

Progesterone and Luteinizing hormone secretion patterns in early pregnant gilts

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Abstract

We studied luteinizing hormone (LH) pulsatility and episodic progesterone release of the corpus luteum (CL) on Day 11 and Day 21 in inseminated gilts and aimed to establish a relationship between these two hormones. Blood was collected at 15-min intervals for 12 hr on Days 11, 16 and 21 from a *vena cava caudalis* catheter. At euthanasia, eight gilts were pregnant and six gilts were not pregnant. Progesterone parameters (basal, mean, pulse frequency and pulse amplitude) did not differ between pregnant and non-pregnant gilts on Day 11, LH pulse frequency and amplitude tended to differ ($p = .07$ and $p = .079$). In pregnant gilts, basal and mean progesterone, progesterone pulse amplitude and frequency declined significantly from Day 11 to Day 21 ($p < .05$). A significant decline was also seen in the LH pulse amplitude from Day 11 to Day 21 ($p < .05$). None of the LH pulses was followed by a progesterone pulse within 1 hr on Day 21. On Day 11 and Day 21 appeared a synchronicity in the LH pulse pattern, as there were two or three LH pulses in 12 hr and these LH pulses appeared in the same time window. We conclude that on Day 11 and Day 21 of pregnancy in gilts, progesterone pulses do not follow an LH pulse within one hour. Further we demonstrated that the successful or not successful formation of a CL of pregnancy is independent of progesterone release on Day 11 after insemination. We confirmed the decline of progesterone from Day 11 to Day 21 in the *vena cava caudalis* and could demonstrate that this decline is partly due to lower progesterone pulse amplitude and frequency and that the decline occurs simultaneously with a decline in LH pulse amplitude.

KEYWORDS

Corpus luteum, early pregnancy, gilt, luteinizing hormone, progesterone

1 | INTRODUCTION

Different hormones along the hypothalamo–pituitary–gonadal (HPG) axis orchestrate sexual function. In the pig, LH secretion supports corpus luteum (CL) function during pregnancy (Peltoniemi, Easton,

Love, Klupiec, & Evans, 1995; Tast, Love, Clarke, & Evans, 2000) and the CL is the main source of progesterone throughout gestation (Anderson, 1967; Bazer & Johnson, 2014). Progesterone at the ending of the HPG axis is released in an episodic manner (Jarry et al., 1990; Parvizi, Elsaesser, Smidt, & Ellendorf, 1976). The veins

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from the ovaries, oviduct and uterus drain blood via the utero-ovarian vein into the *vena cava caudalis*; progesterone content in the *vena cava caudalis* is the result of ovarian progesterone release and of the remaining progesterone after oviductal and uterine uptake.

Virolainen, Love, Tast, and Peltoniemi (2005) and Hoving et al. (2017) demonstrated that progesterone measured peripherally in the *vena jugularis* does not reveal the same pulsatile pattern as measured locally in the *vena cava caudalis*. Progesterone is metabolized in the liver; therefore, its systemic concentration in the *vena jugularis* is lower (Hoving et al., 2017; Prime and Symonds, 1993) and growing evidence suggests that ovarian progesterone production is best studied in blood samples taken locally, near the production site (Athorn et al., 2013).

Transformation of the cyclic CL into the CL of pregnancy between Day 11 and Day 21 maintains progesterone secretion (Bazer & Johnson, 2014; Geisert et al., 2006) and is necessary for the successful embryonic phase. The decline in peripheral progesterone concentration during this transformation period (King & Rajamahendran, 1988; Pharaszyn, Foxcroft, & Aherne, 1991; Tast et al., 2000) was partly explained with the increasing need of the growing embryos (Magness, Reynolds, & Ford, 1986), while Ziecik, Krzymowska, and Tilton (1982) documented the decline of mean LH from Day 12 to Day 24. Hypophysectomy (Anderson, Dyck, Mori, Henricks, & Melampy, 1967) and administration of LH antiserum (Spies, Slyter, & Quadri, 1967) demonstrated that the CL can work without LH support up to Day 12 of the oestrus cycle. After Day 12 of pregnancy, the use of a GnRH-agonist (Peltoniemi et al., 1995), immunization against GnRH (Tast et al., 2000) and GnRH-antagonist treatments (Virolainen, Love, Tast, & Peltoniemi, 2004; Virolainen, Love, et al., 2004) causing an LH decline longer than 48 hr eventually interrupted CL function. However, it is questionable if the CL is fully autonomous until Day 12 as it was demonstrated that passive immunization of the non-pregnant gilt with porcine anti-LH antibodies decreased progesterone concentrations already at Day 8 of the oestrous cycle (Szafranska & Ziecik, 1989). In vitro progesterone secretion was induced by LH in slices of non-pregnant gilts' CLs collected on Days 10–12 (Przygodzka, Lopinska, & Ziecik, 2014) and stimulated from Day 3 to Day 14 of the oestrous cycle (Astiz, Gonzalez-Bulnes, Perez-Solana, Sanchez-Sanchez, & Torres-Rovira, 2013; Tekpetey & Armstrong, 1991). Indications for a direct relationship of progesterone secretion to LH release in vivo were found in sows as early as Day 14 (Hoving et al., 2017) and on Day 21 in one gilt (Virolainen et al., 2005). On the other hand, Brussow, Schneider, Wollenhaupt, and Tuchscherer (2011) did not find such a relationship on Day 11, 13, 15 or 17 in gilts and Haen, Heinonen, Kauffold, et al. (2019) demonstrated on Day 16 and Day 21 that progesterone release is pulsatile even in the absence of LH pulses.

