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Influence of progesterone concentrations on secretory functions of trophoblast and pituitary during the first trimester of pregnancy in dairy cattle

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Abstract

The essential role played by progesterone in the maintenance of pregnancy is unequivocal; however, the effects of progesterone on the secretory patterns of placental and pituitary molecules during the gestation period are not well defined. The objective of this study was to describe pregnancy-associated glycoprotein (PAG) concentrations (measured by RIA-497 and RIA-Pool) in pregnant females with progesterone concentrations lower (low-P4 group, $n = 20$) or higher (high-P4 group, $n = 17$) than the mean of 8.74 ng/mL on Day 21 (AI = Day 0). Luteinizing hormone (LH) and prolactin concentrations were also measured in both groups. Throughout the study period, blood samples were collected on Days 0, 21, 45, 60, and 80 from 37 females that were confirmed to be pregnant. PAG concentrations measured by both RIA-497 and RIA-Pool tended to be higher in high-P4 group than in low-P4 group from Day 30 until Day 80. On Day 80, plasma PAG concentrations that were measured using RIA-497 were observed to be higher ($P < 0.05$) in the high-P4 group than in the low-P4 group (10.2 ± 8.7 ng/mL versus 6.9 ± 3.8 ng/mL). Concentrations of LH on Day 60 and prolactin on Day 80 were observed to be significantly lower ($P < 0.05$) in the high-P4 group. There was a tendency for the concentrations of LH (Days 45 and 80) and prolactin (Days 30, 45, and 60) to be lower in cows in the high-P4 group than in the low-P4 group. Our results suggest the existence of a relationship among the concentration levels of progesterone, PAG, LH, and prolactin during early pregnancy.

Keywords: Pregnancy-associated glycoprotein; Progesterone; Radioimmunoassay; Dairy cattle; Early pregnancy

1. Introduction

During the bovine estrous cycle, luteinizing hormone (LH) is secreted at low levels except for the large preovulatory surge. This surge stimulates follicle

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swelling, ovulation, and formation of the corpus luteum (CL). Also, LH is the principal hormone that stimulates the production of progesterone by the CL [1]. As reviewed by Niswender et al. [2] and Milvae et al. [3], the mechanism whereby LH stimulates the secretion of progesterone involves the formation of cAMP and the activation of protein kinase A system, which in turn phosphorylates the enzymes involved in steroidogenesis and enhances the transportation of cholesterol from the cytoplasm to the inner mitochondrial membrane of small luteal cells. After the transportation of cholesterol to the mitochondrial matrix, the cleavage of the side chain of cholesterol to produce pregnenolone and the conversion of pregnenolone to progesterone can be initiated.

In the nonpregnant cattle, endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) initiates the luteolytic process between Days 15 and 17 after ovulation [4]. In contrast, in the presence of a viable conceptus, the secretion of trophoblastic proteins prevents the pulsatile release of $PGF_{2\alpha}$ by the endometrium [5,6] and facilitates luteal maintenance [7]. Bovine interferon tau is the trophoblast secretory glycoprotein, which is believed to be the antiluteolytic molecule in the cow. It is synthesized by the mononucleate cells of trophectoderm and exclusively secreted at the uterine lumen [8,9]. Although an increase in mRNA concentration of bovine interferon tau has been found to be detectable from Days 9 to 10 of pregnancy [10], large-scale production is restricted to Days 17–19 when the conceptus is undergoing rapid expansion into an elongated form [11]. A number of studies also reported that higher maternal progesterone concentrations might provide a more suitable uterine environment for the developing embryo, resulting in conceptus that is larger and produces more trophoblast proteins such as interferon tau [12–14]. However, these studies failed to follow in parallel the luteal and trophoblast secretory functions over a relatively long period but instead focused on a single interferon tau measurement in uterine flushes after the slaughter of pregnant cows.

Other families of trophoblast glycoproteins are expressed by the bovine conceptus at the same time or even before the expression of interferon tau [15]. Among them are the pregnancy-associated glycoproteins (PAGs), also known as pregnancy-specific protein (PSPB) or pregnancy-serum protein 60 kDa (PSP60). The PAGs are a complex, placenta-expressed family with cDNA coding for 22 distinct bovine PAG molecules (boPAG-1 to boPAG-22) in the mononucleate and binucleate cells of the trophectoderm [16–19]. However, in contrast to interferon tau, at least some boPAG molecules can reach the maternal circulation, and these are being used for pregnancy diagnosis and follow-up of placental secretory function using RIA and ELISA techniques [20–23].

