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Milk production correlates negatively with plasma levels of pregnancy-associated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses

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Abstract

This study was designed to establish possible factors affecting plasma pregnancy-associated glycoprotein (PAG) concentrations during early pregnancy in high producing dairy cows with live fetuses. Blood samples were obtained on days 35, 42, 49, 56 and 63 of gestation from 80 lactating cows in two herds carrying live fetuses. Radioimmunoassay systems were used to determine PAG (RIA-497 and RIA-706) and progesterone concentrations. We evaluated the effects on PAG concentrations of herd, lactation number, sire of fetus, day of gestation, fetus number, plasma progesterone and milk production at each time point established, along with possible paired interactions. Mean milk production per cow approached 41 kg during the study period. PAG concentrations were not affected by herd, lactation number or plasma progesterone concentration. Significant positive effects on PAG concentrations were shown by the gestation day, and the interaction between day of gestation and twin pregnancy. Significant differences between bulls and a significant negative correlation between milk production and PAG values on day 63 of pregnancy were also detected. Proportions of blood samples showing undetectable PAG levels and false negative diagnoses throughout the study period

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were significantly higher ($P < 0.001$) using the RIA-497 system (2.5% and 5.3%, respectively) compared to RIA-706 (0% and 0.8%, respectively). Our findings suggest that PAG concentrations during the early fetal period are related to the day of gestation, milk production, number of fetuses and sire of fetus in high producing dairy cows. Under our working conditions, the RIA-706 method was better at detecting plasma PAG molecules than the RIA-497 system.

Keywords: Pregnancy-associated glycoprotein; Progesterone; Early fetal period; Dairy cattle; Milk production

1. Introduction

In mammals, the successful course of gestation following a normal process of fertilization depends on complex interactions among several biological molecules within the microenvironments of the female genital tract. The mammalian embryo appears to influence maternal endocrine and/or immune systems as early on as the earliest cleavage stages, during its migration through the Fallopian tube to the uterus [1]. Conceptus–maternal interactions then continue beyond the uterus. The trophoectoderm is the first epithelium to form during development and gives rise to extraembryonic structures, while the inner cell mass develops principally into the embryo proper. In cows, trophoblastic binucleate cells from the early conceptus synthesize substantial amounts of glycoproteins related to pregnancy. These pregnancy-associated glycoproteins (PAG), belonging to the aspartic proteinase family [2], were discovered when an early pregnancy test was attempted in cattle [3]. In effect, some PAG (in the PAG-1 subgroup) are released into the maternal circulation at the time of implantation (i.e., day 25) allowing for accurate pregnancy tests, since the presence of PAG in blood serum rises steadily as gestation proceeds and peaks just before parturition [4–6]. Although their function remains unknown, PAG concentrations in maternal blood have been used both for pregnancy diagnosis and as a marker of fetal/placental wellbeing [7–11], or to monitor pregnancy failure during the late embryo and early fetal period [5,12–14].

It is evident that fertility has declined with rising milk yields over the past few decades [15–17]. Dairy herds are under ever-increasing pressure to improve their efficiency and primary attention is usually directed towards postpartum reproductive disorders and failure to correctly detect estrus. However, it is also of great interest to understand the effects pregnancy loss may have on the reproductive cycle once a cow is pregnant. Prenatal loss is probably the single most important factor affecting the profitability of the dairy industry. In the cow, the embryonic period of gestation extends from conception to the end of the differentiation stage (about 42 days), and the fetal period extends from day 42 to parturition [18]. Although most pregnancy losses occur during the early embryonic period [19–21], the incidence of early fetal loss is increasing under the current intensive management systems used for dairy cattle [22]. Indeed, fetal loss per diagnosed pregnancy can exceed 12% [23–25]. Early fetal loss peaks between 45 and 60 days of gestation [26], and several management and cow factors of a non-infectious nature have been previously related to pregnancy loss during this period [26–30]. This is the first of a series of studies based on plasma PAG profiles designed to gain knowledge on early fetal loss under intensive

management systems. The objective of the present study was to determine factors affecting plasma PAG concentrations from day 35 to 63 of gestation in high producing dairy cows carrying live fetuses.