Previous studies that investigated a possible dependence of progesterone production on pituitary support in the period between Day 11 and Day 21 of pregnancy have thus far focused on exploring local progesterone secretion by means of feeding (Athorn et al., 2013; Hoving et al., 2017; Langendijk, Bouwman, Chen, Koopmanschap, & Soede, 2017) or external hormonal stimulation (Brussow et al., 2011; Haen, Heinonen, Kauffold, et al., 2019). Tracking the variation in the

local progesterone pattern and LH and the properties of these two hormones towards each other while aiming to establish a relationship was not carried out before.

We hypothesized that the CLs' pulsatile progesterone secretion is independent of pulsatile LH stimulation on Day 11 of pregnancy. We aimed to study the functioning of the CLs' progesterone secretion and investigated whether an LH decline occurs simultaneously with a progesterone decline between Day 11 and Day 21. Therefore, we studied in the *vena cava caudalis* both hormones LH and progesterone and investigated whether their mean and basal level and their pulse frequency and amplitude are related. Finally, we hypothesized that on Day 21, the progesterone secretion of the LH-dependent CLs is reactive to LH stimulation and that progesterone pulses follow LH pulses.

2 | MATERIALS AND METHODS

2.1 | Ethical permission

The study protocol and all experimental procedures were approved by the Animal Experiment Board ELLA in Finland (permission ESLH-2009-06207/Ym-23).

2.2 | Animals and management

Altogether 14 cross-bred (Finnish Yorkshire x Landrace) gilts aged 6 to 8 months were brought to the trial unit from two commercial farms. The gilts were either selected gilts ($n = 4$) for reproduction or fattening gilts ($n = 10$). The study was conducted in the following three batches: January to February ($n = 3$) (daylight about 7 hr), April to May ($n = 6$) (daylight about 15 hr) and October to November ($n = 5$) (daylight about 10 hr). Daylight through windows was the main source of light. Artificial light was applied only during feeding times and during sampling days.

After arrival at the trial unit, the gilts were weighed (125 ± 12 kg) and kept in single pens separated by iron bars. The pens were at least 7 m² and had a concrete solid floor covered with a mixture of straw and sawdust. The gilts were fed twice daily (0800 and 1430) with a commercial diet of 1.6 kg (Tiineys Pekoni[®], Suomen Rehu, 13 MJ/kg, 14.5% crude protein and 7.4 g lysine), and they had ad libitum access to water. In addition, they received two handfuls of straw daily. The animals were allowed to move freely. Daily oestrous detection was performed from arrival onwards. After all gilts were detected in oestrus, the subsequent oestrus was synchronized using an oral progestagen (altrenogest, Regumate[®], Janssen, 20 mg/gilt/day) for 18 days and a subcutaneous injection of 200 I.U. hCG and 400 I.U. PMSG (Suigonan[®] vet, MSD Animal Health) 48 hr after the last oral progestagen administration.

Oestrous detection was performed twice a day (0900 and 1600) by assessment of a standing response in the presence of a mature boar. Gilts were inseminated artificially (AI) every 12 hr during standing oestrus with a commercial dose of semen (Finnish Animal

Breeding Association, FABAs) containing 3×10^9 sperm cells. AI doses were stored at 18°C and used within 4 days of collection. We considered the day of last insemination as Day 1 of pregnancy.

We inserted an indwelling catheter in the *vena cava caudalis* of the gilts on Day 10 and collected blood samples on Day 11 ± 1, 16 ± 1 and 21 ± 1. We euthanized the gilts between Days 22 and 30 after insemination using pentobarbital intravenously. Their reproductive tracts were recovered, and uteri were cut longitudinally to determine their pregnancy state. The gilts were classified to pregnant ($n = 8$) and non-pregnant ($n = 6$) in retrospect, based on post-mortem findings. If the gilt was pregnant, embryos in the uteri and CLs on the ovaries were counted.

2.3 | *Vena cava caudalis* catheterization and blood sampling

On Day 10, an intravenous catheter (PVC tubing, 1.0 mm i.d., 1.5 mm o.d., Sterihealth Laboratory Products Pty LTD, Australia) was placed into the *vena cava caudalis* of the gilts.