PAGs are detectable in the maternal circulation from Days 24 to 25 after fertilization [24], which increases gradually until Day 240, and then more dramatically during the few days before delivery [25,26]. PAG concentrations have been directly correlated to the placental mass [26–28], which in turn is related to the stage of pregnancy [29,30]. The influence of both maternal environment (breed of the recipient) and embryonic origin (genetic makeup of the embryo and use of nuclear transfer techniques) on PAG concentrations was also shown by Zoli et al. [25] and Chavatte-Palmer et al. [23]. However, these studies did not report on the possible influence of progesterone levels on maternal PAG concentration in early pregnancy period.

Whether luteal function (progesterone concentration) is related to trophoblast secretory properties (PAG secretion) during embryonic and early fetal development remains to be elucidated. Therefore, the objective of this study was to investigate whether progesterone concentrations at early pregnancy (Day 21) could influence the secretion of PAG and affect concentrations of the pituitary hormones, LH, and prolactin in dairy cattle.

2. Materials and methods

2.1. Animals

This study was conducted from February to June 2004 in Kabylie, Algeria (36°43′N, 5°04′W). The experimental protocol was approved by the Faculty Council of the University, Abderahmane Mira (Bejaia, Algeria). Blood sampling of the Friesian Holstein females was carried out following the rules of good veterinary practice under farm conditions.

For this study, 154-Holstein Friesian dairy females were examined for signs of estrus and were artificially inseminated 8–14 h after the detection of estrus. The age of the females were between 17 months and 10 years with mixed parity (0–8). The body condition score (BCS) was determined during the bleeding period using a 5-point scale [31]. The lactation number of the cow was also recorded. The day of artificial insemination (AI) was considered as Day 0.

The first pregnancy diagnosis was conducted on Day 30 after AI by routine laboratory analysis of PAG concentrations (RIA-497) [25]. The threshold of 0.8 ng/mL was used to discriminate between pregnant and nonpregnant cows [21,32]. The pregnancies of females were confirmed by rectal palpation approximately at 2–3

months. Only the samples of those females that were confirmed as pregnant by the PAG-RIA and rectal investigations were analyzed over the whole sampling period.

2.2. Blood sampling

Blood samples were collected on Days 0, 21, 30, 45, 60, and 80. Samples (7.0 mL) collected from the coccygeal vein were transferred into a tube containing EDTA (Sarstedt®, Numbrecht, Germany). The plasma was obtained by centrifugation (1500 × *g* at 15 min) immediately after collection and was stored at −20 °C until the assay was performed.

2.3. Progesterone radioimmunoassay

Progesterone concentrations were determined in plasma using a direct method (without extraction), as described previously in detail [33,34]. The estimated doses (ED) at 20, 50, and 80% of B/B_0 (tracer bound/tracer bound in the zero standard) (mean ± S.D.) were 15.3 ± 4.1, 2.1 ± 0.5, and 0.3 ± 0.2 ng/mL, respectively. The minimum detection limit (MDL) was 0.15 ng/mL. The intra- and interassay coefficients of variation of the progesterone RIA were 8.5% and 9.4%, respectively.

2.4. PAG radioimmunoassays

The measurement of plasma PAG concentrations was carried out by two distinct RIA systems differing in their antisera, as described previously [35]. Antiserum R#497 was raised against a pure bovine $PAG_{67\ kDa}$ preparation (boPAG-1; [36]) and was used as the primary antibody in RIA-497. A mixture of R#497, R#706 (raised against caprine $PAG_{55\ kDa+62\ kDa}$; [37]), R#780 (raised against ovine $PAG_{57\ kDa+59\ kDa}$; [38]), and R#809 (raised against $ovPAG_{55\ kDa}$; [39]) constituted the antiserum named Pool [35]. For RIA-Pool, the aforementioned antisera were mixed in the following proportions: AS#497: one part; AS#706: one part; AS#780: two parts and AS#809: two parts. The first antibody dilutions were 1:200,000 and 1:64,000 for RIA-497 and RIA-Pool, respectively. Pure $boPAG_{67\ kDa}$ was used as standard (0.2–25 ng/mL) and tracer (≈25,000 cpm) for both the RIA-497 and RIA-Pool PAG assays. Iodination (Na-I^{125}, Amersham Pharmacia Biotech, Uppsala, Sweden) was carried out according to the Chloramine T method [40]. The MDL were 0.2 and 0.1 ng/mL for RIA-497 and RIA-Pool, respectively. The intra- and inter-assay coefficients of variation were 3.5 and 6.8% for RIA-497 and 4.6 and 17.2% for RIA-Pool. Samples with PAG concentrations over the estimated point to which the B/B_0 percentage corresponded to 20% (ED-20) were assayed again at a dilution of 1/2.

