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Original article

Peripheral concentrations of a 60-kDa pregnancy serum protein during gestation and after calving and in relationship to embryonic mortality in cattle

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Summary — In order to have a specific marker for studying pregnancy in cattle we examined the characteristics of a pregnancy serum protein produced by the placenta (PSP60). Its profile in peripheral blood was determined by radioimmunoassay in pregnant cows of 3 breeds after artificial insemination (AI): Charolais (n = 24), Normande (*n* = 24) and Holstein (n = 26). From 27 d post-AI to the end of pregnancy the plasma PSP60 concentration increased, especially during the last 2 wk, to reach a peak a few d before calving, which was higher ($P < 0.001$) in the Charolais (1 238 ± 422 ng/ml) than in the other breeds (528 ± 458 and 444 ± 204 ng/ml). With an apparent half-life of ≈ 8 d, this protein was still detectable in the maternal blood from 105, 85 and 87 d *post-partum* in the Charolais, Normande and Holstein breeds, respectively. Fertility results on 1 102 inseminations in the Charolais breed showed that the PSP60 concentration dropped then disappeared after embryonic mortality. Sequential assays of this protein between 28–90 d after AI are useful for studying the course of pregnancy, although they do not allow discrimination between early embryonic mortality and non-fertilization which together constitute 75% of pregnancy failures.

cattle / pregnancy protein / embryonic mortality

Résumé — Évolution des concentrations périphériques d'une protéine sérique de gestation de 60kD pendant la gestation, après le vêlage et en relation avec la mortalité embryonnaire. *Afin de disposer d'un outil spécifique de suivi de la gestation dans l'espèce bovine, nous avons exploré les caractéristiques d'une protéine sérique de gestation sécrétée par le placenta : la PSP60. Nous avons étudié, par dosage radio-immunologique, sa cinétique de sécrétion dans le sang maternel pendant la gestation de vaches de la race allaitante Charolaise (n = 24) et de deux races laitières, Normande (n = 24) et Holstein (n = 26) après insémination artificielle (IA). Détectable à partir de 27 j post-IA, la PSP60 présente une concentration plasmatique croissante pendant la gestation, et surtout dans les 2 dernières semaines qui précèdent le vêlage. Après un pic situé quelques jours avant le vêlage, plus élevé (P < 0,001) en race Charolaise (1 238 ± 442 ng/ml) que dans les races laitières (528 ± 458 et 444 ± 204 ng/ml), la concentration plasmatique de PSP60 décroît progressivement. Avec une demi-vie apparente d'environ 8 j, cette protéine reste détectable dans le sang mater-*

nel pendant 105, 85 et 87 j post-partum *respectivement en race Charolaise, Normande et Holstein. Les résultats de fertilité de 1 102 inséminations en race Charolaise montrent qu'une mortalité en cours de gestation est suivie de la chute, puis l'annulation des concentrations de PSP60. Des dosages sériés de cette protéine entre 28 et 90 j après IA s'avèrent donc intéressants pour suivre la gestation bien qu'ils ne permettent pas de dissocier mortalité embryonnaire précoce et non-fécondation, qui constituent ensemble les 3/4 des échecs de gestation.*

bovins / protéine de gestation / mortalité embryonnaire

INTRODUCTION

In cattle where 45% of artificial inseminations fail (Diskin and Sreenan, 1980; Sreenan and Diskin, 1986), a reliable and specific marker is necessary to study the course of pregnancy in a large number of cows. In this species, the diagnosis of early pregnancy is still frequently based on the use of progesterone assays in milk or plasma which reflects luteal activity but is not a signal specific to pregnancy. To overcome this, studies were directed at utilizing specific placental and/or embryonic signals to detect pregnancy, as has been performed for many years in humans using hCG assay (Marschall *et al,* 1968). As a matter of fact, in cattle as in humans (Bohn, 1985), the placenta synthesizes and secretes several pregnancy-specific proteins, hormones and enzymes (Martal *et al,* 1988).

In the cow, previous studies have shown that after the 16th d of pregnancy the presence of the embryo is essential for maintaining the structural and functional integrity of the corpus luteum (Northey and French, 1980; Heyman *et al,* 1984; Humblot and Dalla Porta, 1984) *via* a trophoblastic signal. As in sheep (Martal *et al,* 1979; Godkin *et al,* 1982; Imakawa *et al,* 1987; Charpigny *et al,* 1988) this signal is an interferon: bTP-1 (bovine trophoblast protein-1) (Helmer *et al,* 1987; Imakawa *et al,* 1989; Stewart *et al,* 1989). bTP-1 interferon is secreted by the conceptus between d 16 and 26 of pregnancy (Bartol *et al,* 1985) when the majority of pregnancy interruptions have already occurred (Roche *et al,* 1981). Unfortunately, this signal which acts locally within the uterus (Helmer *et al,* 1989) is not released in the peripheral circulation as has been reported by Godkin *et al* (1984) for oTP-1 (ovine trophoblast protein-1), a protein of similar function to bTP-1, so it cannot be used to estimate the presence and viability of the embryo.