Gilts were sedated with azaperone (i.m., 4 mg/kg, Stresnil®; Janssen Animal Health), and anaesthesia was induced with an i.m. injection of detomidine hydrochloride (0.08 mg/kg, Domosedan®; Orion Ltd), ketamine (10 mg/kg, Ketaminol®; Merck, Animal Health) and butorphanol (0.2 mg/kg, Torbugesic®; Scan Vet) (Heinonen, Raekallio, Oliviero, Ahokas, & Peltoniemi, 2009).

We followed the procedure of the catheterization as described in Haen, Heinonen, Kauffold, et al. (2019). Between sampling days, we flushed the catheters once a day with heparin NaCl solution (1 ml of 5,000 IU heparin mixed with 100 ml NaCl 0.9%). On the sampling days, 2 ml of blood was withdrawn and discarded before collection of a 5-ml blood sample, which was collected into lithium-heparin tubes every 15 min from 0700 to 1900 (total up to 49 blood samples per gilt during one sampling day). Catheters were flushed after each sample collection with heparin NaCl solution. After collection, the samples were centrifuged within 1 hr and the extracted plasma was stored at -20°C until analysis.

2.4 | Hormone analysis

All blood samples taken on Day 11 and on Day 21 were analysed for LH and progesterone. In addition, the blood samples taken on Day 16 were analysed for progesterone.

2.4.1 | Progesterone

Progesterone concentrations were analysed using a commercial radioimmunoassay (RIA) (Spectria, Orion Diagnostica) validated to measure progesterone in pig plasma (Peltoniemi et al., 1995). Fifty microlitres of plasma sample and 500 µl of buffered ^{125}I label were added into antibody-coated tubes. After vortexing, tubes were incubated at room

temperature for 2 hr. Supernatant was decanted, and tubes were left standing upside down for 5 min. Each tube was counted for 1 min in a γ counter (Wallac, LKB-Wallac, Turku, Finland). The sensitivity of the assay was <0.09 ng/ml, and intra-assay coefficients of variation (CV) were 10.0% and 13.1% and interassay CV were 5.1% and 14.3% for low and high progesterone concentrations, respectively.

2.4.2 | Luteinizing hormone

LH concentrations were analysed in duplicates using a homologous double-antibody RIA according to the method described by Cosgrove, Booth, and Foxcroft (1991) with some modifications as described by Hoving, Soede, Feitsma, and Kemp (2012). Porcine LH was supplied by the National Hormone and Peptide Program (NHPP, NIDDK, Dr Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA). The used assay is reliable for LH concentrations between 0.012 ng/ml and 6.25 ng/ml, and the intra- and interassay CV were 7.0% ($n = 73$) and 6.5% ($n = 15$), respectively.

2.5 | Hormone profile characteristics

For both LH and progesterone, mean levels, basal levels and number and amplitude of pulses were determined. Mean level was defined as the average concentration of all samples. Basal level was defined as the mean of the eight samples with the lowest hormone concentration.

A progesterone pulse started when at least two consecutive samples exceeded the basal concentration by more than three standard deviations and subsequent values above this threshold belonged to the same pulse (Haen, Heinonen, Kauffold, et al., 2019).

An LH pulse started when LH levels exceeded the basal concentration by more than two standard deviations (Langendijk et al., 2017; Tast et al., 2000). Pulse amplitude was reached within two samples from the previous nadir, and there were at least two samples between the pulse amplitude and the return to basal level or the next nadir.

For both LH and progesterone, the pulse amplitude was the difference between the maximum pulse value minus basal level. Mean pulse amplitude was the average of the pulse amplitudes for each sow on each sampling day. Further, a progesterone pulse following an LH pulse within 1 hr was regarded as a response to the LH pulse (Hoving et al., 2017).

When all the LH pulses of pregnant gilts were plotted against time of day, it seemed that LH pulses of the different gilts appeared in specific time windows. The time windows during which a maximum LH pulse level occurred in the pregnant gilts and which were separated by 1 hr without maximum LH pulse levels were defined as pulse periods.

2.6 | Statistical analyses

Statistical tests were conducted using IBM SPSS statistics v25. All variables were checked for normal distribution. Outcome variables

were progesterone basal concentration, mean concentration and number and amplitudes of pulses on Days 11, 16 and 21. For LH, the outcome variables were basal concentration, mean concentration, and number and amplitudes of pulses on Day 11 and Day 21. These outcome variables were not normally distributed and were therefore log-transformed. An initial univariable screening was performed to identify potential confounders of the outcome variables. The main explanatory variable was pregnancy (Yes/No), and other explanatory variables were batch and gilt origin (fattening pig/selected gilt). Possible correlations between each of these explanatory variables were explored using a chi-squared test. Possible correlations between each explanatory and each outcome variable were explored using a univariate analysis of variance (ANOVA). Neither of the explanatory variables was affected by batch or gilt origin and was omitted from the models. A general linear model for each LH and progesterone variable was built for data obtained at Day 11 of pregnancy with pregnancy as a fixed effect.

