for progesterone, estradiol, and luteinizing hormone (LH).

Main outcome measures: Daytime fluctuations in s-progesterone and s-estradiol.

Results: There was a significant positive correlation between median P4 levels and the magnitude of P4 variations—women with median P4 < 60 nmol/l had clinically stable P4 levels throughout the day, while patients with median P4 > 250 nmol/l exhibited periodic P4 peaks of several hundred nanomoles per liter. These endogenous P4 fluctuations were observed irrespective of the type of stimulation protocol or mode of triggering of final oocyte maturation and despite the fact that LH was under the detection limit at the time of measurement. Simultaneously, large fluctuations were seen in s-estradiol.

Conclusion: Monitoring of early to mid-luteal P4 levels in IVF cycles may be valuable in the planning of individualized luteal phase support in the attempt to increase reproductive outcomes. The prerequisite for luteal phase monitoring is, however, that the validity of a single measured P4 value is reliable. We show for the first time, that a single P4 measurement in the low progesterone patient quite accurately reflects the corpus luteum function and that the measurement can be used to detect IVF patients with a need of additional exogenous luteal P4 administration.

Keywords: serum progesterone, in vitro fertilization, serum estradiol, luteal phase, daytime variation
INTRODUCTION

The human corpus luteum (CL) is a transient ovarian endocrine gland, which is active during the luteal phase of the menstrual cycle and in early pregnancy until gestational week 8. The CL produces significant amounts of progesterone (P₄), estradiol (E₂), and androgens as well as growth factors and nonsteroidal hormones. The overall maintenance of CL function is critically dependent upon regular stimulation of pituitary luteinizing hormone (LH) or human chorionic gonadotropin (hCG) to sustain the steroidogenesis from the luteinized granulosa and theca cells (1). A sufficient P₄ production from the CL is an absolute necessity for the decidualization of the endometrium preceding implantation and the establishment of early pregnancy. Progesterone secretion from the CL is maximal during the mid-luteal phase inducing a serum P₄ level of approximately 40–60 nmol/l in the natural cycle (2, 3).

During ovarian stimulation for in vitro fertilization (IVF) supra-physiological levels of E₂ are obtained during the late follicular phase as a result of the multifollicular growth. This hyper-estrogenic state must be counterbalanced in the luteal phase by an increased P₄ load to achieve a receptive endometrium in time for embryo transfer. Previously, Humaidan and co-workers showed that the use of GnRH agonist trigger in IVF cycles followed by a standard vaginal luteal phase support resulted in mid-luteal P₄ levels comparable to levels seen in the natural cycle (39 nmol/l) (4). However, in contrast to what was expected, this P₄ level was too low to secure successful implantation and pregnancy, resulting in an ongoing IVF pregnancy rate of only 6%. Thus, emphasizing the fact that the P₄ requirement during the luteal phase of the stimulated cycle is greater than that of the natural cycle. When the luteal phase support was modified by adding a bolus of 1,500 IU hCG on the day of oocyte retrieval, the mid-luteal P₄ level of the GnRHa triggered cycle increased to 74 nmol/l resulting in a delivery rate of 24% per transfer (5). It seems that a mid-luteal serum P₄ threshold of approximately 80–100 nmol/l exists after IVF treatment followed by fresh embryo transfer, and that this threshold must be surpassed in order to secure a successful reproductive outcome (6). The traditional luteal phase support in artificial IVF cycles with administration of vaginal micronized P₄ induces a luteal serum P₄ level of approximately 40 nmol/l (7–9). Thus, a substantial additional endogenous P₄ production by the CL is mandatory to surpass the P₄ threshold to subsequently optimize the chance of pregnancy following IVF treatment. Traditionally, clinicians do not monitor the luteal phase P₄ levels in the firm belief that the luteal phase support will cover the P₄ need of the cycle. However, we have previously seen that more than 25% of IVF patients in both the hCG and GnRHa triggered group have a mid-luteal serum P₄ below 60 nmol/l despite luteal phase support and the fact that they had more than 14 follicles on the day of aspiration (10). Furthermore, data from non-human species (11, 12) and data from human frozen/thawed embryo cycles (13, 14) have shown that an optimal luteal P₄ range exists and that pregnancy outcome is reduced not only below but also above this optimal P₄ level. Whether this is also the case following IVF and fresh embryo transfer, is still to be explored. If this is the case, monitoring of luteal P₄ levels may help to improve the reproductive outcome in IVF cycles by allowing an individualization of treatment based on the serum P₄ measurements.