Some of the proteins synthesized by the placenta can be found in the maternal circulation during gestation. bPL (bovine placental lactogen), a glycosylated proteic hormone involved in the growth of the foetus and the development of the mammary tissues (Bolander and Fellows, 1976; Kelly *et al,* 1976) can be detected in the blood of pregnant cows at very variable stages during pregnancy (between 26 and 110 d) which prevents it from being used for pregnancy diagnosis (Beckers *et al,* 1982). Another signal of pregnancy in the serum is PSPB (pregnancy specific protein B). This has been partially characterized in cattle by Butler *et al* (1982) and is present in the circulation of the pregnant female from d 24 until the end of the gestation. The

Abbreviations: PSP60, pregnancy serum protein of M_r 60 kDa ; PAG, pregnancy-associated glycoprotein; bPAG, bovine PAG; PSPB (bPSPB), bovine pregnancy-specific protein B.

PSPB family includes 5 acid glycoproteins with an apparent molecular weight of 78 kDa for the major acid glycoprotein and 90, 85, 69, 48 kDa for the others (Crock *et al,* 1988). Radioimmunoassay (RIA) of PSPB enables the course of the pregnancy or late embryonic mortality to be studied (Humblot *et al,* 1988a, b) and it is presently used commercially as a pregnancy test in France. Recently, Zoli *et al* (1991) have purified and characterized a bovine pregnancy-associated glycoprotein (bPAG) with a molecular weight of 67 kDa from foetal cotyledons. This protein has been detected in maternal peripheral blood from d 30 until the end of pregnancy in all pregnant cows (Zoli *et al,* 1992a).

A pregnancy serum protein with molecular weight of 60 kDa (PSP60) has also been purified from extracts of bovine fœtal cotyledons (Camous *et al,* 1988). Its 39-NH_2-terminal amino acid sequence (INRA patent No FR 88 03590, filed March 18, 1988) is identical to that inferred from the bp314 cDNA of bPAG (Xie *et al,* 1991), but is not related to other published reports of placental proteins. Two residues of the NH_2-terminus of PSP60 which correspond to asparagine in the cDNA of bPAG could not be identified because they were linked to oligosaccharides. The difference between the molecular weights of PSP60 and bPAG seems to result from the degree of glycosylation. The amino acid sequence of a bPSPB 64 kDa form was found to be homologous with bPAG (Lynch *et al,* 1992). bPAG PSP60 proteins may correspond to one of the 5 forms of PSPB. The presence of PSP60 in the maternal circulation throughout pregnancy (Camous *et al,* 1991) is the consequence of the migration of secretory binucleate cells from the trophoblast into the endometrium (Wooding and Wathes, 1980) as with bPL (Wooding and Beckers, 1987), PSPB (Eckblad *et al,* 1985; Reimers *et al,* 1985) and bPAG (Zoli *et al,* 1992a). The objectives of this study were to follow the pattern of PSP60 secretion thoughout gestation and its elimination after calving in 3 cattle breeds (Charolais, Norman, Holstein) and to determine the changes in this secretion consecutive to embryonic or foetal mortality. These results, based upon concentrations of a single protein (PSP60), were compared to those obtained with the complex of 5 proteins (PSPB) including one form that was homologous with PSP60.

MATERIALS AND METHODS

Animals and sampling procedure

This study included cows from 2 experimental INRA herds corresponding to the 2 main production systems: milking and suckling cattle. The first was a suckling herd of 349 Charolais heifers and cows inseminated several times during the 1987–1990 period. In the second herd, dairy cows of Normande (n = 24) and Holstein (n = 26) breeds were used in 1990. All the females were artificially inseminated (AI) at natural oestrus.

In order to describe PSP60 secretion during pregnancy and its elimination after calving, 24 pregnant Charolais cows in the suckling herd, 24 Norman de and 26 Holstein pregnant cows in the dairy herd were bled at ≈ 28, 35, 50 and 90 d after the fertilizing insemination and weekly during the *peripartum* period, from 4 wk before calving until the next fertilizing AI. Dairy cows were systematically bled at calving, fortnightly from calving to 8 weeks *post-partum.*

In the suckling herd, the day at which PSP60 was first detected was determined in 299 pregnant heifers sampled once between 20 and 30 d after AI by taking advantage of the variation in the date after AI when heifers were first bled (25.3 ± 2.3 d).