A general linear model for repeated measures was applied to compare the outcome variables within pregnant gilts. The within-subject factor was the 3 (progesterone) or 2 (LH) sampling days. Differences between Day 11 and Day 21 LH outcomes and Day 11, Day 16 and Day 21 progesterone outcomes were evaluated with a pairwise *t* test. Possible relationships between LH and progesterone outcome variables and number of CL and embryos were examined using Pearson's correlation coefficient.

3 | RESULTS

3.1 | Pregnancy status

Out of the 14 inseminated gilts, 8 were pregnant and 6 were not pregnant at euthanasia (pregnancy rate of 57.14%).

The pregnant gilts had an average of 16.25 ± 5.0 (12 – 25) (mean \pm SD [range]) CL and 14.0 ± 4.75 (11–25) embryos, resulting in an embryonic survival rate of 89 ± 24 (44–100).

3.2 | Hormones

Due to difficulties with the catheters in the 8 pregnant gilts, complete sampling could be performed in 5, 6 and 8 gilts on the different sampling days (Table 1).

3.2.1 | Day 11 pregnant versus. non-pregnant gilts

No significant differences were found in basal and mean LH concentration on Day 11 between pregnant and non-pregnant gilts, although non-pregnant gilts tended to have a lower LH pulse frequency (1.17 ± 1.33 versus 2.30 ± 0.51 ; $p = .07$) and mean LH pulse amplitude (0.72 ± 0.92 versus 1.78 ± 0.57 ; $p = .079$) than pregnant gilts (Table 1).

On Day 11, mean and basal progesterone concentrations as well as pulse frequency and pulse amplitude did not differ statistically between pregnant and non-pregnant gilts (Table 1).

3.2.2 | Pregnant gilts, LH and progesterone

The mean and basal LH concentration and pulse frequency of pregnant sows did not change between Day 11 and Day 21 of pregnancy, but the pulse amplitude decreased from Day 11 to Day 21 (Table 1). Basal and mean progesterone concentrations declined, and this decline was significant on Day 11 and 16 to Day 21 (Table 1 and Figure 1). Progesterone pulse frequency did not differ between Day 11 and Day 16 but declined significantly by Day 21. The amplitude of

	Day 11		Day 16	Day 21
	pregnant	non-pregnant	pregnant	pregnant
LH (ng/ml)	<i>n</i> = 6	<i>n</i> = 6		<i>n</i> = 6
Basal	0.35 ± 0.07	0.42 ± 0.17		0.37 ± 0.08
Mean	0.64 ± 0.14	0.61 ± 0.23		0.63 ± 0.14
pulse frequency/12 hr	2.30 ± 0.51^x	1.17 ± 1.33^y		2.67 ± 0.52
Pulse amplitude	1.78 ± 0.57^{xa}	0.72 ± 0.92^y		1.50 ± 0.32^b
P4 (ng/ml)	<i>n</i> = 8	<i>n</i> = 6	<i>n</i> = 5	<i>n</i> = 6
Basal	18.53 ± 1.94^a	19.23 ± 6.08	17.16 ± 5.25^a	12.57 ± 3.28^b
Mean	24.87 ± 4.70^a	24.92 ± 8.14	20.19 ± 4.86^a	13.70 ± 3.04^b
Pulse frequency/12 hr	4.00 ± 1.85^a	3.67 ± 2.81	3.00 ± 1.23^a	2.17 ± 1.33^b
Pulse amplitude	46.10 ± 12.60^a	40.00 ± 8.00	37.90 ± 6.00^{ab}	21.90 ± 12.90^b

TABLE 1 Basal and mean concentrations, pulse frequency and pulse amplitude of LH on Days 11 and 21 and progesterone on Days 11, 16 and 21 after insemination. Data are presented as mean \pm SD

^{ab}Difference between days in pregnant animals, $p \leq .05$.

^{xy}Tendency for a difference between pregnant and non-pregnant animals ($p < .10$).

FIGURE 1 Mean (dashed lined) and basal (dotted line) progesterone concentration (ng/ml) on Day 11 ($n = 8$), Day 16 ($n = 5$) and Day 21 ($n = 6$) of pregnancy, assessed using a 12-hr sampling period with sampling intervals of 15 min. Data are presented as means \pm SD. Means of mean and basal with a different data label differ significantly ($p \leq .05$)