2. Materials and methods

2.1. Cattle and herd management

Only healthy cows free of detectable reproductive disorders and free of clinical diseases during the study period (days 35–63 of gestation) were included. Exclusion criteria were the disorders: mastitis, lameness, digestive disorders and pathological abnormalities of the reproductive tract and/or a fetus detectable on ultrasound. Efforts were made to reduce variation in the general status of the animals, so that plasma PAG changes could be attributed to factors other than the clinical condition of the cows during the study.

We only analyzed data obtained from cows ($n = 80$) whose embryos/fetuses, either singletons or twins, were alive during the experimental period. These cows successfully delivered live calves with the exception of one cow that aborted twins on day 241 of gestation. Cows were diagnosed pregnant in two well-managed, high producing Holstein–Friesian herds over a 6-month period (1 May to 30 September, 2004) in northeastern Spain. The two herds comprised 740 mature animals (500 and 240, respectively). Mean annual milk production of the herds for this period was 11,450 kg per cow. The cows calved all year round, were grouped according to their milk production, were milked three times daily and fed complete rations. Feeds consisted of cotton-seed hulls, barley, corn, soybean and bran, and roughage, primarily corn, barley or alfalfa silages and alfalfa hay. Rations were in line with NRC recommendations [31]. All the animals were tested free of tuberculosis and brucellosis. All cows were artificially inseminated, and the study population only included pregnancies registered in parous cows with no abortions after the last parturition. Only cows inseminated after 80 days in milk were selected to avoid interference with PAG present in the peripheral circulation during the postpartum period [4,5]. The inseminate was semen from 10 independent bulls of proven fertility. The mean annual culling rate for the study period was 28%.

2.2. Pregnancy diagnosis, number and viability of embryos

Pregnancy was diagnosed by transrectal ultrasonography at 35 days of gestation using a portable B-mode ultrasound scanner (Scanner 100 Vet equipped with a 5.0 MHz transducer; Pie Medical, Maastricht, The Netherlands). Scanning was performed along the dorsal/lateral surface of each uterine horn. The presence of twins was established by observation of two embryos in different positions within one uterine horn on two screen scans, two embryos simultaneously present on screen, or one embryo in each uterine horn.

The viability of embryos/fetuses was monitored by ultrasound on days 35, 42, 49, 56, and 63 of gestation, and was determined by detecting the embryonic/fetal heartbeat until day 50 of gestation, and then by heartbeat or fetal movement detection.

2.3. Blood sampling

Heparinized blood samples were withdrawn from each animal from the tail vein immediately before each ultrasound examination. These blood samples were centrifuged (15 min at 1500 × *g*) within 30 min of collection, and plasma was stored at −20 °C until assayed.

2.4. Progesterone radioimmunoassay

The assay buffer used (pH 7.0) contained 9 mM citric acid monohydrate, 180 mM Na_2HPO_4, 154 mM NaCl, 15 mM NaN_3, and 2 mg/ml BSA (INC Biochemicals, Aurora, OH). An ANS solution (8-aniline-1-naphthalene sulfonic acid ammonium salt) was prepared in this buffer (1 mg/ml) and used only to prepare the tracer solution. ANS was used to avoid non-specific binding of progesterone (P4) to serum proteins such as albumin or transcortin.

Progesterone (Sigma Chemical Co., St. Louis, MO) was used as the standard. The tracer progesterone-11-hemisuccinate-2[^{125}I]-iodohistamine was prepared by the mixed anhydride method described by Nars and Hunter [32] and purified by HPLC on a C-18 reverse phase. The primary antibody was raised against progesterone-11-hemisuccinate-BSA injected in multiple sites in a rabbit according to the Vaitukaitis method [33]. Steroid-free serum, prepared by treating a pool of bovine sera with charcoal (10 mg/ml; Merck, Darmstad, Germany), was used to prepare the P4 standard curve of 0.15–20 ng/ml.

