Pregnancy-specific protein B and progesterone concentrations in French Alpine goats throughout gestation

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Summary. The 34 French Alpine dairy goats originated from a single flock and were artificially inseminated 44 h after synchronization of oestrus. They were bled daily at the jugular vein from 15 to 27 days after AI. An early pregnancy diagnosis by RIA of progesterone concentration was performed 21 days after AI. In pregnant goats (>1.5 ng progesterone/ml) daily sampling was extended until 30 days after AI and, from those, 9 were bled every 2 weeks until the end of pregnancy and at 50 and 63 days post partum. Pregnancy-specific protein B (PSPB) was also assayed.

The kidding rate was 67.6% (23/34). PSPB concentrations (ng/ml) in pregnant goats were significantly different from those of non-pregnant goats at 24 days after AI (0.82 ± 0.18 vs 1.78 ± 0.19; mean ± s.e.m.) and rose to 40 ng/ml at the end of pregnancy. From Day 25 and throughout gestation, females with 2 fetuses had higher PSPB concentrations than did those with a single fetus (P < 0.05). In the 2 goats exhibiting late embryonic mortality according to progesterone concentrations, one had a PSPB profile very similar to those of pregnant goats until 30 days while the other did not show any elevation of PSPB concentration. It is concluded that PSPB profiles in goats are similar to those found in cows throughout pregnancy and that PSPB RIA may be useful for pregnancy diagnosis or diagnosis of late embryonic mortality.

Keywords: PSPB; progesterone; pregnancy; embryonic mortality; goat

Introduction

In ruminants during early pregnancy several protein signals originating from the conceptus and of an antiluteolytic (ewe: Martal et al., 1979; Godkin et al., 1982, 1984; Vallet et al., 1988; Charpigny et al., 1988; cow: Knickerbocker et al., 1986) or immunosuppressive (ewe: Segerson, 1981; cow: Fisher et al., 1985; Klima et al., 1987) nature have been identified. None of those signals has yet been found in the peripheral circulation. It is therefore not possible to use these signals to determine the presence of a living conceptus or to diagnose embryonic mortality.

Several proteins have been identified at later stages of pregnancy (Sasser et al., 1986; Beckers et al., 1988; Godkin et al., 1988; Martal et al., 1988). Among those, PSPB (pregnancy-specific protein B) has been isolated from cattle embryos at Day 24 to Day 40 (Butler et al., 1982) and found in the peripheral circulation of some pregnant animals as early as Day 20 (Sasser et al., 1986). This protein has been found in various ruminant species (mountain goat: Houston et al., 1986; sheep: Ruder et al., 1988; deer: Wood et al., 1986) and has been extensively tested in cattle for pregnancy determination and diagnosis of embryonic death (Humblot et al., 1988a, b).

This study with French Alpine dairy goats was undertaken to determine PSPB concentrations after AI throughout pregnancy or when pregnancy failed.
Materials and Methods

Animals and samples. A total of 34 French Alpine goats from a single flock were artificially inseminated in July 1986, i.e. before the breeding season. Oestrous cycles were previously synchronized by an 11-day insertion of a vaginal sponge impregnated with 45 mg fluorogestone acetate (Chronogest: Intervet, Angers, France) and intramuscular injections of 400 i.u. PMSG (Folligon: Intervet) and 200 µg cloprostenol (Estrumate: Coopers, Meaux, France) 2 days before sponge removal. Artificial inseminations (AI) were performed with frozen semen 44 h after sponge removal.

All goats were bled daily from Day 15 to 27 after AI. An early pregnancy diagnosis was performed 21 days after AI by measurement of progesterone concentrations. In goats found pregnant at that time (i.e. with progesterone concentrations >1.5 ng/ml) daily sampling was extended until 30 days after AI and pregnancy was tested again at 40 days after AI by real time ultrasonic scanning (Toshiba SAL 32 A). Of those females found to be pregnant, 9 were bled every 3 weeks until the end of pregnancy and at 50 and 63 days post partum.

Peripheral blood (15 ml) was collected from the jugular vein into heparinized vacutainers (Beckton Dickinson, Grenoble, France). Plasma was separated by centrifugation (W.O.Og) and stored at -20°C until assays for progesterone and PSPB.