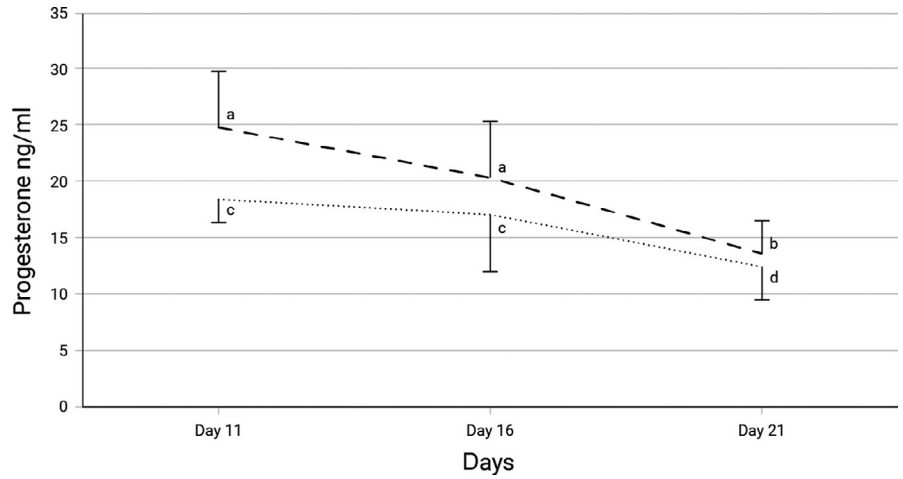
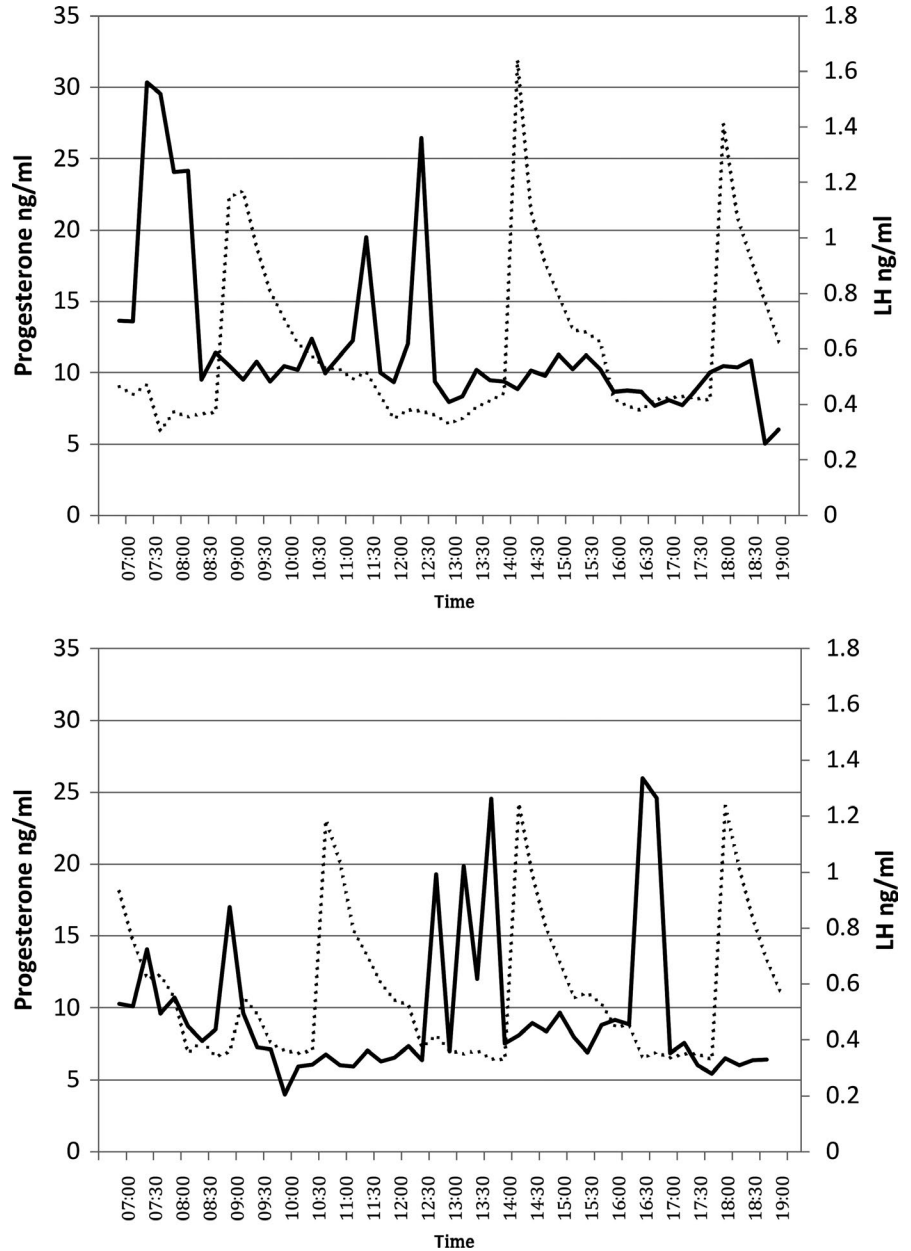


FIGURE 2 Progesterone (solid line) and LH (dotted line) of a 12-hr sampling period in two representative gilts on Day 21 of pregnancy. Progesterone pulsatility is seen when LH secretion is basal



these pulses significantly decreased from Day 11 to Day 16 and from Day 16 to Day 21 (Table 1).

On Day 11, three of the 14 LH pulses (21.4%) were followed by a progesterone pulse within 1 hr and nine out of 21 progesterone pulses (42.9%) were followed by an LH pulse.