Progesterone concentrations were determined in plasma using a direct method, as described by Ronayne and Hynes [34] but slightly modified. Briefly, 0.1 ml of standard and sample were dispersed in crystal polystyrene (75 mm × 12 mm) tubes in duplicate, to which tracer (0.3 ml) diluted in ANS solution (25,000 cpm) and 0.1 ml of antiserum (AS#43, 1/15,000) were added. After vortexing for a short period, the tubes were incubated for 4 h at 4 °C.

Following incubation, 1.0 ml of a secondary antibody precipitation system (sheep anti-rabbit immunoglobulin (0.83% v/v), normal rabbit serum (0.17% v/v), polyethylene glycol 6000 (20 mg/ml; Merck, Vel, Leuven, Belgium), microcrystalline cellulose (0.05 mg/ml; Merck, Darmstadt, Germany) and BSA (2 mg/ml; INC Biochemicals, Aurora, OH) diluted in phosphate buffer) was added to all tubes except those reserved for total counts (TC). After incubating for 30 min at room temperature, 2.0 ml of phosphate buffer were added and the tubes were centrifuged at 1500 × *g* for 20 min. The pellet, containing bound ^{125}I-P4, was counted using a gamma counter (LKB Wallac 126 Multigamma counter, Turku, Finland) with a counting efficiency of 75%. Intra-assay and inter-assay coefficients of variation of the P4-RIA technique were 5.05% and 10.52%, respectively.

The accuracy for P4 RIA was determined by adding increasing concentrations of P4 (0.5, 1.0, 1.5 and 2.0 ng) to bovine sera (Table 1). Parallelism was assessed by serially diluting pregnant cow serum with serum P4-free (Table 2).

2.5. PAG radioimmunoassay

Pregnancy-associated glycoprotein concentrations in the plasma samples were determined using two radioimmunoassay procedures (RIA-497 and RIA-706), differing in terms

Table 1
Recovery of different P4 concentrations added to bovine serum samples

Amount of P4 added (ng/ml)	Serum sample P4 concentration (ng/ml)	Theoretical P4 concentration (ng/ml)	Obtained P4 concentration (ng/ml)[a]	Recovery (%)[b]
0.50	2.51	3.01	2.84 ± 0.12	94.4
1.00	2.51	3.51	3.65 ± 0.21	104
1.50	2.51	4.01	3.94 ± 0.30	98.3
2.00	2.51	4.51	4.40 ± 0.26	97.6

[a] Mean (±S.D.) concentrations were determined in four duplicates in the same assay.
[b] (Observed value/expected) × 100.

Table 2
Parallelism of serial dilutions (mean ± S.D.) of a serum sample containing detectable P4 concentrations

Dilution of sample[a]	P4 concentration[b] (ng/ml)
1/1	3.31 ± 0.26
1/2	1.54 ± 0.06
1/4	0.72 ± 0.06

[a] Sample from pregnant female diluted in bovine P4 serum free.
[b] Mean (±S.D.) concentrations were determined in four duplicates in the same assay.

of the antiserum used [35,36]. A pure bovine PAG-1 preparation (boPAG-$1_{67\,kDa}$), purified according to the protocol of Zoli et al. [3], was used as the standard and tracer in each assay. Iodination (Na-I^{125}, Amersham Pharmacia Biotech, Uppsala, Sweden) was carried out according to the chloramine T method [37]. Rabbit polyclonal antisera AS#497 and AS#706 were raised, respectively, against bovine $PAG_{67\,kDa}$ (accession number Q29432) and caprine $PAG_{55\,kDa+62\,kDa}$ (accession numbers P80935 and P80933) preparations according to the Vaitukaitis method [33]. The first antibody titer was determined to obtain a tracer binding ratio for the zero standard of approximately 20–30% (initial dilutions of 1:200,000 for AS#497 and 1:80,000 for AS#706). The second antibody precipitation system was very similar to that used for the progesterone assay except that Tris–HCl buffer (25 mM Tris, 10 mM $MgCl_2$, and 0.02% w/v NaN_3, pH 7.5) was used instead of phosphate buffer.