However, mid-luteal P₄ measurements are complicated by the pulsatile nature of hormone secretion from the CL. Filicori and co-workers (15) showed that plasma P₄ concentrations exhibit large and rapid fluctuations during the mid-luteal phase of naturally cycling women. Thus, P₄ levels ranged from values as low as 7 nmol/l to peaks of 128 nmol/l within minutes during a 24-h study period. In the natural cycle, two distinguishable types of P₄ pulses exist during the mid-luteal phase: those preceded by an LH pulse and others emerging at time of LH quiescence; the latter being a result of an autonomous steroid secretion by the CL independent of LH activity. During the mid-luteal phase of the stimulated IVF cycle, the pituitary is suppressed by the negative feedback from supra-physiological steroid levels and s-LH is significantly reduced to levels much lower (0.5–0.7 IU/l) than seen in the mid-luteal phase of the natural cycle (16–18). How this diminished LH pulse activity influences the secretory pattern of ovarian steroidogenesis during the mid-luteal phase of an IVF cycle is until now unknown.

The present study was performed to explore whether mid-luteal serum P₄ levels in an IVF cycle exhibit a similar high-pulsatile pattern as seen during the natural cycle, knowing that the LH pulse activity is distinctly reduced. From a clinical point of view, we wanted to investigate whether a single morning P₄ measurement provided a reliable index of mid-luteal CL function following IVF treatment.

MATERIALS AND METHODS

Study Population

Ten female patients undergoing IVF/ICSI at the Fertility Clinic in Skive, Denmark, from December 2014 to December 2015 volunteered to participate in the study. Clinical information regarding age, body mass index (BMI), smoking habits, biochemical reproductive profile, cause of infertility, prior IVF attempts, course of stimulation, and laboratory results were recorded. Baseline characteristics of participants are provided in Table 1. Written informed consent was obtained from all patients prior to study participation. Participants were chosen so as to represent both the long GnRH agonist cycle as well the GnRH antagonist cycle and different types of triggering for final oocyte maturation (hCG or GnRH agonist).

Protocols for Ovarian Stimulation

Six patients were treated in a long GnRH agonist cycle with pituitary suppression using SC injection of Buserelin 0.8 mg (Suprefact®; Sanofi, Denmark) starting in the mid-luteal phase of the preceding cycle. A daily dose of 0.4 mg Buserelin was administered until the day before ovulation triggering. On day 2 of the cycle, a transvaginal ultrasound examination was carried out, and in case of an endometrial thickness < 4 mm, ovarian stimulation started with corifollitropin-alfa (Elonva®; MSD, Denmark) in combination with either r-FSH/rLH (Pergoveris®; Merck Biopharma, Denmark) or hMG (Menopur®, Ferring Pharmaceuticals, Denmark). The gonadotropin dosage was...
| Patient | Age (years) | Body mass index (kg/m²) | Basal FSH (IU/l) | Cause of infertility | Protocol | Total FSH sum (IU) | Duration of FSH stimulation (days) | Ovulation trigger | Luteal phase support | No. of follicles > 14 mm | No. of oocytes | No. of MII | Mid-luteal P₄ (nmol/l) |
|---------|-------------|--------------------------|-----------------|---------------------|----------|-------------------|-------------------------------|-----------------|---------------------|---------------------|--------------|-----------|---------------------|
| 1       | 38          | 24.4                     | 6.5             | Male factor         | Long GnRHa | 3,225             | 11                            | hCG 10,000 IU   | Lutinus 300 mg daily | 5                  | 5            | 1        | 89                  |
| 2       | 37          | 22.8                     | 6.6             | No male partner/   | GnRH antagonist | 1,725             | 9                            | Suprefact 0.5 mg | Lutinus 300 mg daily + 1,500 hCG (OPU) + 1,000 hCG (OPU + 5) | 10              | 11          | 10      | 283                |
| 3       | 39          | 30.0                     | 6.4             | No male partner    | Long GnRHa   | 3,450             | 12                            | hCG 10,000 IU   | Lutinus 300 mg daily | 14              | 12           | 12      | 277                |
| 4       | 34          | 22.2                     | 14.7            | Unexplained        | Long GnRHa   | 3,600             | 12                            | hCG 10,000 IU   | Lutinus 300 mg daily | 9                | 8            | 8       | 213                |
| 5       | 28          | 19.9                     | 5.9             | Male factor        | Long GnRHa   | 2,925             | 11                            | hCG 10,000 IU   | Lutinus 300 mg daily | 9                | 6            | 3       | 97                 |
| 6       | 37          | 25.3                     | 8.3             | Unexplained        | Long GnRHa   | 3,600             | 12                            | Ovitrelle 6,500 IU | Lutinus 300 mg daily | 11              | 8            | 8       | 376                |
| 7       | 28          | 31.8                     | 3.9             | Endometriosis      | GnRH antagonist | 2,025             | 9                            | Suprefact 0.5 mg | Crinone 180 daily + 1,500 hCG (OPU) | 17              | 11           | 11      | 36                 |
| 8       | 36          | 25.6                     | 4.9             | Tubal factor       | GnRH antagonist | 1,913             | 9                            | Suprefact 0.5 mg | Crinone 180 daily + 1,500 hCG (OPU) | 19              | 13           | 13      | 55                 |
| 9       | 40          | 34.5                     | 7.2             | Unexplained        | Long GnRHa   | 2,250             | 9                            | hCG 10,000 IU   | Lutinus 300 mg daily | 7                | 6            | 6       | 54                 |
| 10      | 36          | 29.4                     | 7.2             | Male factor        | GnRH antagonist | 3,938             | 15                            | Suprefact 0.5 mg | Lutinus 300 mg daily + 1,000 hCG (OPU) + 500 (OPU + 5) | 20              | 14           | 8      | 161                |

