Plasma bovine pregnancy-associated glycoprotein concentrations throughout gestation in relationship to fetal number in the cow

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Abstract

This study characterized the peripheral plasma bovine pregnancy-associated glycoprotein (bPAG) profile throughout gestation and examined the effect of stage of gestation and fetal number on this profile in Holstein cows after non-surgical embryo transfer. Cows (n = 12) were divided into three groups: group 1 = normal singleton pregnancies (n = 5); group 2 = normal twin pregnancies (n = 5); group 3 = abnormal twin pregnancies (n = 2). Blood was collected about every third day from day 0 (defined as the first day of standing estrus), then daily for the last 10 days of gestation, and sampling was stopped one day postpartum. The time-related changes in plasma bPAG concentrations were significantly (P < 0.01) affected by the stage of gestation and fetal number (P < 0.01), except during the last 10 days of gestation. In both normal pregnancy groups, bPAG concentration increased rapidly during the first trimester (0.5 ± 0.1 to 14.6 ± 1.7 ng/ml and 1.0 ± 0.6 to 21.8 ± 4.8 ng/ml in singleton and twin-bearing groups respectively), then progressively between days 160 and 20 prepartum (31.6 ± 6.2 to 114.3 ± 31.3 ng/ml and 41.6 ± 7.4 to 155.8 ± 36.6 ng/ml in singleton and twin-bearing cows respectively). The mean concentration between days 20 and 10 prepartum approximately tripled (P < 0.001) in both these groups of cows (114.3 ± 31.1 to 493.0 ± 75.3 ng/ml and 155.8 ± 36.6 to 409.3 ± 114.7 ng/ml in singleton and twin-bearing cows respectively), but between days 10 prepartum and parturition the values increased about threefold (P < 0.01) in the singleton group (493.0 ± 75.3 to 1352.8 ± 286.5 ng/ml) and fivefold (P < 0.001) in the twin-bearing group (409.3 ± 114.7 to 2154.0 ± 505.7 ng/ml). The two cows in group 3 that gave birth prematurely to a stillborn calf or to a schistosomus reflexus calf exhibited an aberrant bPAG profile. Our results indicate that peripheral bPAG concentrations are correlated to the stage of gestation and fetal number, and that the profile of the peripheral plasma concentrations provides a useful indication of the feto–placental status.

European Journal of Endocrinology \textbf{137} 423–428

Introduction

In domestic ruminants, communication between the conceptus and maternal tissue is initiated before implantation, to maintain the structural and functional integrity of the corpus luteum (1, 2). The major classes of compounds that may serve as signals for feto–maternal interactions include proteins, prostanoids and steroids (1, 2). Characterization of these signals, notably the steroidal and proteinaceous compounds, has been used for many decades both in humans and in horses, to diagnose pregnancy and assess its prognosis (3, 4). In recent decades, proteins implicated as having a luteoprotective role in the ruminants have been identified and characterized (5). Unfortunately, these proteins act locally and are not released into the peripheral circulation (5). However, a number of other proteins synthesized later by the placenta and secreted into the peripheral circulation have been identified, including: bovine placental lactogen (bPL), pregnancy specific protein-B (PSPB), pregnancy serum protein-60 (PSP-60) and bovine pregnancy-associated glycoprotein (bPAG) (6–9). The assay systems for these proteins have been validated, methods for their quantification established and their profiles during gestation in different breeds of cattle defined (7–10).
In humans, monitoring of pregnancy-specific proteins is an invaluable index of conceptus status or number, or both, and is used as a yardstick for planning antenatal and neonatal care (3). In ruminants, there is evidence that PSPB and PSP-60 are related to the stage of gestation and fetal number and are indicators of fetoplacental viability (7, 11–13). However, there is only a single report in the literature on the peripheral concentrations of bPAG throughout gestation in the cow (9), and no data on the effect of conceptus number on the peripheral concentration of this protein in any species. The objectives of this study were therefore twofold: (i) to characterize the time-related profile of changes in peripheral concentrations of bPAG throughout gestation in the cow, and (ii) to determine the relationship of this protein to the stage of gestation and the number of fetuses being carried.