Plasma PAG concentrations were determined according to the method described by Perényi et al. [35] with some modifications. The standard curve ranged from 0.2 to 25 ng/ml. Standard and plasma samples (0.1 ml) were diluted in 0.2 and 0.3 ml of Tris–BSA buffer (1 mg/ml of BSA; pH 7.5), respectively. To minimize non-specific interference of plasma proteins, 0.1 ml of PAG-free plasma was added to all standard tubes. Samples and standard tubes were incubated with 0.1 ml of the diluted first antibody (AS#497 at 1:200,000 or AS#706 at 1:80,000). On the next day, 0.1 ml of I^{125}-PAG-1 (25,000 cpm) was added and the tubes incubated for a further 4 h. All incubations were performed at room temperature (20–22 °C). The assay volume was 0.6 ml.

After the tubes had been incubated for 30 min with 1.0 ml of the second antibody PEG solution, 2.0 ml of Tris–BSA buffer was added and the tubes were centrifuged (20 min at 1500 × *g*). The supernatant was aspirated and a second wash was undertaken using 3.0 ml of Tris–BSA buffer. After centrifugation (20 min at 1500 × *g*), the tubes were aspirated and the pellet containing the ^{125}I-PAG bound to the antibodies was counted using a LKB Wallac 126 Multigamma counter (Turku, Finland).

The intra-assay variation was assessed as the coefficient of variation (CV) of 20 replicate determinations in the same assay. The inter-assay variation was assessed as the CV of 10 duplicate determinations in different assays. Intra-assay and inter-assay CV were, respectively, 3.48% and 6.76% (2.59 ± 0.09 and 2.58 ± 0.17 ng/ml) for RIA-497 and 3.08% and 10.25% (3.43 ± 0.11 and 3.29 ± 0.34 ng/ml) for RIA-706. A cut-off value of 0.8 ng/ml plasma PAG used previously for pregnancy tests [7,8] was taken as the reference value in the present study.

2.6. Data collection and analysis

The effects of herd, lactation number, sire of fetus, milk production, plasma progesterone levels, twin pregnancy, day of gestation (35, 42, 49, 56 and 63) and possible interactions of paired factors on PAG concentration were analyzed by GLM repeated measures analysis of variance using the SPSS package, version 11.5 (SPSS Inc., Chicago, IL, USA). Progesterone concentrations and milk production were introduced as covariables in the analysis. Since a significant interaction effect between milk production and day of gestation on PAG concentration was included in the main model by use of RIA-497, we also explored relationships between milk production and PAG levels for each time point using both the RIA-497 and RIA-706 systems through the regression analysis procedures implemented in the SPSS program. The proportions of samples showing low PAG values were compared using the Chi-squared test.