The information provided by different pregnancy diagnostic methods and PSP60 concentrations was analyzed jointly in the Charolais herd when studying the secretion of PSP60 in relation to pregnancy failure. The diagnostic methods included an early pregnancy diagnosis made by progesterone RIA from plasma sam-

ples 22–23 d after AI, an ultrasonic examination at 50.4 ± 4.4 d post-AI and the observation of a return to oestrus in some cases. Rectal palpation was performed at 89.9 ± 3.5 d post-AI to confirm gestation. Pregnancy was confirmed by calving. The females were systematically bled at 27.5 ± 6, 33.7 ± 1.1, 49.5 ± 3.1 and 89.9 ± 4.4 d after AI for determination of PSP60 concentrations unless oestrus had been observed meanwhile. In total, the results of 1 102 inseminations performed in 349 females were studied.

PSP60 and progesterone assays

Peripheral blood (5 ml) was collected from the caudal vein into heparinized or EDTA vacutainers in order to determine the PSP60 concentration at different stages of pregnancy. Plasma was separated by centrifugation and stored at –20°C until assayed. PSP60 plasma concentrations were measured with a RIA developed by Camous *et al* (INRA patent No FR 88 03590, filed March 18, 1988). PSP60 assays were performed on 4 100-µl aliquots of pure or diluted (with plasma from a non-pregnant cow) plasma. Rabbit antiserum against PSP60 was used at a final dilution of 1 : 1 800 000. There was no cross-reactivity with either ovine luteinizing hormone (oLH) or ovine follicle stimulating hormone (oFSH). Cross-reactivity with ovine placental lactogen (oPL) was minimal (< 0.003%). Sensitivity of the assay was 0.2 ng/ml plasma. Intra- and inter-assay coefficients of variation were 6 and 12%, respectively, when estimates were run for a 1.3 ng/ml sample of reference plasma.

Progesterone concentrations were assayed on 100-µl samples of plasma with a rabbit antiserum used at a final dilution of 1/25 000 according to a RIA procedure previously described by Terqui and Thimonier (1974). An early pregnancy diagnosis was then performed as described in Thimonier (1973).

Data analysis

Secretion and elimination of PSP60

Secretion patterns during pregnancy seemed to present 2 phases in all 3 breeds; a first phase with a slow increase in concentration and a second phase characterized by a sharp increase in concentration. At each phase, linear and exponential functions of time were tested in models including the breed effect. The duration of each phase was computed after the breaking point between them was determined. Between-breed differences in the parameters for each phase and in breaking-point concentrations were tested in these models with Student's *t*-test. The relationship between calf birthweight and dam PSP60 concentration at calving was studied, calculating the within-breed correlation coefficient. After parturition, PSP60 elimination appeared to follow a regular function of time. The half-life of PSP60 was estimated during the *post-partum* period when this protein was no longer secreted; indeed, the number of binucleate cells starts to decline shortly before calving (Gross *et al*, 1985).

Fertility groups

The AI performance of the Charolais females was assigned to 5 fertility groups according to the information from early pregnancy diagnosis by progesterone assay, ultrasonic examination, rectal palpation and date of oestrus (table I). There were 2 extreme groups. The first group (pregnancy at 90 d) included females in which all pregnancy diagnoses were positive and no œstrus was observed before 90 d. The second extreme group (non-fertilized/ early embryonic mortality) included females with a negative progesterone diagnosis and showing oestrus between 16 and 25 d after AI. The grouping of females into the 3 intermediate fertility groups was based on ultrasonic examination and observation of oestrus. The same female could be inseminated several times a year and could then be assigned to different groups.

RESULTS

Concentration of PSP60 in cows achieving pregnancy

The detailed study of the beginning of PSP60 secretion in the 299 pregnant Charolais heifers has been reported in table II

Table I. Determination of the fertility groups according to the pregnancy diagnosis methods.

Fertility groups	*Diagnostic methods*					
	Progesterone d 22	*Ultrasound d 50*	*Palpation d 90*	*Oestrus (d)*	*Inseminations* N	*(%)*
Non-fertilized/early embryonic mortality	–			>16 and <25	333	(30)
Late embryonic mortality: 16–35 d	+			>24	47	(4)
Very late embryonic mortality: 25–50 d	+	– Or ?		>37	62	(6)
Foetal mortality: 50–90 d	+	+	–(+)	>52	10	(1)
Pregnancy at 90 d	+	+	+	None or >90	650	(59)
Total					1 102	(100)

and was undertaken to determine the earliest day on which PSP60 was detectable in the maternal blood. The results showed that the PSP60 concentration was high enough (>0.2 ng/ml) to be detected in almost 100% of the pregnant females no earlier than d 27 of pregnancy.