2.5. LH radioimmunoassay

Concentrations of LH were determined by the technique described previously by Ectors et al. [41] with some modifications. The LH preparation [42] used in the standard curve (0.2–25 ng/mL) and for iodination [43] was of porcine origin. The first antibody (L#34) was raised in rabbits against an ovine LH preparation (NIH-LH-S_{15}; NIH, Bethesda, MD, USA), as described by Vaitukaitis et al. [44]. This antibody was used at an initial dilution of 1:100,000. The double antibody precipitation system was composed of a mixture of sheep antirabbit immunoglobulin (0.83%, v/v), normal rabbit serum (0.17%, v/v), polyethylene glycol 6000 (20 mg/mL; Vel, Leuven, Belgium), cellulose microcrystalline (0.05 mg/mL; Merck, Darmstad, Germany), and BSA (2 mg/mL; ICN Biochemicals Inc., Aurora, OH) diluted in Tris–HCl buffer (0.025 M Tris and 0.01 M $MgCl_2$; pH 7.5).

In brief, 0.1 mL of each sample and standard dilution were aliquoted into duplicate assay tubes containing 0.2 and 0.1 mL of Tris–BSA buffer, respectively. To minimize the nonspecific interference of plasma proteins, 0.1 mL of LH-free plasma was added to all standard tubes. Following this, 0.1 mL of the diluted LH antiserum (L#34) was added and the tubes were incubated overnight at room temperature (20–23 °C). The following day, 0.1 mL of radiolabeled ^{125}I-LH (≈25,000 cpm) was added to all tubes and further incubated for 4 h before the addition of the double antibody precipitation system (1.0 mL). After 30 min incubation, 2.0 mL of Tris–BSA buffer was added to all except the total count (T_c) tubes. Bound and free LH were separated by centrifugation (1500 × *g* for 20 min, 4 °C). The supernatants were discarded and the radioactivity of the pellet was determined using a gamma counter with a counting efficiency of 75%. The MDL for LH-RIA was 0.25 ng/mL. The intra- and inter-assay coefficients of variation of LH radioimmunoassay were 3.9 and 4.4%, respectively.

2.6. Prolactin radioimmunoassay

A double antibody radioimmunoassay procedure was carried out according to the methodology described by Malven and McMurtry [45] and was used to measure plasma concentrations of prolactin in bovine samples. Bovine prolactin (NIH-B5 bPRL, NIH, Bethesda, MD,

USA) was diluted in assay buffer (Tris–BSA) and was used as standard (0.8–200 ng/mL) and tracer. The iodination (Na-I^{125}, Amersham Biosciences, Uppsala, Sweden) was carried out according to the Chloramine T method [40]. The first antiserum (R#144) was raised against ovine prolactin (NIH-S8 oPRL, NIH, Bethesda, MD, USA) and was used at an initial dilution of 1:100,000.

In brief, a volume of 50 μL of each sample and 0.1 mL of standard preparation were diluted in Tris–BSA buffer. Following this, 0.1 mL of radiolabeled prolactin (25,000 cpm) and 0.1 mL of the diluted antiserum (R#144) were added to all the tubes followed by 4 h incubation at room temperature (20–23 °C). As previously described for the LH assay, bound and free prolactins were separated after the addition of the double antibody precipitation system. The intra- and inter-assay coefficients of variation of prolactin radioimmunoassay were 7.4 and 12.3%, respectively, and the MDL was 0.3 ng/mL.