3. Results

Herds 1 and 2 provided 36 and 44 cows, respectively. The mean lactation number and mean number of days in milk at conception (mean ± S.D.) were 2.5 ± 1.6 (1–8) lactations and 155 ± 65 (81–340) days, respectively. Twin pregnancies were recorded in 14 animals (17.5%). Table 3 shows PAG and progesterone concentrations, milk production, and the proportions of animals showing undetectable PAG levels and values lower than 0.8 ng/ml (false negative diagnoses) throughout the early fetal period. These proportions of undetectable PAG values and false negatives were significantly higher ($P < 0.001$) when we used the RIA-497 system (2.5% and 5.3%, respectively) compared to the RIA-706 method (0% and 0.8%, respectively). Only in the samples collected on day 63 of gestation were all the PAG values recorded higher than the cut-off value of 0.8 ng/ml established for a positive pregnancy diagnosis using RIA-497. In contrast, using the RIA-706 system, all samples obtained on days 35, 42 and 63 gave PAG values higher than the 0.8 ng/ml cut-off. For the RIA-497 method, of the total number of 320 samples taken between days 35 and 56 of gestation, 10 samples (3.1%) corresponding to five cows and 21 samples (6.6%) derived from 10 cows showed undetectable PAG levels or levels lower than 0.8 ng/ml, respectively. However, when the RIA-706 system was used during the same period, between days 35 and 56 of gestation, only three samples (0.9%) from two cows yielded PAG concentrations lower than 0.8 ng/ml on days 49 (one sample) and 56 (two samples) of pregnancy.

Table 4 provides the variables included in the final model for factors affecting PAG concentrations. Significant positive effects of both the day of gestation and the interac-

Table 3
Mean values (mean ± S.E.M.) for milk production, progesterone, pregnancy-associated glycoproteins and animals showing undetectable levels of PAG or levels lower than 0.8 ng/ml (cut-off for positive pregnancy diagnosis) during the early fetal period ($n = 80$ cows)

Days of gestation	Milk (kg)	Progesterone (ng/ml)	RIA-497			RIA-706		
			PAG (ng/ml)	Undetectable PAG (%)	PAG values < 0.8 ng/ml (%)	PAG (ng/ml)	Undetectable PAG (%)	PAG values < 0.8 ng/ml (%)
D35	40.9 ± 0.77	7.09 ± 0.31	1.67 ± 0.07	1.3	7.5	3.29 ± 0.16	0	0
D42	41.5 ± 0.90	7.41 ± 0.32	1.67 ± 0.09	5	8.8	2.91 ± 0.17	0	0
D49	41.1 ± 0.98	8.01 ± 0.36	1.76 ± 0.09	5	7.5	2.94 ± 0.15	0	1.25
D56	40.2 ± 1.00	8.19 ± 0.36	2.12 ± 0.10	1.3	2.5	3.34 ± 0.18	0	2.5
D63	39.3 ± 1.03	7.84 ± 0.29	2.72 ± 0.13	0	0	4.02 ± 0.21	0	0
Total	40.6 ± 0.99	7.71 ± 0.31	1.99 ± 0.09	2.5 a	5.3 b	3.30 ± 0.18	0 c	0.8 d

Different letters denote significant differences when compared in 2 × 2 contingency tables by the Chi-squared test (a–c: $P = 0.0001$; b–d: $P < 0.0001$).

Table 4
Main model of the GLM repeated measurement analysis for factors affecting pregnancy-associated glycoprotein (PAG) concentrations

Subject effects	Factor	RIA-497			RIA-706		
		d.f.	*F*	*P*	d.f.	*F*	*P*
Within	Days of pregnancy	4	12.1	<0.0001	4	3.11	0.016
	Days × bull interaction	36	0.7	0.92	36	1.01	0.46
	Days × twins interaction	4	14.0	<0.0001	4	7.69	0.0001
	Days × milk interaction	4	3.3	0.01	4	0.56	0.69
Between	Bull	9	3.1	0.0003	9	3.20	0.003

tion between day of gestation and twin pregnancy were observed on PAG concentrations using both the RIA-497 and RIA-706 systems. Significant differences between bulls were also detected. Mean PAG values during the study period ranged from 1.2 ± 0.26 to 2.6 ± 0.79 ng/ml (RIA-497) and from 1.87 ± 0.35 to 4.3 ± 1.91 ng/ml (RIA-706) for the different bulls. A significant effect of the interaction day of gestation × milk production was