Radioimmunoassays. An assay procedure previously described and validated by Thibier & Saumande (1975) was used to determine progesterone concentrations. A monoclonal antibody (531/b; Clonatec, Paris, France) raised against progesterone-11α-succinyl-BSA was used at a final dilution of 1:80 000. This antiserum displayed very low cross-reactivity with steroids susceptible to interference in goat plasma; cross-reactivity with 17α-hydroxyprogesterone was <0.5%. The sensitivity of the assay was 0.05 ng/ml. Inter- and intra-assay standard deviations were 0.08 and 0.02 when duplicate estimates were run (6 assays) for a 0.4 ng/ml sample of reference plasma.

[Diagram: Log R37 PSPB (ng/tube) vs. % 125I-labelled PSPB bound]
A heterologous system (Willard et al., 1987) derived from the method previously described by Sasser et al. (1986) was used to measure PSPB. Rabbit antiserum to sheep PSPB (RGS 45-1) was used at a final dilution of 1:100 000. Bovine PSPB (preparation R 37) was used as the labelled and standard protein in the RIA. This heterologous system was used to assay PSPB in goat plasma because the parallelism between the standard curve and serial dilutions of goat plasma was better than with the homologous bovine system used routinely for PSPB assay in cattle plasma or serum. Effectively, for successive dilutions of the same goat plasma sampled at the end of pregnancy (i.e. with expected high concentrations), the displacement in PSPB binding was much wider when using the rabbit antiserum to sheep PSPB than when using the antiserum to bovine PSPB (Fig. 1). The PSPB concentrations for the dilutions of this plasma with the antiserum to sheep PSPB were in the range of 0·4 (dilution 1/10) to 2·5 ng/tube (undiluted) which corresponds to the zone of maximum slope for this system, whereas with the antiserum to bovine PSPB, the estimated amounts of successive dilutions were included between 0·2 (dilution 1/10) and 0·5 ng/tube (undiluted).

With the antiserum to ovine PSPB, mean sensitivity of the standard curve was 200 pg per assay tube. Intra- and inter-assay variation were similar: inter-assay standard deviations (4 assays) were 0·34 and 6·3 respectively for reference goat plasma samples of 1 ng/ml and 41 ng/ml.

Data analysis. At all stages of pregnancy, PSPB concentrations were considered significantly different from 0 when the two B/Bo replicates were <90% of buffer control. Data are presented as mean ± standard error of the mean (s.e.m.). Differences between pregnant and non-pregnant animals for progesterone and PSPB concentrations between Days 15 and 27 of pregnancy were analysed by Student’s *t* tests. Finally, the effects of the number of conceptuses and gestational age on progesterone and PSPB concentrations were studied by a two-way non-orthogonal analysis of variance (Dagnelie, 1984).

Results

The results of the progesterone and PSPB radioimmunoassays, real-time ultrasonic scanning and kidding data are given in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Progesterone* (ng/ml)</th>
<th>PSPB†</th>
<th>Ultrasonic scanning</th>
<th>Return to oestrus (days)</th>
<th>Kidding</th>
<th>No. of goats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;1·5</td>
<td>Low</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7 (20·6%)</td>
</tr>
<tr>
<td>2</td>
<td>&gt;1·5</td>
<td>Low</td>
<td>Not pregnant</td>
<td>None</td>
<td>1</td>
<td>2 (2·9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Elevated</td>
<td>Pregnant</td>
<td>76</td>
<td>1</td>
<td>2 (2·9%)</td>
</tr>
<tr>
<td>3</td>
<td>&gt;1·5</td>
<td>Elevated</td>
<td>Pregnant</td>
<td>131, 141‡</td>
<td>2</td>
<td>23 (67·6%)</td>
</tr>
<tr>
<td></td>
<td>&gt;1·5</td>
<td>Elevated</td>
<td>Pregnant</td>
<td>None</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

*Between Days 21 and 24.
†Between Days 24 and 27.
‡Fetuses were seen at abortion.