2.7. Data analysis

Holstein Friesian dairy cows and heifers used in the present investigation were selected on the basis of five main criteria: (1) progesterone concentrations below 1.0 ng/mL on the day of AI; (2) presence of a functional CL on Day 21 after AI, as determined by progesterone concentrations; (3) positive pregnancy diagnosis on Day 30 after AI, as determined by PAG concentrations; (4) confirmation of ongoing pregnancy by rectal palpation at around 2–3 months after AI; and (5) absence of embryonic/fetal mortality during the bleeding period (until Day 80 after AI), as determined by analysis of both progesterone and PAG profiles and rectal palpation. After all the analyses were completed, the data file from 37 out of 154 Holstein Friesian females was retained for the investigation of a potential effect of progesterone concentrations on Day 21 after AI on peripheral concentrations of PAG, progesterone, LH, and prolactin measured on Days 30, 45, 60, and 80 after AI.

First, concentrations of progesterone in confirmed pregnant females were classified into following two groups depending on the level of progesterone (P4) on Day 21: females with P4 concentrations higher than the mean of 8.74 ng/mL: high-P4 group, and females with P4 concentrations lower than the mean: low-P4 group.

Data from both groups were analyzed using a repeated measure design from the mixed procedure of SAS [46]. In each group (high P4 and low P4), progesterone levels on Day 21, sampling period (Days 30, 45, 60, and 80 after AI) and their interactions were considered as factors of variation for the different measured molecules (PAG measured by RIA-497 and by RIA-Pool, LH, and prolactin). The concentrations at the different sampling periods were compared with those of progesterone on Day 21. All statistical tests with a value of $P < 0.05$ were considered as significant. The results were expressed as mean ± S.D.

3. Results

After the completion of all analyses, 37 out of 154 Holstein Friesian females were retained for the study. Sixteen females (10.4%) were excluded because their progesterone concentrations were >1.0 ng/mL on Day 0, and 66 (42.8%) were excluded because their progesterone concentrations were <1.0 ng/mL on Day 21. Forty (26.0%) of the remaining 72 females were diagnosed to be pregnant on Day 30 (routine PAG RIA-497 diagnosis). Thirty-seven (24.0%) of 40 were confirmed to be pregnant by rectal palpation 2–3 months after AI. Three others were excluded because they suffered embryonic loss as demonstrated by PAG and progesterone analysis (data not shown). Mean BCS and lactation number in the 37-Friesian Holstein pregnant females were 2.3 ± 0.5 and 1.9 ± 1.3, respectively. With regard to the total population, a significant effect of both BCS and lactation number was observed on progesterone concentrations ($P < 0.05$). However, these parameters had no effect on PAG, LH, and prolactin levels.

Mean (±S.D.) concentrations of progesterone, PAG (measured by RIA-497 and RIA-Pool), LH, and prolactin in the 37-pregnant females are shown in Table 1. The progesterone concentrations increased ($P < 0.0001$) from Day 0 (0.2 ± 0.1 ng/mL) to Day 21 (8.7 ± 3.5 ng/mL), with a large range of individual concentrations (3.3–20.7 ng/mL) being observed on Day 21. After a slight decrease in progesterone concentrations on Day 45 ($P < 0.05$), they remained relatively constant until Day 80. Individual LH concentrations were highly variable on the day of AI, probably because of the presence of the preovulatory surge in some of the females. Mean LH concentrations decreased ($P < 0.05$), from Day 0 until Day 30 and remained relatively constant thereafter. Plasma concentrations of PAG measured by both RIA-497 and RIA-Pool remained below 0.7 ng/mL until Day 21. On Day 30, they were higher than the threshold of pregnancy diagnosis (0.8 ng/mL) in all pregnant females (higher than 0.9 and 1.7 ng/mL as measured by RIA-497 and RIA-Pool, respectively). From Day 30 to 80, PAG concentrations were higher ($P < 0.05$), when measured by RIA-Pool than by RIA-497. There

Table 1
Plasma concentrations of progesterone, PAG (determined by RIA-497 and RIA-Pool), LH, and prolactin obtained in 37 confirmed pregnant Holstein Friesian dairy cattle on Days 0 (Day of AI), 30, 45, 60 and 80