Profiles of plasma PSP60 concentrations in the pregnant Charolais, Normande and Holstein cows sampled from the 4th wk of pregnancy to the 14th wk after calving are shown in figure 1. Two phases clearly appeared during gestation, where linear functions better fitted the evolution of concentrations and explained 47% and 48% of the observed variances in the first and second phase, respectively. The first phase was much longer than the second which corresponded to the last 2 wk of pregnancy (table III).

During the first phase of secretion, the PSP60 concentration increased slowly, by ≈ 0.3 ng/ml per day. During the second month of pregnancy, between 35 and 50 d, a slight slowing down in the curve appeared. Significant differences between breeds ($P < 0.05$) were observed in secretion (0.35, 0.32 and 0.25 ng/ml/day in the

Table II. Early detection of PSP60 in the plasma of pregnant Charolais heifers ($N = 299$).

Sampling interval after AI (d)	*No of sampled heifers* N	*Heifers with detectable PSP60 (concentration > 0.2 ng/ml)* N	*(%)*	*Mean PSP60 concentration (ng/ml)*
20 + 21	12	0	0	0.00
22	34	2	6	0.03
23	35	7	20	0.13
24	29	6	21	0.13
25	24	22	92	0.64
26	44	38	86	0.50
27	69	68	99	0.68
28	42	42	100	0.68
29 + 30	10	10	100	1.30

Table III. Parameters of secretion during gestation and *post-partum* elimination of PSP60.

Breed	*1st secretion phase*	*Inflexion point*		*2nd secretion phase*		*Maximum point*		*Elimination phase*		
	α^* *(ng/ml/day)*	*d before calving*	*PSP60 (ng/ml)*	*duration (d)*	α^* *(ng/ml/day)*	*d before calving*	*PSP60 (ng/ml)*	α^{**} *(ng/ml/day)*	*Half -life (d)*	*Cancelling (d)*
Charolais ($N = 24$)	0.35[a]	−17	80[a]	16	73[a]	−1	1 238[a]	0.083[a]	8.4[a]	105
Norman ($N = 24$)	0.25[b]	−18	56[c]	14	33[b]	−4	528[b]	0.088[a]	7.9[a]	85
Holstein ($N = 26$)	0.32[a]	−18	69[b]	15	26[b]	−4	444[b]	0.085[a]	8.2[a]	87

* Regression coefficient in a linear model: $C = C_0 + \alpha t$; ** regression coefficient in an exponential model: $C = C_0 e^{-\alpha t}$; [a,b,c] means with different superscripts in the same column differ significantly ($P < 0.05$).

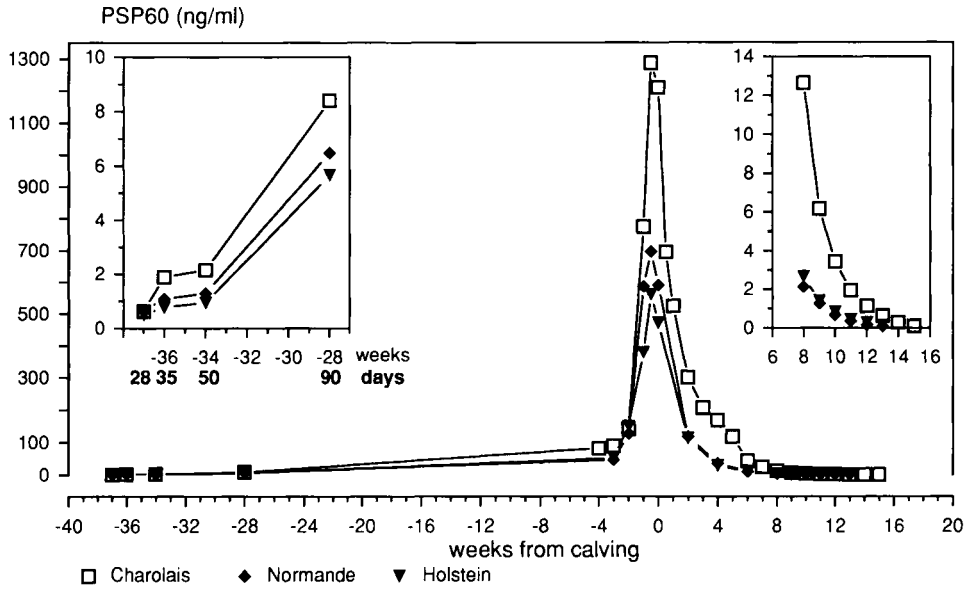

Fig 1. Concentration of PSP60 in plasma of Charolais ($N = 24$), Normande ($N = 24$) and Holstein ($N = 26$) cows during pregnancy and after calving.