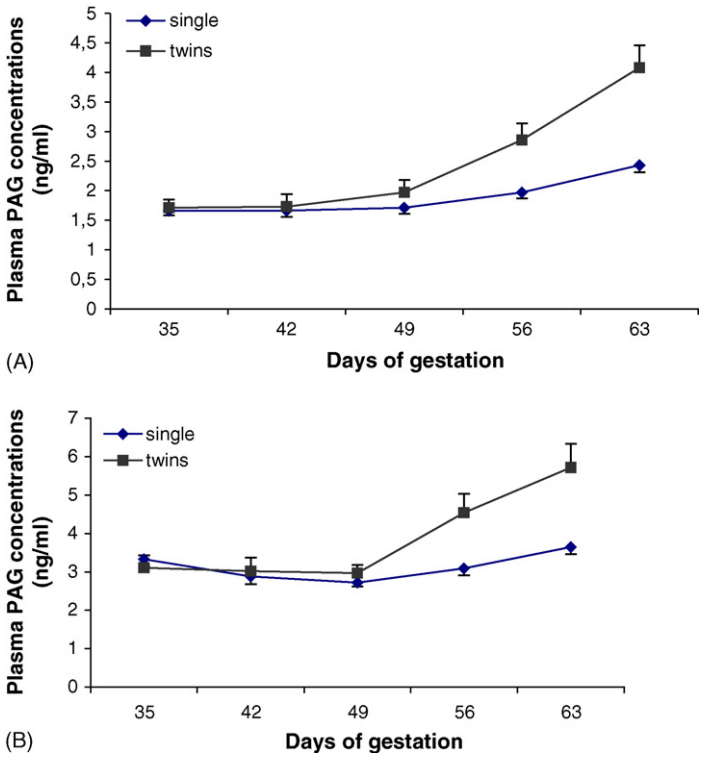

Fig. 1. Mean plasma concentrations (±S.E.M.) of pregnancy-associated glycoproteins detected by RIA-497 (A) and RIA-706 (B) during the early fetal period for singleton and twin pregnancies.

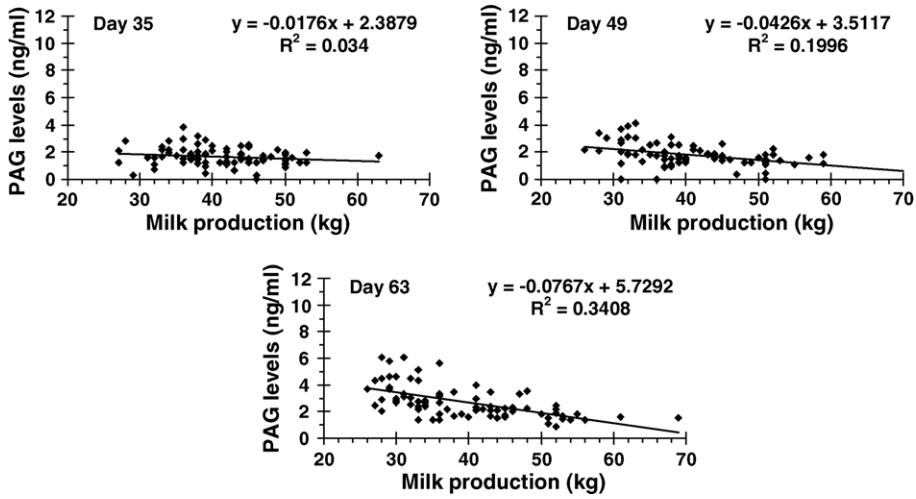

Fig. 2. Plasma pregnancy-associated glycoprotein concentrations measured by RIA-497 according to milk production on days 35, 49 and 63 of pregnancy ($P = 0.1$, $P = 0.07$ and $P < 0.0001$, respectively).

observed using RIA-497. Herd, lactation number and plasma progesterone concentration produced no effects on plasma PAG concentrations.