Day	Mean ± S.D. (ng/mL) (minimum-maximum)				
	Progesterone	PAG-497	PAG-Pool	LH	Prolactin
Day 0	0.2 ± 0.1 a (0.2–0.8)	0.2 ± 0.1 a (0.2–0.6)	0.1 ± 0.1 a (0.1–0.5)	6.2 ± 5.6 a (2.8–25.0)	29.9 ± 24.1 (4.4–107.6)
Day 21	8.7 ± 3.5 b (3.3–20.7)	0.2 ± 0.1 a (0.2–0.7)	0.2 ± 0.2 a (0.1–0.7)	4.0 ± 1.1 b (2.5–7.2)	23.2 ± 14.9 (5.4–53.8)
Day 30	8.4 ± 2.3 b (4.5–15.5)	1.8 ± 1.2 b (0.9–7.8)	4.7 ± 2.7 b (1.7–13.5)	3.4 ± 0.9 c (2.5–6.5)	20.1 ± 13.1 (5.3–48.2)
Day 45	6.7 ± 2.4 c (3.8–12.3)	3.1 ± 1.3 c (1.2–7.3)	5.2 ± 4.1 b (1.3–19.5)	3.3 ± 1.1 c (2.0–7.1)	22.1 ± 13.2 (7.1–50.0)
Day 60	7.3 ± 2.8 c (2.4–13.6)	4.8 ± 2.5 d (1.5–10.3)	8.0 ± 5.4 c (3.0–21.8)	3.2 ± 1.1 d (1.2–6.5)	26.2 ± 9.9 (8.5–43.0)
Day 80	7.1 ± 3.6 c (3.2–18.3)	8.8 ± 7.1 e (2.6–29.2)	18.0 ± 11.0 d (4.2–40.1)	3.5 ± 1.1 c,e (2.2–5.6)	25.0 ± 13.5 (6.7–49.8)

Different letters (a–e) in the same column indicate a significant difference of at least $P < 0.05$ in the concentrations measured at different days of pregnancy.

was no variation in the concentration of prolactin during pregnancy ($P = 0.46$).

Twenty animals were allotted to the group of females presenting progesterone concentrations lower than the mean (8.74 ng/mL) on Day 21 (low-P4 group; mean 6.3 ± 1.7 ng/mL, range 3.3–8.7 ng/mL), whereas 17 females were assigned to the other group because the concentrations was found to be higher than the mean (high-P4 group: 11.1 ± 3.8 ng/mL, range 8.9–20.7 ng/mL). In general, progesterone concentration on Day 21 had no effect on the plasma concentrations of progesterone, PAG, LH, and prolactin during the later stages of pregnancy. A significant effect of the interaction day of gestation and progesterone concentration on Day 21 was observed only for progesterone ($P < 0.0005$).

Taking into consideration the whole sampling period, concentrations of progesterone, PAG, LH, and prolactin did not differ between low- and high-P4 groups. When the data for the two different groups were compared, it was found that the mean progesterone concentrations were higher ($P < 0.0001$) in the high-P4 group than in the low-P4 group on Day 21, similar at Days 30 and 45 in both groups, then higher in the high-P4 group on Day 60 ($P < 0.001$) (Fig. 1). Plasma profiles of PAG were similar when measured by RIA-497 and RIA-Pool. Concentrations increased from Days 21 to 30 and tended to be higher in the high-P4 group than in the low-P4 group until Day 80. As shown in Fig. 2, plasma PAG concentrations

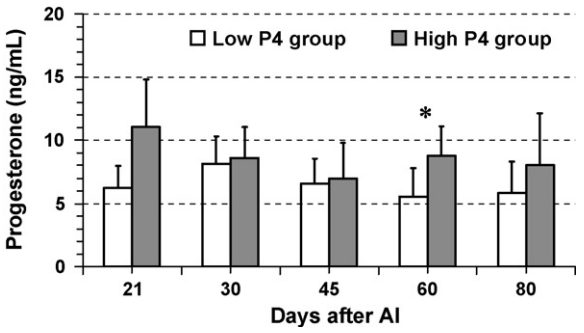

Fig. 1. Plasma concentrations (mean ± S.D.) of progesterone at different days after AI in females presenting low (low-P4 group: <8.74 ng/mL) and high progesterone concentrations on Day 21 after AI (high-P4 group: >8.74 ng/mL). A significant difference in mean concentrations between the low-P4 group and high-P4 group from Days 30 to 80 after AI is indicated by asterisk (*$P < 0.001$).