Charolais, Holstein and Normande breeds respectively) and consequently in the PSP60 concentration at the end of the first phase (80, 69 and 56 ng/ml respectively). At each stage of pregnancy during this phase, PSP60 concentrations varied widely amongst these pregnant females with observed within-breed coefficients of variation between 50% and 130%.

PSP60 secretion during the last 2 wk of gestation was 80 to 200-fold greater than at previous stages. The increase in concentrations in the Charolais breed (73 ng/ml/day) was significantly higher ($P < 0.001$) than in the dairy breeds (33 and 26 ng/ml/d in the Normande and Holstein breeds, respectively). Duration of the second phase was similar in the 3 breeds. It ended when PSP60 concentration reached a peak, generally a few d before parturition as observed in 17 Normande and 17 Holstein cows. This maximum was significantly higher ($P < 0.001$) in the Charolais breed (1238 ng/ml) than in the dairy breeds (528 and 444 ng/ml in the Normande and Holstein breeds respectively). At this stage of pregnancy, the within-breed individual variation coefficients of the PSP60 concentration amongst cows were very high, *ie,* between 36 and 87%. In the Charolais breed, the maternal PSP60 concentration at calving appeared to be related to the calf birthweight ($r = +0.51$; $P < 0.001$). PSP60 concentration was highest in the Charolais breed ($P < 0.001$), which might be partly due to a higher calf birthweight ($P < 0.05$) than in the 2 dairy breeds (48.0 *vs* 43.0 and 43.3 kg in the Charolais, Holstein and Normande breeds respectively). However, such a high level of PSP60 secretion does remain specific to the Charolais breed (fig 2).

After parturition, the decrease in the plasma concentration *(C)* of PSP60 was shown to follow an exponential function ($C = Co\ e^{-\alpha t}$) that explained 69% of the observed variances. A linear relationship was obtained after the logarithmic transformation and the apparent half-life for PSP60 was computed as follows $t1/2 = (\text{Log } 2)/\alpha$. Half-life estimates for PSP60 were 8.4 d in the Charolais 7.9 d in the Normande and 8.2 d in the Holstein breeds (table III). Differences between breeds were not significant ($P > 0.05$). Using the regression function the expected time required to reach a residual concentration < 0.2 ng/ml was 105 d *post-partum* in the suckling Charolais breed, 85 d in the Normande and 87 d in the Holstein dairy breeds. The actual time when residual concentrations of PSP60 became undetectable was observed in 18, 21 and 26 cows from the Charolais, Normande and Holstein breeds. The mean and extreme values were : 107 d (91 to 119 d) in the Charolais breed, 84 d (56 to 105 d) in the Normande breed and 88 d (63 to 126 d) in the Holstein breed.

PSP60 plasma concentration in females exhibiting pregnancy failure

Only heifers and cows of the Charolais herd were examined in this part of the study (table IV, fig 3). The distribution of AI results over the different fertility groups defined for these females is reported in table I.

PSP60 concentration in non-fertilized females or in females exhibiting early embryonic mortality

In all except 7 heifers of this group (total $N = 123$), mean PSP60 concentrations remained below the detection limit of the assay. On the other hand, at the first 2 stages of sampling, the cows ($N = 210$) had a detectable mean concentration of PSP60 which declined gradually with time. This indicated that residual concentrations were present in the blood of some cows during the *post-partum* period.

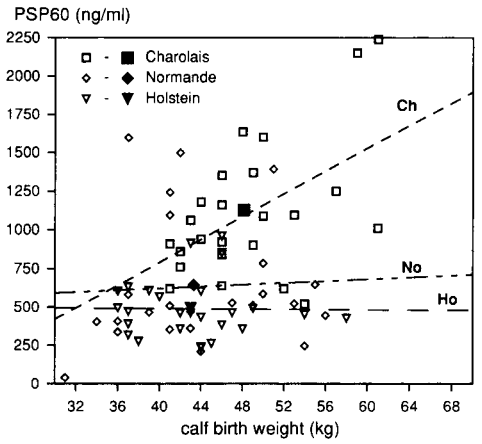

Fig 2. Across- and within-breed relationship between PSP60 concentrations in plasma of cows at calving and calf birthweight.