The kidding rate was 67·6% (23/34): 7 goats (20·6%) were found to be non-pregnant by progesterone assay 21 days after AI and 4 goats were diagnosed as pregnant at 21 days but did not kid. Of these 4 goats, 2 aborted, expelling dead fetuses on Days 131 and 141 and returning to oestrus soon after. Progesterone and PSPB profiles of those females between Days 15 and 30 were analysed with those of the pregnant goats. In the other 2 goats, pregnancy was interrupted sooner as shown by the results of ultrasonic scanning or return to oestrus.

Mean progesterone profiles from animals of Groups 1 and 3 are illustrated in Fig. 2. In non-pregnant animals of Group 1, the mean progesterone concentration at Day 15 was 5·25 ng/ml. This concentration decreased quickly to mean values <2 ng/ml at Day 20. Low concentrations were observed until Day 25 and increased to reach 3 ng/ml at Day 27. Although constantly lower, mean progesterone concentrations in non-pregnant animals were significantly different from those observed in pregnant females at Day 18 (3 ± 0·67 vs 6·81 ± 0·45; P < 0·05).

There was no trend for an increase or a decrease of mean progesterone concentrations throughout pregnancy in pregnant animals (Group 3). No difference in terms of progesterone concentrations was found between females with one or several conceptuses.
In the 2 goats which had a prolongation of luteal function and subsequently found to be non-pregnant (Group 2), progesterone concentrations between Days 15 and 27 were similar to those of pregnant goats.

Figure 3 illustrates mean PSPB concentrations in non-pregnant (Group 1) and pregnant goats (Group 3). In non-pregnant goats mean PSPB concentrations were always ≤ 1·1 ng/ml. The mean concentration in pregnant animals was significantly different from the concentration observed in non-pregnant ones at Day 24 (0·82 ± 0·18 and 1·78 ± 0·19 ng/ml; P < 0·05). In pregnant animals, mean concentration was doubled at Day 26 (3·37 ± 0·29 ng/ml). Maximum mean concentrations of 40 ng/ml were observed at Days 107 and 121 and at these stages varied among individuals from 20 to 80 ng/ml. PSPB values fell to basal concentrations within 50 days after parturition.

From Days 22 to 30, individual PSPB concentrations of pregnant animals were highly correlated to the concentrations measured over the following days (Table 2). At each stage studied no correlation was found between progesterone and PSPB concentrations.

Goats which had 2 conceptuses or more presented higher PSPB concentrations than did those carrying a single fetus (P < 0·05). An interaction between the so-called type of animal (1 or 2 fetuses present) and the stage of pregnancy was found (P < 0·01), showing that this difference was limited to the period between Day 25 and birth (Fig. 3). In the 2 goats of Group 2 that were diagnosed as non-pregnant by 40–76 days, Female 336 (diagnosed as non-pregnant by ultrasonic scanning) exhibited a PSPB profile similar to those in goats that were diagnosed non-pregnant by the progesterone RIA as early as 21 days after AI. By contrast, Female 469 found pregnant at 40 days and observed in oestrus 76 days after AI had a rise of PSPB concentrations similar to that of the pregnant goats during the first 30 days of pregnancy.

Discussion

The kidding rate was very close to that usually reported for French dairy goats after synchronization of oestrus (Corteel et al., 1984). Late embryonic mortality and abortions represented the lower
Fig. 3. Mean PSPB concentrations (± s.e.m.) in non-pregnant goats of Group 1 (N = 7, \(\triangle \cdots \triangle\)) and in pregnant females with a single conceptus (N = 4, \(\bigcirc \bigcirc\)) or several conceptuses (N = 21, \(\bullet \bullet \bullet\)).