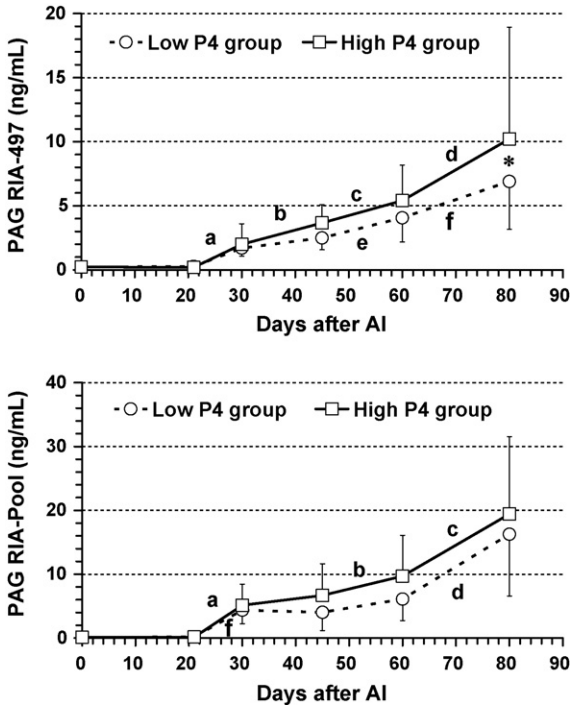

Fig. 2. Plasma concentrations (mean ± S.D.) of PAG as measured by RIA-497 and RIA-Pool at different days after AI in females presenting low and high progesterone concentrations at Day 21 after AI (low-P4 group and high-P4 group, respectively). Asterisk indicates a significant difference (*$P < 0.05$) in mean concentrations between the low-P4 group and high-P4 group. Significant differences in mean concentrations between two consecutive days in the same group are indicated by letters ($^{a,b,c,e}P < 0.05$; $^{f}P < 0.005$; $^{d}P < 0.0001$).

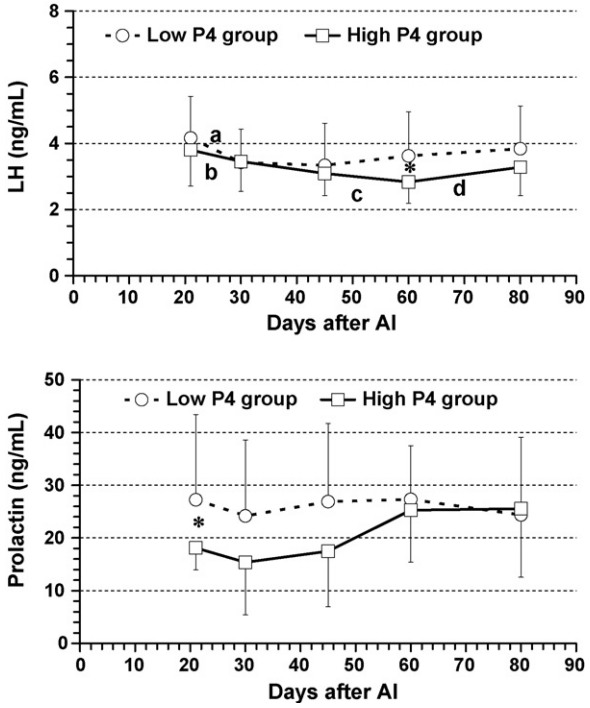

Fig. 3. Plasma concentrations (mean ± S.D.) of LH and prolactin at different days after AI in females presenting low and high progesterone concentrations on Day 21 after AI (low-P4 group and high-P4 group, respectively). Asterisk indicates a significant difference ($^{*}P < 0.05$) in mean concentrations between the low-P4 group and high-P4 group. A significant difference in mean concentrations between two consecutive days in the same group is indicated by letter ($^{a,b,c,d}P < 0.005$).

were higher ($P < 0.05$) in high-P4 group (10.2 ± 8.7 ng/mL) than in low-P4 group on Day 80 for RIA-497 (6.9 ± 3.8 ng/mL). Concentrations of LH from Days 21 to 45 and concentrations of prolactin on Days 60 and 80 were very similar between the groups (Fig. 3), whereas on the other days, there was a tendency of LH and prolactin concentrations to be lower in the high-P4 group than in the low-P4 group and prolactin concentrations on Day 21 were lower ($P < 0.05$) in high-P4 group than in low-P4 group (18.1 ± 4.2 ng/mL versus 27.2 ± 16.2 ng/mL).