Mid-luteal P₄ = median progesterone level (nmol/l) 7 days after oocyte retrieval.  
hCG, human chorionic gonadotropin; OPU, oocyte pick-up.
determined individually based on patient age, BMI, baseline FSH, previous response to gonadotropins, and antral follicle count and adjusted by monitoring follicular size by transvaginal ultrasound during treatment. Final oocyte maturation was induced with either hCG 10,000 IU SC (Pregnyl®, MSD, Denmark) or 6,500 IU SC (Ovitrelle®, Merck Biopharma, Denmark) when two or more leading follicles reached a mean diameter of 17 mm. Oocyte retrieval was carried out 36 h after hCG administration. IVF/ICSI procedures and embryo culture were performed according to normal clinical practice. A maximum of two embryos were transferred on day 3 or day 5 after oocyte retrieval. Luteal phase support was given as vaginal micronized P4 (Lutinus® 300 mg daily, Ferring Pharmaceutical, Denmark) or Crinone® 180 mg daily, Merck Biopharma, Denmark) starting 1 day after oocyte pick-up (OPU).

In four patients the GnRH antagonist protocol was used. On day 2 of the cycle ovarian stimulation commenced with either r-FSH (Gonal-F®; Merck Biopharma, Denmark) or hMG (Menopur®, Ferring Pharmaceuticals, Denmark) after a vaginal ultrasound examination. Daily GnRH antagonist co-treatment (Orgalutran® 0.25 mg/day, MSD, Denmark) was added at a follicle size of 12 mm. The FSH dose was individually adjusted according to the ovarian response. Final oocyte maturation was induced with SC Buserelin 0.5 mg (Suprefact®; Sanofi, Denmark) as soon as two or more follicles of ≥17 mm were present. Oocyte retrieval was carried out 36 h later. A maximum of two embryos were transferred on day 3 or day 5 after OPU. Luteal phase support was given in an individualized regimen consisting of vaginal administration of 300 mg micronized P4 daily (Lutinus®, Ferring Pharmaceuticals, Denmark) in combination with a bolus of hCG (1,000–1,500 IU) on the day of oocyte retrieval (5, 10). Based on the individual ovarian response to stimulation, some patients received an additional hCG bolus on OPU + 5 (500–1,000 IU) (10). See Table 1 for details. Vaginal P4 administration continued until the day of pregnancy testing (hCG trigger) or until seventh gestational week (GnRHa trigger).

### Blood Sampling

Blood sampling was conducted during the mid-luteal phase, i.e., 7 days after OPU (OPU + 7). Patients were admitted to the fertility unit early in the morning and stayed at the clinic for the subsequent 12 h. The starting time for blood sampling was between 6 a.m. and 9 a.m. for all patients. Participants were allowed normal daily life activities during the study period.