PSP60 concentration in females exhibiting late embryonic or foetal mortality

Heifers whose pregnancy had been interrupted by an embryo death between 16 and 35 d were defined as those which had a positive progesterone assay and a time of return to oestrus between 25 and 37 d. Amongst 16 such heifers, 8 never had a concentration of PSP60 higher than the detection limit of the assay. For the other 8 heifers, 4 had detectable PSP60 at 28 d and not at 35 d; 3 others presented a late secretion which was only detectable at 35 d. The wide variation in PSP60 concentrations at 28 and 35 d in this group reflects the variation in the time of embryo death from 16 to 35 d. In cows exhibiting mortality during the same period (31 interrupted pregnancies), 10 never had a detectable

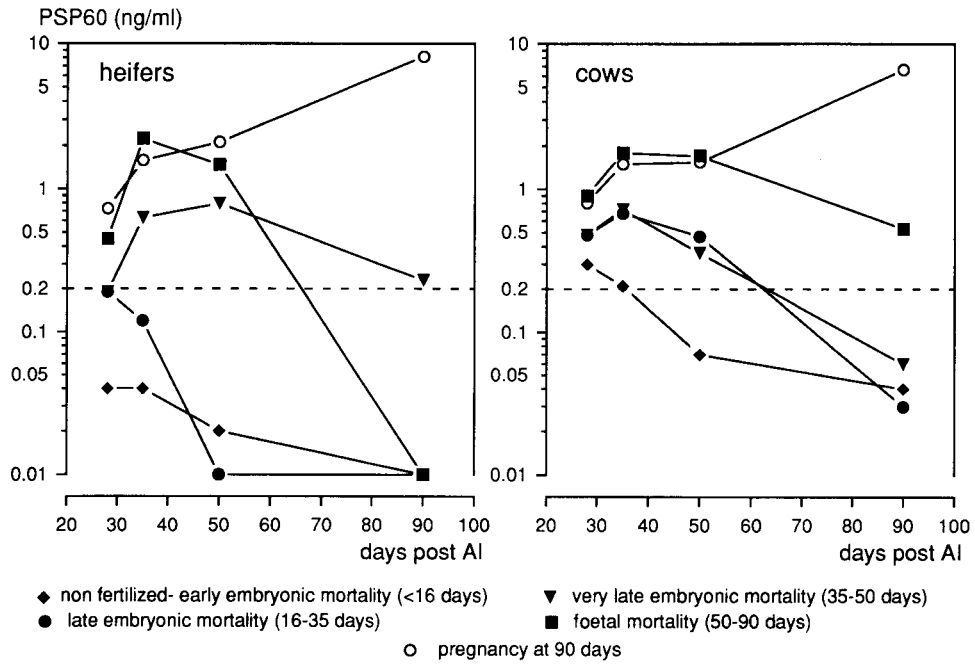

Fig 3. Concentration of PSP60 in plasma of Charolais heifers and cows according to fertility groups (note the logarithmic scale on the Y axis).

Table IV. PSP60 concentrations (ng/ml) in Charolais females in the 5 different fertility groups.