On Day 21, none of the 14 LH pulses were followed by a progesterone pulse within 1 hr and eight out of 13 (61.5%) progesterone pulses were followed by an LH pulse.

On Day 21, in 4 of the 6 gilts a specific pattern in LH and progesterone secretion was observed, in which pulsatile secretion of one hormone was present, while pulsatile secretion of the other hormone was absent (Figure 2).

3.2.3 | Pregnant gilts, LH synchronicity pattern

The gilts either had three ($n = 7$) or two ($n = 5$) LH pulses over 12 hr. When gilts had three pulses, these pulses fell into three distinctive periods (between 0730 hr to 1,030 hr, 1215 hr to 1515 hr and 1630 hr to 1830 hr) (Figure 3a). On average, one pulse period lasted 113 ± 38 min. When gilts had two LH pulses, they fell into periods between 1000 hr to 1200 hr and 1530 hr to 1845 hr (Figure 3b). On average, their pulse period lasted 79 ± 62 min.

3.3 | Pregnant gilts, correlations of LH and progesterone with embryo and CL numbers

The number of CL or embryos was not related to any of the LH variables on Day 11 or Day 21. The number of CL was negatively correlated with mean progesterone concentration ($r = -0.78$, $p = .02$), number of progesterone pulses ($r = -0.80$, $p = .02$), progesterone pulse amplitude ($r = -0.85$, $p = .007$) on Day 11 and with the progesterone pulse frequency on Day 16 ($r = -0.90$, $p = .04$). There was no correlation between number of CL with any of the progesterone variables on Day 21.

On Day 21, the number of embryos was negatively correlated with progesterone pulse frequency ($r = -0.84$, $p = .04$) and positively with progesterone pulse amplitude ($r = 0.93$, $p = .02$).

4 | DISCUSSION

We accepted our hypothesis that pulsatile progesterone release before maternal recognition is independent of pulsatile LH stimulus. On Day 21 as none of the progesterone pulses was reactive to LH pulses, we observed that progesterone release appeared to occur when pituitary LH release was basal. Therefore, our hypothesis that progesterone pulses are stimulated by LH pulses within one hour on Day 21 (after the establishment of pregnancy) was refuted.

Unexpectedly, we found that episodic LH release appeared in pulse periods that occurred synchronously among the gilts, both on Day 11 and on Day 21 of pregnancy.