Fig. 1 shows the PAG values from day 35 to 65 after AI. Whereas plasma PAG concentrations increased slowly and continuously by use of RIA-497, a non-significant decrease was observed from day 35 to 42 by use of RIA-706. However, PAG levels were higher by use of RIA-706 even at day 42. In both RIA systems, cows carrying twins had higher PAG concentrations than cows carrying singletons, with this difference increasing as gestation progressed.

Figs. 2 and 3 show relationships between milk production and PAG levels for days 35, 49 and 63 of gestation. Significant negative correlations between milk production and PAG values measured on day 63 of pregnancy ($P < 0.0001$) were observed using both RIA-497 and RIA-706. Each 1 kg increase in milk yield was associated with decreases of 0.08 and 0.1 ng/ml PAG concentrations using the RIA-497 and RIA-706 systems, respectively.

4. Discussion

As far as we are aware, the possible effects of high milk production on plasma PAG concentrations have not been previously explored. This study was performed on healthy cows with live fetuses throughout the experimental period (35–63 days of gestation) to ensure that plasma PAG variations could be attributed to factors other than the clinical status of the cow and/or fetus. Daily mean milk production per cow was close to 41 kg during the study period. Among our findings, the following points warrant attention: (a) the interaction day of gestation × milk yield significantly affected PAG concentrations measured using the

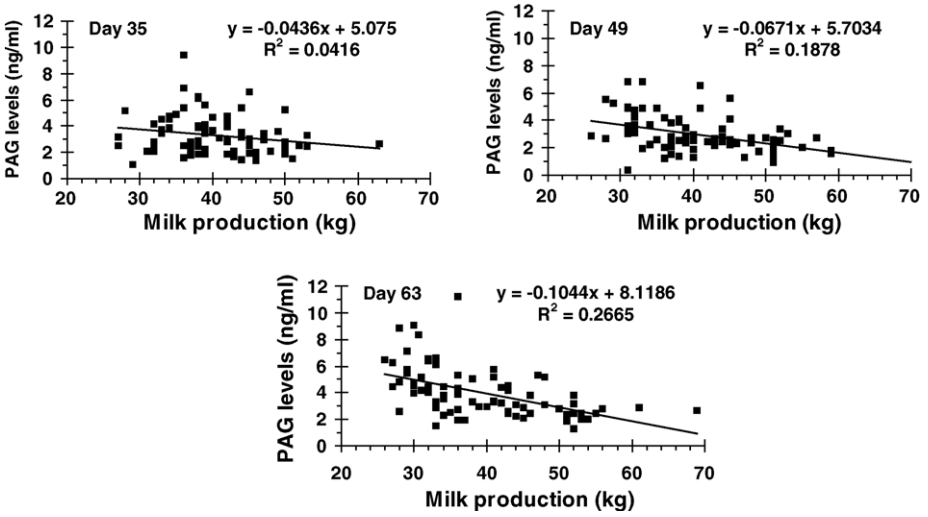

Fig. 3. Plasma pregnancy-associated glycoprotein concentrations measured by RIA-706 according to the milk production on days 35, 49 and 63 of pregnancy ($P = 0.07$, $P < 0.0001$ and $P < 0.0001$, respectively).

RIA-497 system; (b) using both systems, the increased milk yield observed on day 63 of gestation was associated with diminished PAG concentrations; (c) using the RIA-706 system, with the exception of three samples (0.8% of the total number of samples) obtained on days 49 and 56, all plasma samples yielded PAG values higher than 0.8 ng/ml, the cut-off value for a positive pregnancy diagnosis; (d) using the RIA-497 system, it was not until 63 days into gestation that PAG values for all cows were higher than 0.8 ng/ml. Moreover, time point analyses from 35 to 56 days of gestation revealed that 3.1% of samples showed undetectable PAG levels and 6.6% showed false negative diagnoses.