Fertility groups	N		*Time interval after AI*							
			n †	*d 28* *Mean ± SD*	n	*d 35* *Mean ± SD*	n	*d 50* *Mean ± SD*	n	*d 90* *Mean ± SD*
Non-fertilized	123	Heifers	37	0.04 ± 0.10^{a}	19	0.04 ± 0.11^{a}	9	0.02 ± 0.07^{a}	7	0.00 ± 0.00^{a}
early embryonic mortality	210	Cows	114	0.30 ± 1.11^{a}	148	0.21 ± 0.79^{a}	66	0.07 ± 0.16^{a}	46	0.04 ± 0.11^{a}
(<16 d)	173	(Cows*)	76	0.07 ± 0.15^{a}	122	0.04 ± 0.12^{a}	56	0.03 ± 0.09^{a}	40	0.04 ± 0.12^{a}
Late embryonic mortality	16	Heifers	7	0.19 ± 0.24^{a}	12	0.12 ± 0.23^{a}	2	0.00 ± 0.00^{a}	2	0.00 ± 0.00^{a}
(16–35 d)	31	Cows	20	0.48 ± 0.62^{ab}	28	0.68 ± 1.74^{b}	9	0.47 ± 0.80^{a}	6	0.03 ± 0.08^{a}
	23	(Cows*)	13	0.27 ± 0.40^{b}	21	0.25 ± 0.53^{ab}	6	0.17 ± 0.21^{a}	6	0.03 ± 0.08^{a}
Very late embryonic	20	Heifers	9	0.19 ± 0.25^{a}	20	0.63 ± 0.59^{b}	12	0.79 ± 0.83^{a}	9	0.23 ± 0.63^{a}
mortality (35–50 d)	42	Cows	26	0.48 ± 0.72^{ab}	40	0.72 ± 0.72^{b}	36	0.36 ± 0.42^{a}	26	0.06 ± 0.18^{a}
	34	(Cows*)	20	0.31 ± 0.31^{b}	32	0.63 ± 0.66^{b}	28	0.25 ± 0.37^{a}	23	0.03 ± 0.10^{a}
Foetal mortality	3	Heifers	2	0.45 ± 0.07^{a}	3	2.23 ± 0.42^{c}	3	1.47 ± 1.16^{ab}	3	0.00 ± 0.00^{a}
(50–90 d)	7	Cows	3	0.90 ± 0.66^{ab}	7	1.79 ± 1.52^{c}	7	1.70 ± 1.18^{b}	7	0.53 ± 0.83^{a}
	5	(Cows*)	1	0.20	5	1.76 ± 1.81^{c}	5	1.52 ± 1.18^{b}	5	0.70 ± 0.95^{a}
Pregnancy at 90 d	303	Heifers	121	0.73 ± 0.43^{b}	238	1.58 ± 0.88^{c}	244	2.11 ± 1.36^{b}	301	8.13 ± 4.14^{b}
	347	Cows	221	0.80 ± 0.77^{b}	320	1.50 ± 1.09^{c}	339	1.54 ± 1.28^{b}	334	6.63 ± 4.72^{b}
	283	(Cows*)	156	0.65 ± 0.38^{c}	260	1.36 ± 0.94^{c}	276	1.51 ± 1.28^{b}	272	6.87 ± 5.00^{b}

(Cows*): cows with a calving-blood sampling interval ≥ 110 d; *N*: total number of females in each fertility group; *n*: number of sampled females at each stage (PSP60 sampling ceased after return to oestrus); †: sampling time included in the interval: $27 \leq \leq 31$ d after AI; ^{a,b,c} fertility group means, within heifers, cows or (cows*), with different superscripts in the same column differ significantly ($P < 0.05$).

concentration of PSP60, 6 had a slight increase in secretion between 28 and 35 d; the 15 others had a declining concentration from 28 to 35 d.

Heifers and cows exhibiting a very late embryonic mortality, *ie* between 35 and 50 d after AI (20 + 42 = 62 interrupted pregnancies) generally showed an increasing concentration of PSP60 between 28 and 35 d and a drop in concentration between 50 and 90 d. Between 35 and 50 d the PSP60 concentration remained constant or declined. Nevertheless in some cases, although the embryo was already dead at 50 d according to ultrasonic scanning (and sometimes return to oestrus), the concentration increased steadily between 35 and 50 d and decreased only thereafter. As expected, the concentration at 50 d in females of that group was significantly lower than that in pregnant females ($P < 0.001$). At the previous stages (28 and 35 d) before mortality had occurred, the concentration was already significantly lower. Moreover, it should be noted that amongst the 35 females in this group, 14 had no detectable PSP60 at the d 28 stage.

When foetal death occured between 50 and 90 d, a decrease in the concentration of PSP60 was noted between these 2 stages. In some cases, this decrease started before 50 d, although, according to ultrasonic examination, the embryo was still alive at this stage.

DISCUSSION

The time at which 60-kDa bovine pregnancy serum protein (PSP60) appeared in the maternal blood varied relatively little, *ie* between 22 and 28 d after AI. Sasser *et al* (1986) detected PSPB in 3 out of 15 pregnant cows as early as 15 d of pregnancy. It is possible that one of the PSPB proteins appears in the maternal blood earlier than PSP60 does, but its flow in the maternal circulation at a moment when implantation has not yet begun remains an open question. However, these early detected concentrations may correspond to residual protein from a previous pregnancy. Almost all females had a detectable plasma PSP60 concentration 27 d after AI. First detected during the 4th wk of pregnancy, PSP60 was present throughout pregnancy at an increasing concentration level. The plasma profile of PSP60 during pregnancy was very similar to the PSPB (Sasser *et al*, 1986) and bPAG profiles (Zoli *et al*, 1992b), which tends to confirm that PSP60 and bPAG might correspond to a form of PSPB. The lesser secretion between 35 and 50 d could result from a change in the regulation of the development of the foetoplacental unit and/or reflect the unknown function of PSP60. We have no explanation for this phenomenon at present.