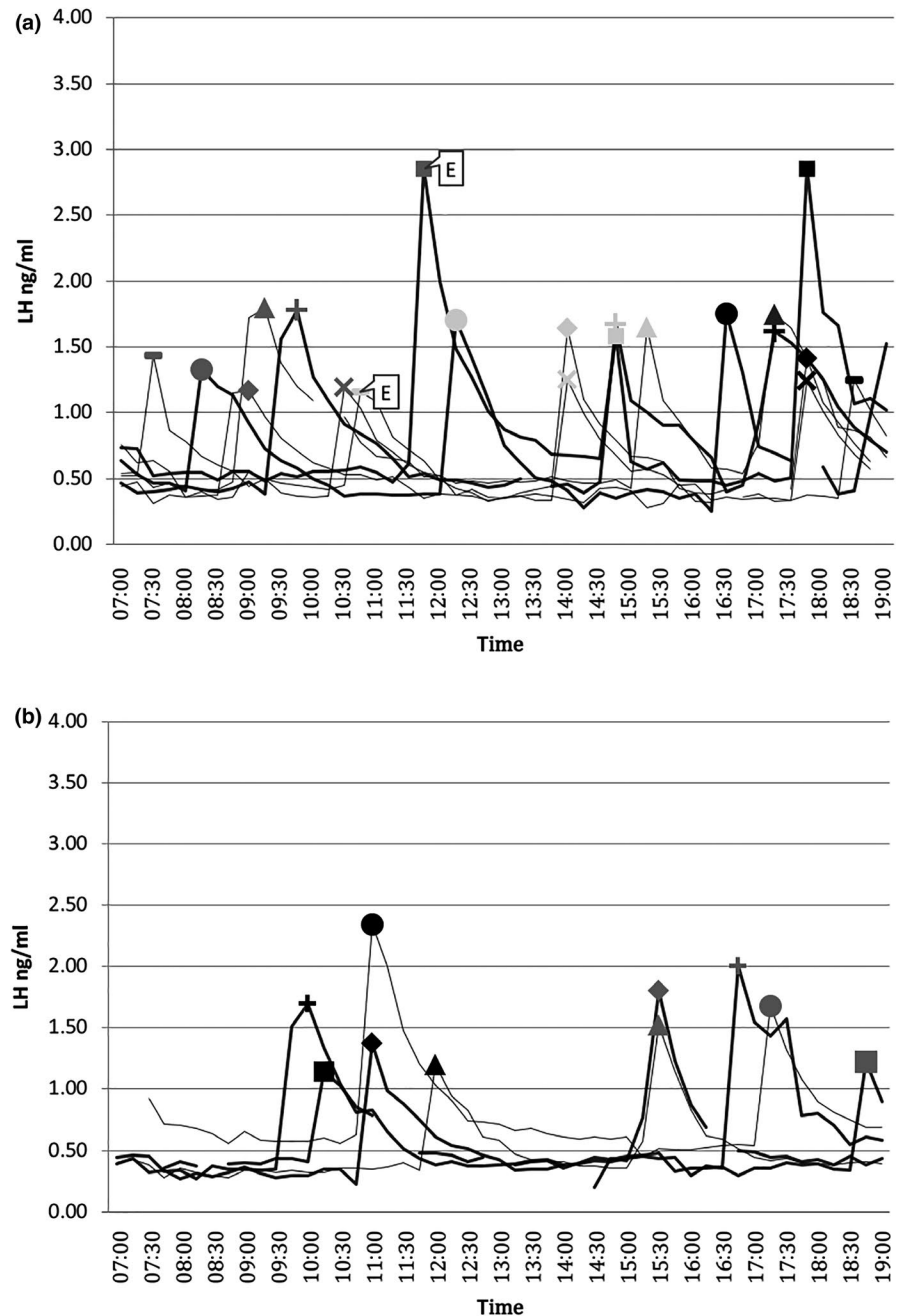
We confirmed a decline in both basal and mean *vena cava caudalis* progesterone values from Day 11 to Day 16 to Day 21 of pregnancy and could demonstrate that causative for this decline is the regressing progesterone pulse frequency and amplitude. A decline was also observed in studies that measured systemic progesterone (Ford & Christenson, 1979; King & Rajamahendran, 1988; Pharazyn et al., 1991) and is consistent with the decline in CL size after Days 12 to 13 to about Day 20 of pregnancy in the pig (Langendijk & Peltoniemi, 2013). Progesterone concentration measured in a single blood sample per sampling day up to Day 16 of gestation was higher in veins draining the ovary and oviduct than in veins draining the uterine venous system, whereas no difference was found between progesterone concentration in the *vena jugularis* and veins draining the uterus (Pharazyn et al., 1991). The high progesterone in the ovarian and oviductal venous drainage might be taken up by the uterus and the embryos (Kephart, Hagen, Griel, & Mashaly, 1981; Magness et al., 1986). Stefańczyk-Krzyszowska, Grzegorzewski, Wąsowska, Skipor, and Krzymowski (1998) demonstrated in gilts from Day 12 to Day 35 of pregnancy that progesterone concentration in the arterial blood supply of the oviduct and the uterus was significantly higher than in the jugular vein. Progesterone pulses most probably do not survive the uterine passage (Hoving et al., 2017; Pharazyn et al., 1991), and the decline in progesterone pulse amplitude and frequency seen in the current study might be related directly to the ageing CLs' secretory capacity (Gregoraszczyk, 1992). Supportive for this idea is the observation that from Day 11 to 21 of pregnancy only LH pulse amplitude decreased in the current study, but no decline in mean and basal LH or LH pulse frequency could be observed. Therefore, the reason for the decline in progesterone could be sought in the CLs or CLs downstream and not in a decreasing LH stimulation.

Reason for this decline might be the inhibitory effect of PGF2 alpha and oxytocin on the CL on Days 12–14 (Wuttke, Theiling, Hinney, & Pitzel, 1998) or a change in LH receptor density (Gebarowska, Ziecik, & Gregoraszczyk, 1997; Phoophitphong, Srisuwatanasagul, & Tummaruk, 2017).

We found significant negative correlations between number of CL and progesterone parameters (mean progesterone, progesterone pulses and progesterone amplitude) on Day 11 and Day 16 (progesterone pulse frequency) and on Day 21 between progesterone pulse frequency and number of embryos (negative correlation) and progesterone pulse amplitude and number of embryos (positive correlation). Athorn et al. (2011) showed the importance of local progesterone supply for embryo survival. Thus, whether positive or negative, the aforementioned correlations in the current study could indicate that the CL secretion is reactive to an embryonic and uterine need of progesterone through an unknown communication process. This communication might only take place locally and not on the hypothalamic–hypophyseal–gonadal axis, excluding LH.

In the current study, *vena cava caudalis* progesterone was low on all sampling days (basal from 18.53 ± 1.94 to 12.57 ± 3.28 ng/ml and mean from 24.87 ± 4.70 to 13.70 ± 3.04 ng/ml) in comparison with

FIGURE 3 Figure 1a, b presents LH profiles of gilts after classifying according to the number of LH pulses. LH frequency of three (a) and two pulses (b) in a 12-hr (0700–1900) sampling period on Day 11 (thick lines) and Day 21 (thin lines) of pregnancy in gilts. Same amplitude marker is given for the same gilt. Pulses labelled with an E are considered outliers in the described synchronicity pattern. a, LH frequency of seven gilts having three pulses in 12 hr; first pulse period: grey markers, second pulse period: light grey markers, third pulse period: black markers. b, LH frequency of five gilts having two pulses in 12 hr; first pulse period: black markers, second pulse period: grey markers



other studies (Brussow et al., 2011, Day 11 to Day 17; Langendijk et al., 2017, Day 11). Reasons might be found in the radioimmunoassay for progesterone (Lawrenz et al., 2018) but also breed can affect both peripheral (Wettemann, Johnson, & Omtvedt, 1980) and local (Brussow, Schneider, Tuchscherer, Egerszegi, & Ratky, 2008) progesterone concentration. Lower progesterone concentrations may also be due to feeding, but results are controversial (Athorn et al., 2013; Langendijk et al., 2017).