Materials and methods

Twelve regularly cycling Holstein cows of mixed age and parity, housed and managed in the National Institute of Animal Industry, were used as recipient animals. The animals were divided into three groups: normal singleton pregnancies (group 1; n = 5); normal twin pregnancies (group 2; n = 5) and abnormal twin pregnancies (group 3; n = 2).

Japanese Black cow embryos, generated by in vitro fertilization, were obtained from a commercial supplier (Tokyo Bio-tech. Center, Tokyo, Japan) and details of their production are given elsewhere (14). In all cows, the embryos were non-surgically transferred on day 7 of the estrous cycle (day 0 was defined as the first day of standing estrus). A singleton pregnancy was established by transfer of a single embryo into the uterine horn ipsilateral to the corpus luteum-bearing ovary. A twin pregnancy was established by transfer of individual embryos into each uterine horn. Pregnancy was diagnosed at day 30 by ultrasonography and confirmed by birth of the calf or calves. In the present paper, results are reported for cows that maintained pregnancies and gave birth to either a single calf (group 1) or twin calves (groups 2 and 3).

Blood was collected via jugular venepuncture into a 50 ml heparinized polypropylene tube, placed immediately on ice and centrifuged (1800 g at 4 ºC for 1 h) within 1 h of collection. The harvested plasma was stored at −20 ºC until required for assay. Blood was collected about every third day from day 0, then daily until pregnancy was established by transfer of individual embryo transfer and day 10 prepartum were approximated to the nearest tenth day with the following model:

\[ Y_{ijk} = \mu + T_i + C_{(ij)} + S_k + TS_{ik} + E_{ijk} \]

where \( \mu \) = overall mean; \( T_i \) = effect of fetal number; \( C_{(ij)} \) = cow nested in fetal number; \( S_k \) = effect of day of gestation; \( TS_{ik} \) = interaction between fetal number and day of gestation; \( E_{ijk} \) = error term associated with \( Y \).

Results

The time-related profile of changes in plasma bPAG concentrations (Fig. 1) was significantly (\( P < 0.01 \)) affected by the stage of gestation and fetal number, except during the last 10 days of gestation (Table 1). In both normal pregnancy groups, the mean concentration increased rapidly between days 20 to 90 postestrus (0.5±0.1 to 14.6±1.7 ng/ml and 1.0±0.6 to 21.8±4.8 ng/ml; means±S.E.M. in singleton and twin-bearing groups respectively), and then progressively between days 160 and 20 prepartum (31.6±6.4 to 114.3±31.3 ng/ml and 41.6±7.4 to 155.8±36.6 ng/ml in singleton and twin-bearing cows respectively). The mean concentration between days 20 and 10 prepartum approximately tripled (\( P < 0.001 \)) in both these groups of cows (114.3±31.1 to 493.0±75.3 ng/ml and 155.8±36.6 to 4093±114.7 ng/ml in singleton and twin-bearing cows respectively), but between days 10 prepartum and parturition, bPAG values increased about threefold (\( P < 0.01 \)) in the singleton group (493.0±75.3 to 1352.8±286.5 ng/ml) and fivefold (\( P < 0.001 \)) in the twin-bearing group (4093±114.7 to 2154.0±505.7 ng/ml). The peak concentrations were reached 3 days before parturition and on the day of parturition in the singleton and twin-bearing groups respectively (Fig. 1c).