4. Discussion

This study investigated whether the concentrations of progesterone during early pregnancy could be related to trophoblastic secretory function (as measured by two PAG-RIA systems), based on the classification of pregnant females as having relatively high or low progesterone concentrations than the mean on Day 21 of pregnancy (high-P4 group and low-P4 group, respectively). We demonstrated for the first time that PAG concentrations were higher in high-P4 group than in low-P4 group on Day 80 when measured with RIA-497, and tended to be higher at other times. This lack of significant difference in PAG concentrations at different time points appears to be possibly attributed to the high individual variation in PAG concentrations than to an inadequate model of evaluation of luteal–placental interactions. A high variability of individual PAG concentrations has been documented in the literature in both inseminated cows [24,25] and in recipients carrying somatic clones [23]. Further studies with a larger number of animals, a more discriminative classification according to progesterone concentrations (e.g. low-quartile-, middle-half-, and high-quartile groups) or even the use of progestagen implants at different pregnancy periods are envisaged in future.

The progesterone measured in our study would have been produced by the CL, because CL is the major source of progesterone during most of the pregnancy period in cattle (until Day 200) [47,48]. An influence of BCS on progesterone concentrations from Days 28 to 90 after AI was also described by Santos et al. [49], whereas no relationship between lactation number and progesterone synthesis by the CL have been clearly reported. Concerning the effect of maternal parameters on PAG secretory pattern, our results agree with those from Lopez-Gatius et al. [34] who did not find any effect of lactation number on PAG secretion in Holstein Friesian dairy cows.

The tendency for LH concentrations to be lower during pregnancy in the high-P4 group might be possible because of the negative feedback of progesterone on pituitary gonadotropins (LH and FSH). It has been established that the principal luteotrophic hormone in cattle is LH and treatment with either a GnRH antagonist [50] or LH anti serum [51] during luteal development reduces progesterone production.

The lower prolactin concentrations in high-P4 group compared to low-P4 group in the study on Day 21 might indicate a negative feedback effect of progesterone on prolactin secretion in cows. The mechanism of this potential effect is unclear because prolactin has been assumed to have no luteotrophic activity in cows [51]. As a result, only a few studies on ovarian prolactin receptors have been developed in the bovine species [52,53], unlike in species such as the rat [54], hamster [55], sow, and sheep [56].

An examination of Fig. 1 shows interesting data on progesterone secretion during early pregnancy in cows. First, it was observed that the concentrations were largely variable on Day 21 when the two groups were

constituted. Thereafter (Days 30 and 45), the levels of progesterone became very similar. Finally, on Day 60 and to a lesser extent on Day 80, the progesterone concentrations became different once again according to the initial classification of both groups. This finding suggests that an intermediate factor might influence the secretion of progesterone in later pregnancy period. We hypothesize that the second necessary pregnancy signal on around Day 36 in cows found by Bridges et al. [57] could be represented by one of the multiple trophoblast-expressed PAG, which are abundantly expressed from Days 7 to 14 after fertilization until term [15,18,58,59]. As demonstrated by Bridges et al. [57] in experiments, in which the CL was removed by supra-vaginal luteotomy and replaced by exogenous progestagen treatment, a second signal from the embryo to the cow on around Day 36 may be required to complete maternal recognition of pregnancy, as interferon tau is expressed exclusively between Days 12 and 25 of pregnancy [5,6,11]. Interestingly, boPAG-2, one of the PAG molecules with the oldest origin [60], has been considered to represent a chorionic gonadotrophic-like (CG-like) molecule, which can bind to the LH receptor in the bovine CL [61,62]. This CG-like glycoprotein is a 372-amino acid polypeptide, having 58% amino acid sequence identity with boPAG–1 [16]. Expression of boPAG-2 mRNA is detected from Days 17 to 19 of pregnancy [16] until term [18]. However, as there is no availability of highly purified boPAG-2 preparation, no biological or immunological models could be developed to investigate a hypothetical influence of this protein on progesterone concentrations during the first trimester of pregnancy.

In conclusion, this study presents a first report on the relationship between progesterone and PAG concentrations during the first trimester of pregnancy in dairy cattle. It was observed that PAG concentrations on Days 30, 45, 60, and 80 tended to be higher in pregnant females with progesterone concentrations higher than the mean (8.74 ng/mL) on Day 21 of pregnancy, when compared with those having lower progesterone concentrations. Further studies including large-scale investigations are needed to confirm our results, as well as to investigate maternal concentrations of both progesterone and PAG in pregnant females experiencing interrupted pregnancies.

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