An intravenous cannula was inserted into a vein in the antecubital fossa and blood samples (4 ml) were drawn every 60 min for 12 h (n = 10) and for two of these hours every 15 min (n = 8 because of difficult venous access in two patients). After coagulation at room temperature, blood samples were centrifuged and serum was isolated and stored at −80°C until analysis.

### Hormone Measurement

Serum P4 (nmol/l), E2 (pmol/l), and LH (IU/l) concentrations were measured using automated electro chemiluminescent immunoassays (Cobas® Modular analytics E170, Roche Diagnostics, Switzerland) routinely used for analysis at Department of Biochemistry, Viborg Regional Hospital, Denmark. All measurements were performed according to manufacturer’s instructions using a commercially available chemiluminescent immunoassay kit intended for measurements in serum.

The detection limit of hormones was 0.2 nmol/l, 18.4 pmol/l, and 0.1 IU/l for P4, E2, and LH, respectively. All serum samples from each patient were measured within the same assay run. All hormone concentrations above the assay detection limit were measured in duplicate. The intra-assay coefficients of variation for P4, E2, and LH were all below 4%.

**FIGURE 1** | Individual mid-luteal serum profiles of progesterone over a 12-h interval in 10 patients undergoing controlled ovarian stimulation for in vitro fertilization treatment.
Statistics
Data are presented as mean ± SD or median and range when appropriate. The maximum absolute variation (MAV) in serum P4 over a 12-h period is given as the maximum P4 concentration – minimum P4 concentration during the time of sampling for each patient.
Spearman’s correlation coefficient (r) was calculated to correlate median steroid levels with the maximum absolute hormone variation during the day (MAV). A p value < 0.05 was considered to be statistically significant. All analyses were performed using STATA, version 13.

Ethics
The study was conducted according to the declaration of Helsinki for Medical Research and approved by the local Ethics Committee of the Central Denmark Region. ClinicalTrials.gov registration number NCT02673034.

RESULTS

Patient Characteristics
Patients had a mean age of 35.3 ± 4.2 years, mean BMI of 26.6 ± 4.7 kg/m² and 1.9 ± 2.0 prior IVF attempts. Median level of FSH for all patients was 6.55 UI/l (interquartile range 5.9;7.2 IU/l). All participants were non-smokers. In four patients the cause of infertility was non-female (male factor or no male partner), in three patients the cause was female (tubal factor or endometriosis) and in three patients the cause of infertility was idiopathic (unexplained). See Table 1 for details.

Overall Mid-Luteal Progesterone Values
Three patients had median mid-luteal P4 levels below 60 nmol/l. Two of these patients (#7 and #8) were triggered with a GnRH agonist and received luteal phase support with 1,500 IU hCG (OPU) and vaginal P4 (Crinone 180 mg daily). Despite having 19 and 17 follicles ≥ 14 mm and 11–13 mature oocytes retrieved at the day of OPU they presented with a mid-luteal P4 of only 55 and 36 nmol/l, respectively. The other patient (#9) with P4 < 60 nmol/l was triggered with 10,000 IU hCG and had seven follicles > 14 mm at the day of aspiration.

Overall Mid-Luteal LH Values
None of the patients downregulated in a long GnRH agonist protocol (n = 6) had s-LH levels above the detection limit of the assay at any point during measurement (i.e., LH < 0.1 IU/l). In three of the four patients stimulated in the GnRH antagonist protocol a modest LH pulse activity was seen with LH amplitudes ranging from 0.2 to 2.8 UI/l. In all patients, the LH peak was followed by an increase in serum P4, ranging from 4 to 36 nmol/l.

Daytime Variation in Serum Progesterone
As seen during the natural cycle, large fluctuations in mid-luteal P4 were also present during daytime in some of the women undergoing IVF treatment (Figure 1). Fluctuations in luteal steroids were seen independent of the choice of stimulation protocol, the mode of final oocyte maturation and the type of luteal phase support.