During the course of this study, one twin-embryo recipient cow (cow F) delivered stillborn calves (male weighing 27 kg, female weighing 18 kg) on day 254 of gestation (Fig. 2). Another twin-embryo recipient cow (cow S) gave birth to only one calf, a monstrous...
schistosomus reflexus calf (67.5 kg), on day 274 of gestation (Fig. 3). Cow F expelled a grossly discolored placenta. The plasma bP AG concentration profile of cow F from day 20 to about day 200 postestrus paralleled the mean profile of that of the twin-bearing group (Figs 1 and 2), but thereafter the concentration started to decline until, between days 210 (day 70 prepartum) and 250 (day 30 prepartum), it was about 50% less than that in the twin-bearing group (70.0 and 69.5 ng/ml in cow F; 114.2 ± 27.3 and 143.6 ± 40.4 ng/ml in the twin-bearing group at day 250). In addition, the dramatic prepartum increase in the plasma bP AG concentrations in normal pregnancies (Fig. 1c) was not observed in cow F (Fig. 2).

Cow S had a peripheral plasma bP AG concentration profile comparable to that of the twin-bearing group up to about day 150 postestrus (Figs 1a and 3), but thereafter the concentrations increased rapidly in cow S, in contrast to the progressive increase seen in the twin-bearing group, and was 134.5 ng/ml by day 90 prepartum (compared with 80.4 ± 17.2 ng/ml in the twin-bearing group), 294.9 ng/ml by day 50 prepartum (144.3 ± 40.5 ng/ml in the twin-bearing group) and 424.5 ng/ml by day 20 prepartum (155.8 ± 36.6 ng/ml in the twin-bearing group). The change in bP AG concentrations between day 10 prepartum and parturition in cow S (from 557.7 to 1164 ng/ml) was comparable to

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**Table 1** Least square means (± S.E.M.) of plasma bPAG concentrations in singleton and twin-bearing cows throughout gestation.

<table>
<thead>
<tr>
<th></th>
<th>1st trimester</th>
<th>2nd trimester</th>
<th>3rd trimester</th>
<th>Last 10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singleton</td>
<td>5.7(0.57)</td>
<td>29.9(1.4)</td>
<td>77.9(4.6)</td>
<td>1322.7(58.4)</td>
</tr>
<tr>
<td>Twin-bearing</td>
<td>10.4(0.59)</td>
<td>43.8(14)</td>
<td>129.9(5.2)</td>
<td>1342.9(64.4)</td>
</tr>
</tbody>
</table>

n, Number of animals. a Trimester represents 90 days of pregnancy. b Excluding last 10 days of gestation. c Including parturition day. All four values within the singleton group are significantly different (P < 0.001), as are those within the twin-bearing group (P < 0.001). At each stage of pregnancy except the last 10 days of gestation, values for the singleton group differ significantly (P < 0.001) from those for the twin-bearing group.
that in the singleton group (from 493.0±75.3 to 1352.8±286.5 ng/ml; Figs 1c and 3).

Discussion
To the best of our knowledge, this study is the first to characterize bP AG profiles from the day of embryo transfer to early postpartum, with frequent and consistent sampling in singleton and twin-bearing cows. The time of detection and biphasic profile of bP AG in singleton cows was consistent with the initial report (9). There are no reports in the literature on the interaction between the stage of gestation or fetal number and peripheral concentrations of bP AG. The present investigation has demonstrated a significant interaction between the stage of gestation and concentrations of bP AG (P<0.001), and has revealed that fetal number does influence the peripheral concentrations of bP AG (P<0.001), except in the last 10 days of gestation, in cattle. Comparison of the basic structures of PSPB and bP AG (16, 17) suggests that these proteins are related and, correspondingly, the present findings, together with those of our earlier study involving PSPB (13), show that the peripheral profiles of these two hormones are parallel in singleton and twin-bearing cows, although our results also reveal that the concentrations of these hormones in the peripheral circulation of the cow are different. Likewise, the influence of the fetus on the prepartum concentrations of these proteins is also diverse, particularly with regards to PSPB (13). These disparate concentrations and fetal influences thus suggest a discrete role for the two proteins in their conjectured association with the parturition process.