Table 2. Correlation coefficients between PSPB concentrations at Days 22–30 after AI for goats in Group 3

<table>
<thead>
<tr>
<th>Stage of pregnancy (days)</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
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<tbody>
<tr>
<td>22</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>0.64</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.58</td>
<td>0.69</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.51</td>
<td>0.78</td>
<td>0.75</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>0.45*</td>
<td>0.70</td>
<td>0.64</td>
<td>0.73</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>0.48*</td>
<td>0.58</td>
<td>0.68</td>
<td>0.76</td>
<td>0.84</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>0.53</td>
<td>0.64</td>
<td>0.68</td>
<td>0.79</td>
<td>0.86</td>
<td>0.92</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>0.51</td>
<td>0.56</td>
<td>0.69</td>
<td>0.70</td>
<td>0.83</td>
<td>0.92</td>
<td>0.90</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.43*</td>
<td>0.47*</td>
<td>0.60</td>
<td>0.53</td>
<td>0.65</td>
<td>0.74</td>
<td>0.82</td>
<td>0.81</td>
<td>1</td>
</tr>
</tbody>
</table>

All coefficients significant at \(P < 0.01\) except *\(P < 0.05\).
part of pregnancy failures. Most of the non-pregnant goats (>60%) at the end of the experiment were detected as such by progesterone RIA at 21 days. When considering information obtained in the cow, on the effect of embryonic death on corpus luteum function (Northey & French, 1980; Humblot & Dalla Porta, 1984) it seems reasonable to assume that those goats had not been fertilized or had experienced early embryonic death. The frequency (20-60%) of females in this condition is very similar to the data reported by Lyngset (1968). As previously shown by Thorburn & Schneider (1972) and Thibier et al. (1980), progesterone values between 21 and 24 days in those goats were close to or lower than 1 ng/ml.

Although constantly low, plasma PSPB concentrations in the 7 non-pregnant goats were detectable, contrary to findings for cows (Humblot et al., 1988a). The goats were sampled a long time after the previous kidding and this rules out possible interference with post-partum secretion of PSPB. Detectable levels of PSPB in some non-pregnant animals may be explained by cross-reactivity of antiserum with serum proteins.

In pregnant animals, mean progesterone concentrations were in the range of those found by Thorburn & Schneider (1972) and Irving et al. (1972) but no elevation of progesterone concentrations with the stage of gestation was noticed. As reported for the ewe with the bovine PSPB assay (Ruder et al., 1988), Day 24 was the first day at which mean PSPB concentrations were different in pregnant (Group 3) and non-pregnant animals from Group 1. For sheep, the first day of detection, using the current heterologous assay, was Day 21 (Willard et al., 1987). Large differences in the potential of pregnant goats to produce PSPB existed. This is illustrated by the first day at which PSPB concentrations were higher than in Group 1 females and further shown by correlations of PSPB concentrations between Days 22 and 30 of pregnancy.

No relationship was found between progesterone and PSPB concentrations in plasma, suggesting that in these physiological conditions there is no effect of each compound on the production of the other. Similar data for all those points were previously reported for the cow (Sasser et al., 1986; Humblot et al., 1988a).

Finally, PSPB production seems to be related to the number of conceptuses. Goats with 2 or more conceptuses expressed an earlier rise in PSPB concentrations and presented mean concentrations higher than in those with a single embryo. Despite this, individual variation in PSPB concentrations was too high to suggest that PSPB assay could be used as an accurate method for twinning diagnosis.

Contrary to what has been shown for the mountain goat breed and with a less sensitive assay (Houston et al., 1986), detectable PSPB values were found in this study at 50 days after kidding, especially in females which gave birth to 2 or more kids. This is very similar to earlier results obtained for the cow (Humblot et al., 1988c). PSPB values fall earlier in the ewe, by 25–30 days post partum (Willard et al., 1987).

Only 2 goats (i.e. 6% of all the goats) expressed late embryonic mortality according to the progesterone profiles. This percentage is low and explains the high accuracy of the positive pregnancy diagnoses by progesterone RIA generally reported for goats (De Montigny et al., 1982; Jardon et al., 1984). Conversely to what has been reported for the cow (Humblot et al., 1988a), progesterone concentrations of these females between 15 and 30 days after AI were similar to those of pregnant ones.

As in the cow (Humblot et al., 1988a) these results show that two types of PSPB profiles exist in cases of late embryonic death. This may reflect the different times at which late embryonic mortality occurs.

In conclusion, distinct patterns of circulatory PSPB are found in goats after AI according to their physiological status and advantage of this fact may be taken for monitoring early pregnancy or for detecting failures of gestation.

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