The largest variation in P4 levels was seen in patients with median P4 > 250 nmol/l. In patient #2 with a median P4 of 283 nmol/l, P4 fluctuated from 293 nmol/l at 11 a.m. to 448 nmol/l at 12 p.m.—i.e., an increase of 155 nmol/l within 1 h. This fluctuation in P4 level was present even though s-LH was under the detection level throughout the day (Figure 2A). The increase in P4 was accompanied by a comparable increase in E2 (Figure 2A). Serum P4 concentrations during the 12-h period for that specific patient ranged from 183 nmol/l early in the morning to 448 nmol/l during the day—thus, a MAV during the study period of Δ265 nmol/l. In patient #6 (median P4 376 nmol/l) and #3 (median P4 277 nmol/l) a rapid elevation of P4 levels (Δ 70–75 nmol/l, respectively) was seen within a period of only 15 min without any concomitant LH activity (LH < 0.1 IU/l). In comparison, patient #7 had a median P4 of only 36 nmol/l and showed only minor fluctuations throughout the day with P4 levels ranging from 25 to 48 nmol/l following a small detectable increase in LH secretion (see Table 2 for complete daytime P4 values).

There was a positive correlation between median P4 levels and MAV in P4 during daytime (Spearman’s r = 0.9273, p = 0.0001).
### Table 2: Mid-luteal serum progesterone concentrations during daytime in 10 women undergoing in vitro fertilization treatment.

| Patient | Median $P_4$ (range) |
|---------|----------------------|
| 1       | 8.00 a.m. 9.00 a.m. 9.15 a.m. 9.30 a.m. 9.45 a.m. 10.00 a.m. 11.00 a.m. 12.00 p.m. 13.00 p.m. 14.00 p.m. 15 p.m. 16 p.m. 17 p.m. 17.15 p.m. 17.30 p.m. 17.45 p.m. 18 p.m. 19 p.m. 20 p.m. 21 p.m. 22 p.m. 23 p.m. | 126 106 91 93 88 97 95 99 107 102 89 72 78 73 67 63 89 (63–126) |
| 2       | 8.00 a.m. 9.00 a.m. 10.00 a.m. 10.15 a.m. 10.30 a.m. 10.45 a.m. 11.00 a.m. 12.00 p.m. 13.00 p.m. 14.00 p.m. 15 p.m. 16 p.m. 17 p.m. 17.15 p.m. 17.30 p.m. 17.45 p.m. 18 p.m. 19 p.m. 20 p.m. 21 p.m. 22 p.m. 23 p.m. | 183 224 280 277 266 283 293 300 448 249 248 244 240 235 232 283 (183–448) |
| 3       | 6.00 a.m. 7.00 a.m. 7.15 a.m. 7.30 a.m. 7.45 a.m. 8.00 a.m. 9.00 a.m. 10.00 a.m. 11.00 a.m. 12.00 p.m. 13.00 p.m. 14.00 p.m. 15 p.m. 16 p.m. 17 p.m. 17.15 p.m. 17.30 p.m. 17.45 p.m. 18 p.m. 19 p.m. 20 p.m. 21 p.m. 22 p.m. 23 p.m. | 370 320 323 301 272 347 281 244 235 301 249 248 240 232 294 283 (183–440) |
| 4       | 8.00 a.m. 8.15 a.m. 8.30 a.m. 8.45 a.m. 9.00 a.m. 10.00 a.m. 11.00 a.m. 12.00 p.m. 13.00 p.m. 14.00 p.m. 15 p.m. 16 p.m. 17 p.m. 17.15 p.m. 17.30 p.m. 17.45 p.m. 18 p.m. 19 p.m. 20 p.m. 21 p.m. 22 p.m. 23 p.m. | 216 228 224 228 226 226 220 202 240 238 230 225 225 222 283 262 (183–440) |
| 5       | 9.00 a.m. 10.00 a.m. 11.00 a.m. 12.00 a.m. 13.00 a.m. 14.00 a.m. 15.00 a.m. 16.00 a.m. 17.00 a.m. 18.00 a.m. 19.00 a.m. 20.00 a.m. 21.00 a.m. 22.00 a.m. 23.00 a.m. 24.00 a.m. 25.00 a.m. 26.00 a.m. 27.00 a.m. 28.00 a.m. 29.00 a.m. | 131 122 110 119 136 97 92 89 70 77 97 (70–136) |
| 6       | 7.00 a.m. 8.00 a.m. 9.00 a.m. 10.00 a.m. 11.00 a.m. 11.15 a.m. 11.30 a.m. 11.45 a.m. 12.00 p.m. 13.00 p.m. 14.00 p.m. 15.00 p.m. 16.00 p.m. 17.00 p.m. 18.00 p.m. 19.00 p.m. 20.00 p.m. 21.00 p.m. 22.00 p.m. 23.00 p.m. 24.00 p.m. | 416 296 311 299 365 314 312 312 382 413 414 376 (275–440) |
| 7       | 7.00 a.m. 8.00 a.m. 9.00 a.m. 10.00 a.m. 11.00 a.m. 11.15 a.m. 11.30 a.m. 11.45 a.m. 12.00 p.m. 13.00 p.m. 14.00 p.m. 15.00 p.m. 16.00 p.m. 17.00 p.m. 18.00 p.m. 19.00 p.m. 20.00 p.m. 21.00 p.m. 22.00 p.m. 23.00 p.m. 24.00 p.m. | 27 25 32 36 40 39 36 34 29 29 36 (25–48) |
| 8       | 7.00 a.m. 8.00 a.m. 9.00 a.m. 9.15 a.m. 9.30 a.m. 9.45 a.m. 10.00 a.m. 11.00 a.m. 12.00 a.m. 13.00 a.m. 14.00 a.m. 15.00 p.m. 16.00 p.m. 16.15 p.m. 16.30 p.m. 16.45 p.m. 17.00 p.m. 18.00 p.m. 19.00 p.m. 20.00 p.m. 21.00 p.m. 22.00 p.m. 23.00 p.m. | 51 55 50 50 50 49 50 53 59 59 55 (49–62) |
| 9       | 7.00 a.m. 8.00 a.m. 9.00 a.m. 10.00 a.m. 11.00 a.m. 11.15 a.m. 11.30 a.m. 11.45 a.m. 12.00 a.m. 13.00 a.m. 14.00 a.m. 15.00 p.m. 16.00 p.m. 16.15 p.m. 16.30 p.m. 16.45 p.m. 17.00 p.m. 18.00 p.m. 19.00 p.m. 20.00 p.m. 21.00 p.m. 22.00 p.m. 23.00 p.m. | 65 64 63 68 59 60 59 62 56 56 54 (51–65) |
| 10      | 7.00 a.m. 8.00 a.m. 8.15 a.m. 8.30 a.m. 8.45 a.m. 9.00 a.m. 10.00 a.m. 10.15 a.m. 10.30 a.m. 10.45 a.m. 11.00 a.m. 12.00 p.m. 13.00 p.m. 14.00 p.m. 15.00 p.m. 16.00 p.m. 17.00 p.m. 18.00 p.m. 19.00 p.m. 20.00 p.m. 21.00 p.m. 22.00 p.m. 23.00 p.m. | 185 178 187 182 181 180 112 108 117 124 161 (108–189) |