As expected, the day of gestation showed a significant positive effect on plasma PAG levels [4,6,8,9,38], although levels were lower than values previously published for RIA-497. Thus although a PAG concentration of 3 ng/ml on days 30–35 days of gestation is a commonly accepted figure [4,35,39], this concentration was not recorded here until 56–63 days of gestation, and only in cows carrying twins. Further, as noted above, several cows showed undetectable PAG levels between days 35 and 56 of gestation. In previous studies using the same RIA-497 system, PAG was already detected in the mother's peripheral blood by gestation day 30 in all pregnant cows [4,7,35]. Since the difference between previously reported values and lower values reported in the current study, a principal question must concern the manner in which high milk production can be associated with lower PAG values. A possibility is that high producing dairy cows undergo higher metabolic clearance of PAG molecules measured by this RIA system. Substantial amounts of PAG could also be drained through the mammary glands into the milk.

High feed intake related to high production increases liver blood flow and the metabolism of at least progesterone and estradiol in dairy cattle [40]. Although transport mechanisms from the maternal circulation to the milk are unknown, PAG has been detected in milk samples during the postpartum period in cattle [41,42] and in pregnant dairy goats, in

which milk PAG levels were relatively high and could be used as a pregnancy diagnosis test [43]. The main issue here is that plasma PAG levels detected by the RIA-497 system during the early fetal period are reduced or even undetectable in high producers. Thus, if PAG concentrations reflect fetus/placental wellbeing, the next question can be addressed by determining whether possible PAG actions take place at the placental interface rather than outside the uterine lumen. It cannot be precluded that the presence of such placental proteins in the maternal circulation could be an unexpected consequence of the invasive nature of trophoblast binucleate cells and their release close to maternal capillaries [44]. A further point should consider that PAG molecules released into the maternal blood during early pregnancy could be slightly different in high producing dairy cows. The ability of different RIA systems to recognize differentially PAG molecules in the maternal blood can be improved by carefully selecting the antiserum [35].

We observed an apparent decline in PAG at day 42 of gestation by using the RIA-706 system. This finding was already observed by Perenyi et al. [35] who hypothesised that this system better recognises some PAG molecules secreted during the early pregnancy period, when compared to the RIA-497 system. This is not surprising in view of the fact that PAG molecules are a family of closely related proteins and that their expression patterns vary temporarily during early (as well as later) pregnancy periods [45]. Additional investigations are in progress in order to better clarify this observation.

Mean progesterone concentrations on days 35–42 of gestation (7 ng/ml) were slightly lower than those previously reported (>12 ng/ml) [46]. Although milk production was not provided in the latter study, the lower progesterone values presented here could be also attributable to the high metabolism rate linked to high production [40]. Despite this, no correlation was found between progesterone and PAG levels during the early fetal period, in agreement with previous findings in cattle [46] and sheep [47] whereby, under physiological conditions, there was no correlation of pregnancy-specific proteins with progesterone production throughout the gestation period.

The positive correlation observed between peripheral PAG concentrations and fetal number is consistent with previous reports for cattle [48,49], sheep [50,51], goats [52,53], and deer [54]. Because the differences in PAG levels between cows carrying twins and singletons became greater as gestation progressed (notably on days 56 and 63), it may be evaluated in future more extensive studies whether fetal number would be of predictive value during the early fetal period.

A substantial amount of variation in the PAG level can be accounted for by the individual cow [35,38], breed of the mother [4,5], fetal sex [4] and fetal number [48,49]. It would also be reasonable to expect a significant paternal effect. Herein we noted very significant differences among bulls. This strongly suggests that the sire's genotype is related to the complex embryo-maternal cross-talk that occurs in the early gestation period. Further work on PAG concentrations will help to extend our knowledge on the widely documented links between early fetal loss and specific bulls [24,26,28].

As an overall conclusion, our results indicate that PAG concentrations during the early fetal period are correlated with the day of gestation, milk production, number of fetuses and with the sire of fetus in high producing dairy cows. Under our work conditions, PAG molecules released in the maternal circulation during the early fetal period were better recognized by the RIA-706 system.

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