Morphological changes signaling implantation in the cow commence at about day 20 near the embryo (18). Subsequently, the high (P = 0.06) bP AG concentrations measurable from day 30 onwards in the twin-bearing cows are a direct result of dual attachment points and enhanced synthetic activity of twin placentas. The reduction in bP AG concentrations near term in the singleton group corresponds with the antepartum reduction in the binucleate cell population that is observed in ruminants (19). This decline in concentration is indistinct in the twin-bearing group, most probably because of the presence of twin placentas. Although the progesterone concentration is identified commercially as an aid to the diagnosis of pregnancy in the cow, it is not pregnancy-specific and its accuracy is variable (20). In addition, reports on the effect of the number of fetuses being carried on progesterone concentration are conflicting (11, 21–23), suggesting that bP AG, in common with PSPB (8, 12, 13) and PSP-60 (7), offers a better serological test for the diagnosis of pregnancy and for distinguishing the number of fetuses being carried. The large individual variation in bP AG concentrations at all stages of gestation in the present study could be attributable to the limited number of animals used. Hence, further studies in a greater number of animals are needed to justify the use of bP AG concentration as an accurate criterion for commercially predicting fetal number in cattle.

Hormones of feto–placental origin, notably glyco-proteins, are used widely in humans to monitor fetal growth and conformity (3), and abnormal concentrations are a characteristic finding in women carrying malformed fetuses (3). The exact time or cause of fetal loss in our cow S is uncertain; however, some reports indicate that most fetal losses occur after day 35 in cows carrying multiple fetuses (22, 24). In addition, our earlier work (13) together with that of Dobson et al. (11), found that loss of a single fetus in a multiple gestation did not significantly alter the peripheral PSPB profile. Similarly, loss of a single fetus in this particular cow appeared not to influence the peripheral
concentrations of bPAG. The fetus directly regulates the source of PSPB, bPAG and PSP-60 in ruminants (19), therefore the abnormal concentrations of this protein in cow S are a direct reflection of either mutated signals from the fetus or the gigantic size of the fetus. Currrently, no biochemical markers have been identified in cows that could allow sequential monitoring of fetal well-being. Although our present findings, together with those concerning PSPB (13), are based on an individual case, they indicate the possibility of gauging fetal conformity, as in humans, by the characterization of pregnancy-specific glycoproteins. The abrupt deviation in the bPAG concentration profile after day 200 of gestation in cow F, together with the gross discoloration of the placenta, suggest that in this animal fetal death was secondary to a compromised placenta. Some abortifacient agents alter the binucleate cell morphology (25), thereby affecting synthetic output without any characteristic gross lesions in either the fetus or the placenta. However, we lack definite proof of a link between the premature birth, fetal death and placental discoloration in cow F and one of these agents. The profile of changes in bPAG concentration is nevertheless as potent an indicator of feto–placental viability as are those of PSPB (12, 13) and PSP-60 (7).

To conclude, our findings indicate that peripheral bPAG concentrations are correlated to the stage of gestation and number of fetuses, and that the profile of peripheral bPAG concentrations is a useful indication of the feto–placental status.

Acknowledgements

We thank Drs N Takenouchi (Chugoku National Agricultural Experimental Station), K Iwama (Kyoritsu Shoji Co., Ltd), M Kobayashi (Morinaga Milk Industry Co., Ltd), N Kusakari (Takikawa Animal Husbandry Experimental Station) and T Iwahori (Shizuoka Agricultural Experiment Station) for their assistance with sample collection, T Tomizuka, T Kojima and K Hashizume of the National Institute of Animal Industry for their help, and H Kamomae of Tokyo University of Agriculture and Technology for valuable advice. Part of this work that was carried out at the University of Liege was kindly supported by grants from IRSIA and FNRS. O V Patel was receiving a PhD fellowship from the Japanese Ministry of Education (Monbusho).

References


Received 4 February 1997
Accepted 22 May 1997