$\Delta P_4$ = individual maximum absolute variation in $P_4$ during daytime = maximum $P_4$ – minimum $P_4$ concentration (nmol/l).

$P_4$ SI conversion factor: nmol/l = 3.18*ng/ml.

The magnitude of $P_4$ pulses and thus the maximum variation is dependent on the median mid-luteal $P_4$ concentration (Figure 3). In patients with median $P_4 > 250$ nmol/l, very large fluctuations in serum $P_4$ were seen during daytime with a median MAV of 165 nmol/l (range 145–265 nmol/l). Patients with median $P_4$ between 89 and 213 nmol/l had median MAV of 68 nmol/l (range 63–81 nmol/l), whereas patients with very low mid-luteal $P_4$ levels (median $P_4 < 60$ nmol/l) had fairly constant serum levels.
P₄ levels throughout the day (median MAV 14 nmol/l, range 13–23 nmol/l).

There was no common general daytime rhythm for P₄ in the 10 women examined, suggesting that the luteal phase is patient specific. Some patients had their highest hormone levels in the morning—others peaked during the day or in the early evening (see Figure 1). The time of P₄ acrophase (zenith) and P₄ nadir was before noon in half of the patients and after noon in the other half of patients (Figure 4).

**Daytime Variation in Serum Estradiol**

Large fluctuations in mid-luteal serum E₂ were also seen during the 12-h sampling time. In patient #2, E₂ increased from 3,480 to 4,664 pmol/l in 1 h (Δ1,184 nmol/l) (Figure 2B). Patients had individual maximum E₂ variations (Max E₂ − Min E₂) over 12 h ranging from Δ404 to Δ1,481 pmol/l. There was no correlation between median E₂ levels and MAV in mid-luteal E₂ (Spearman’s r = 0.4424, p = 0.20).