A major PSP60 increase occurs at the end of pregnancy, as has also been described by Sasser *et al* (1986) for PSPB and by Zoli *et al* (1992b) for bPAG. However, we may note that bPAG levels are consistently higher (Zoli *et al*, 1992b) than those reported for PSPB (Sasser *et al*, 1986) and for PSP60 (this paper). We cannot explain this sudden increase in blood levels. Does this evolution in concentrations mean that these proteins are involved in the induction of parturition or is it only a result of certain hormonal or physical changes in the placenta at the end of pregnancy?

In this study, the levels reached at calving were higher in the Charolais than in the Normande and Holstein breeds; this difference could depend on herd management and location, but certainly also depends on the breed (suckling/dairy). A higher calf birthweight in the Charolais than in dairy breeds (+ 5 kg) only explains part of the gap between the 2 maternal PSP60 levels at calving associated with a possible varia-

tion in placental size and consequently in the number of binucleate cells. Guilbault *et al* (1991) have investigated bPAG concentrations at calving in relation to calf birthweight in Holstein and Hereford cows both carrying pure-bred Holstein foetuses. Lower peak concentrations of bPAG in Holstein recipients (3.0 *vs* 7.6 µg/ml) were associated with higher calf birthweight (50.0 *vs* 43.3 kg). This suggests that peripheral concentrations of protein of placental origin are also probably affected by the dam weight, which is higher in Holstein cows because of the dilution in blood volume. Indeed, in our experiment the Charolais cows presented the lowest weight just after calving (633 *vs* 664 and 685 kg in Holstein and Normande breeds respectively). These differences could also be due to differences in metabolism between dairy and suckling females.

The decrease in PSP60 concentrations initiated before calving may correspond to a similar decline in the binucleate cell count at this moment, as was shown in sheep by Wooding (1983) and in cattle by Gross *et al* (1985). The *post-partum* elimination rate of PSP60 was similar in all 3 breeds. However, residual plasma concentrations after calving were detectable over a 15-wk period in the Charolais breed compared to 13 wk in both dairy breeds, due to higher levels at calving. Similar residual concentrations of PSPB were found in dairy cows over 100 d *post-partum* (Humblot *et al,* 1988c). The decrease of PSP60 concentrations is exponential; hence, residual concentrations cannot be explained as a release by the endometrium after parturition as was suggested by Humblot *et al* (1988c).

The study of PSP60 secretion in females exhibiting pregnancy failure showed different patterns according to the time of failure. In the non-fertilization and early embryonic mortality (before 16 d) group, which represented 75% of pregnancy failures, there was no or little PSP60 secretion. This phenomenon combined with the non-extension of the luteal lifespan reflected the limited development of the trophoblastic tissue, which secretes bTP-1 involved in the maintenance of corpus luteum activity in cattle (Knickerbocker *et al,* 1984, 1986). When there was a late embryonic mortality (between 16 and 50 d) or foetal mortality, the PSP60 concentration curve became straight, determined by the moment of embryonic or foetal death. These different patterns and their respective frequency are consistent with the data previously reported in dairy (Humblot *et al,* 1988a) or in beef breeds (Humblot *et al,* 1990). However, it should be noted that PSP60 concentrations dropped and disappeared some time after embryonic death on account of the long half-life of this protein. In the 3 groups of embryonic and foetal mortalities, PSP60 concentrations declined between 28 and 50 d of pregnancy but remained detectable until 50 d, especially in cows.

On the other hand, in cases of very late embryonic mortality (after 35 d), we found both a delay in the secretion of this protein since at 28 d it was not always detectable, and a low level of secretion. This can probably be explained by a delay or anomaly in the development of the conceptus. As a consequence, in 67% of the heifers and 81% of the cows with a PSP60 concentration below 0.6 ng/ml at 35 d, pregnancy was interrupted before 50 d. Humblot (1991) also reported that with one PSPB assay at ≈ 30 d post-AI, it is possible to detect > 85% of the non-pregnant females. However, as there is a wide individual variability of the concentrations at a given stage, it seems difficult to accurately predict the future course of pregnancy *via* a single concentration measurement. Nevertheless, the secretion kinetics between different points in time (at least 3) provide more information on the time of embryonic mortality than a single concentration.

In conclusion, assaying this pregnancy serum protein (PSP60) in the peripheral circulation of the cow has proven useful as a new method for studying the course of the pregnancy. It has to be utilized within the limits fixed by the patterns of its secretion, *ie* from 27 d after AI, and not before 13 to 15 wk after the previous calving. Comparing secretions between several stages provides complementary information on embryo viability.

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