Furthermore, housing and environmental factors may also play a role, although we do not know to what extent environmental factors influence progesterone secretion. The gilts in our study did not need to fight for their hierarchy in the group while being in a spacious and enriched environment that allowed social and physical activity. In other studies that investigated local progesterone, animals were

commonly housed in individual stalls (Athorn et al., 2013; Hoving et al., 2017; Langendijk et al., 2017; Virolainen et al., 2005). Although group housing did not interfere with peripheral progesterone concentrations or embryonal survival (Tsuma et al., 1996), mimicking stress by means of ACTH injection led to an increase in serum progesterone concentration in cyclic gilts (Brandt, Lundeheim, Madej, Rodriguez-Martinez, & Einarsson, 2007) and in the first and second trimester in pregnant gilts (Brussow, Schneider, Kanitz, Otten, & Tuchscherer, 2005). Whether the low progesterone in the current study might account for the high number of non-pregnant gilts is unclear. Progesterone parameters in non-pregnant and pregnant gilts were similar on Day 11. We were interested to see whether there might be indications in the CLs' progesterone secretion before maternal recognition of pregnancy whether a pregnancy is

established or not. We could not find such indications. At the same time, the LH parameters of the non-pregnant group tended to differ from the pregnant group. We conclude on the basis of our results that the CLs' progesterone secretion on Day 11 is independent of LH stimulation and independent of the pregnancy status. However, due to the limited number of gilts in the non-pregnant group and their unclear pregnancy status on Day 11, this conclusion is rather weak.

On Day 11, CLs' pulsatile progesterone secretion was not reactive to pulsatile LH stimulation within 1 hr.

Also on Day 21, we did not observe progesterone pulses responding to LH pulses within 1 hr. This observation is consistent with the results of Haen, Heinonen, Kauffold, et al. (2019). Although LH pulsatility on Day 21 was abolished by means of a long-acting GnRH agonist implant, progesterone pulses were still apparent.

It is unlikely that progesterone pulses play a role in the progesterone feedback on LH. Unless progesterone pulses are mediated to the pituitary gland through an unknown mechanism, they do not pass through the metabolism process in the liver (Hoving et al., 2017). Therefore, LH pulses most probably cannot be reactive to progesterone pulses. However, we observed that CL progesterone secretion was active when LH concentration was at a basal level on Day 21 and that LH surged when progesterone secretion was basal (Figure 2). It is possible that the current study's definition of one progesterone pulse being reactive to one LH pulse within 1 hr (Hoving et al., 2017) needs to be reconsidered.

Since this study indicates that progesterone pulses are secreted independently of LH pulses, it remains unclear why progesterone secretion is pulsatile. Whether the CLs are a homogenous population such that an individual CL reflects the characteristics of its cohorts (Ottobre, Eyster, & Stouffer, 1984) or not (Rao & Edgerton, 1984) is unclear. Further research is warranted to understand functioning of pulsatile progesterone release.

Interestingly, the LH pulses of the pregnant gilts appeared synchronized. The time window when LH pulses surged was 113 ± 38 or 79 ± 62 min in gilts with a pulse frequency of 3 and 2 per 12 hr, respectively. These surges thus exhibit synchronicity among gilts (Figure 3a, b).

External stimulators acting on the GnRH-LH pathway usually generate frequency and amplitude. Melatonin as the connective link between photoperiod and hypothalamus (Tast et al., 2001), metabolic cues transmitted via leptin or insulin (Barb, Hausman, & Lents, 2008) and sustained elevation of cortisol (Turner, Hemsworth, Canny, & Tilbrook, 1999) act on frequency and amplitude of LH pulses. It is a plausible explanation that a group of individuals experiencing the same external stimulators synchronizes its LH-release pattern as seen in the group of the pregnant gilts. One stable repeating external factor during this study was the timing of feeding and the associated joint activity and rest. It might be that this circadian rhythm was the pacemaker for the synchronized LH release.

Although LH levels tended to differ on Day 11 progesterone secretion did not differ between gilts that were pregnant and gilts that were not pregnant at euthanasia in our study. Furthermore, pulsatile progesterone secretion declined from Days 11 and 21 of pregnancy.

However, in the same period, the LH secretion did not decline and a relationship between LH pulsatility and an episodic release of progesterone could not be demonstrated. Therefore, although it has been established that LH is vital to CL survival, pulsatile progesterone release by the CL seems to be independent of LH pulses from Day 11 to Day 21 of pregnancy in the gilt.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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