As expected, P₄ and E₂ seem to be co-secreted from the CL showing similar patterns of fluctuations over time (Figure 5). Patients with median P₄ < 60 nmol/l had E₂ ranging from 541 to 1,552 pmol/l (median E₂ 1,457 pmol/l) whereas patients with median P₄ between 89 and 213 nmol/l had E₂ levels from 659 to 4,884 pmol/l (median E₂ 2,843 pmol/l). In patients with median P₄ > 250 nmol/l, E₂ ranged from 3,471 to 3,919 pmol/l (median E₂ 3,874 pmol/l). There was a significant correlation between median P₄ levels and median E₂ levels during mid-luteal phase of the stimulated cycle (Spearman’s r = 0.8424, p = 0.002).

**DISCUSSION**

To the best of our knowledge, this is the first study to explore a possible daytime variation in P₄ secretion during the mid-luteal phase in a group of women undergoing IVF treatment.

We found that the magnitude of mid-luteal P₄ fluctuations following IVF treatment was dependent on the median P₄ level. The largest P₄ variations were seen in patients with median P₄ exceeding 250 nmol/l (median MAV 165 nmol/l), whereas patients in the low P₄ group (median P₄ < 60 nmol/l) had relatively constant P₄ levels throughout the day (median MAV 14 nmol/l). Patients showed a highly individual hormone secretion pattern without any obvious common daytime rhythm in P₄ secretion. Serum E₂ showed similar fluctuations in the mid-luteal phase with patients having individual E₂ variations ranging from Δ404 to Δ1,481 pmol/l during the 12-h study time.

Earlier studies described the highly variable pattern of P₄ secretion during the mid-luteal phase of naturally cycling women (15, 19, 20). These studies reported the presence of two distinguishable types of luteal P₄ pulses—some preceded by a LH pulse and others non-concomitant to LH seen during time of pituitary quiescence. The latter seems to be the result of an autonomous P₄ secretion from the CL, triggered and maintained by intraovarian concentrations of E₂, oxytocin, and PGF₂α (21, 22). The CL consists of two types of steroidogenic cells, i.e., the small luteal cells (SLCs) derived from follicular theca cells and the large luteal cells (LLCs) originating from follicular granulosa cells. Both the small and the large cells have extensive capacity to produce P₄. Moreover, both cells have unique steroidogenic functions and the “two-cell” mechanism of E₂ biosynthesis appear to operate in the human CL analogous to the preovulatory follicle (23). Thus, the LLCs contain P450-aromatase essential for E₂ synthesis whereas SLCs express P450c17 for androgen production (24, 25). Both types of luteal cells express E₂ receptors (26) and E₂ stimulation is a powerful trigger of P₄ release from either cell type (27).

Isolation of large and SLCs from human corpora lutea has shown that once induced by the LH peak, the LLCs exhibit the greatest basal P₄ production (28) and that this production is not increased by further LH stimulation (29). The LLCs produce P₄ at a constant rate and are the dominant source of P₄ during the
During the natural cycle both late follicular E₂ levels, follicular diameter at the time of ovulation as well as area under the LH surge curve correlate poorly to the subsequent luteal phase P₄ level (3). Thus, predicting patients with insufficient luteal P₄ levels is troublesome based on the follicular development as abnormal luteal phases can be seen in cycles characterized by normal folliculogenesis (35). In the present study, the two patients with the lowest P₄ levels (36 and 55 nmol/l) had 17 and 19 follicles, respectively, on the day of OPU, showing that a large number of CLs do not warrant secretion, but this effect is overridden by the concomitantly triggered increase in E₂ which will elicit a pronounced P₄ release. In this way, the LH pulse will stimulate an intra-luteal circuit involving auto-and paracrine effects of E₂, oxytocin, PGF₂α—and possible a variety of other regulatory peptides, i.e., Substance P—and the net effect is the generation of a P₄ pulse. This circuit functions for hours without further gonadotropic support, thus generating several P₄ pulses with gradually decreasing amplitude until the next LH pulse sets off the intra-luteal E₂/P₄ loop again. In contrast, in women with hypothalamic deficiency with suppressed LH levels and no LH pulses, mid-luteal P₄ shows a non-pulsatile pattern, underlining the need for an initial high LH/hCG load to trigger the P₄ circuit (21). The oxytocin induced P₄ release can be prevented by treatment of the CL with tamoxifen—an estrogen receptor blocking agent—underlining the E₂ regulation of the autonomous P₄ pulses (27). This independent intra-luteal P₄ pulse generator might serve as an additional biological safety mechanism preventing declining P₄ levels in between LH pulses and might explain the function of the substantial E₂ production during the luteal phase in humans.

In the stimulated IVF cycle, LH pulses are absent during the mid-luteal phase and serum LH levels are distinctly suppressed (33). The hCG bolus administered for ovulation induction or as luteal phase support exerts a tonic and constant stimulation on the luteal tissue due to the prolonged half-life of hCG and, therefore, cannot account for the rapid P₄ fluctuations seen during the mid-luteal phase in this study. The standard vaginal P₄ supplementation reaches steady state during the early luteal phase and contributes with remarkably constant serum P₄ levels though out the day despite multiple daily vaginal doses (34). The very large fluctuations in serum P₄ seen in the present study are, therefore, likely to be the result of the autonomous intraovarian P₄ circuit. This is further emphasized by the fact that P₄ peaks are accompanied by concomitant E₂ rises and exogenous E₂ was not provided as part of the luteal phase support.

We were not able to detect a common general pattern of P₄ secretion during daytime in the 10 patients examined. The peak and nadir P₄ levels occurred at different times in different patients, and the course of hormone levels during the day showed highly individual rhythms. This is in agreement with studies performed during the mid-luteal phase of the natural cycle (3, 19). In a study of seven women studied over 24 h in the mid-luteal phase of the natural cycle, the P₄ acrophase varied from 10.31 a.m. to 11.33 p.m. (16). Based on the lack of a diurnal reproducible pattern for mid-luteal P₄ in the IVF cycle the accuracy of the P₄ measurement is not improved by a fixed timing of blood sampling and, thus, the P₄ measurement could be performed at any time during clinic opening hours.

FIGURE 5 (A) Daytime variation in mid-luteal s-progesterone and s-estradiol in patient #10. Median P₄ = 161 nmol/l, median E₂ = 3,606 pmol/l. (B) Daytime variation in mid-luteal s-progesterone and s-estradiol in patient #1. Median P₄ = 89 nmol/l, median E₂ = 659 pmol/l.
a high $P_4$ output in the luteal phase. For this reason monitoring of luteal phase $P_4$ could be of value to detect patients with low $P_4$ levels, who might benefit from additional exogenous $P_4$ therapy. However, the prerequisite for easy luteal phase monitoring is that the validity of a single measured $P_4$ value is reliable and gives a reasonable estimate of the CL capacity of the patient.

We acknowledge that the small sample size of this study may limit the validity of general interpretations. However, we consider this explorative preliminary study to be pioneering as part of basic research and, importantly, it is the first to explore the mid-luteal $P_4$ fluctuations in different types of IVF cycles. The autonomous LH-independent $P_4$ bursts from the ovaries during the mid-luteal phase were seen in both GnRH analog types (GnRH antagonist and long GnRHa protocol) as well as after different types of triggering of final oocyte maturation (hCG or GnRH agonist). Thus, it seems that these autonomous episodic luteal $P_4$ peaks are generated independently of the choice of treatment regimen and may, therefore, also apply to other IVF stimulation protocols.

**CONCLUSION**

Based on the 10 women examined in this study, we state that the accuracy of a single mid-luteal serum progesterone measurement as an approximation of mean $P_4$ levels throughout the day depends on the $P_4$ concentration and that women with low $P_4$ levels ($P_4 < 60$ nmol/l) exhibit clinically stable $P_4$ levels during daytime. Thus, a single $P_4$ measurement in the low progesterone patient reflects quite accurately the CL function and a measured low $P_4$ value can, therefore, be regarded as a “true low value.” Future studies should clarify, whether additional exogenous $P_4$ support administered to the low luteal $P_4$ patient group can improve the reproductive outcome.

**DATA AVAILABILITY**

The raw data supporting the conclusion of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

**ETHICS STATEMENT**

The study was conducted according to the declaration of Helsinki for Medical Research and approved by the local Ethics Committee of the Central Denmark Region. All patients provided written informed consent to participate in the study.

**AUTHOR CONTRIBUTIONS**

LT designed and conducted the study. LT drafted the manuscript and UK, CA, and PH all contributed to the interpretation of data and critically reviewed the manuscript. All coauthors accepted the final draft.

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