CAPRINE PLACENTAL LACTOGEN:
LEVELS OF PROLACTIN-LIKE AND GROWTH HORMONE-LIKE
ACTIVITIES IN THE CIRCULATION OF PREGNANT GOATS
DETERMINED BY RADIORECEPTOR ASSAYS

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SUMMARY

Radioreceptor assays for prolactin-like (lactogenic) activity and growth hormone (GH)-like activity have been used to study concentrations of caprine placental lactogen (PL) in the circulation during pregnancy. Both lactogenic and GH-like activities increased from less than 100 ng/ml (ovine prolactin- and human GH-equivalents) about 60 days after mating to reach peak levels (400–1600 ng/ml) between days 110 and 130 of pregnancy. The levels of both activities increased in essentially the same fashion but during the last 15 days of pregnancy, lactogenic activity declined less than GH-like activity. This divergence was most pronounced at parturition when levels of lactogenic activity increased (∼ 700 ng/ml) despite very low (< 200 ng/ml) levels of GH-like activity being measured and this probably reflected increased secretion of pituitary prolactin near parturition. When serum from a pregnant goat or a simple alkaline extract of placental cotyledons was fractionated on a column packed with Sephadex G-100, lactogenic and GH-like activities eluted together with distribution coefficients of approximately 0.5–0.6. The possibility that caprine PL serves physiologically as a luteotrophin and/or mammotrophin during pregnancy in goats is discussed.

INTRODUCTION

Using an in-vitro bioassay procedure for prolactin and other lactogenic hormones, Buttfe, Forsyth & Knaggs (1972) were able to detect, in pregnant goats, high levels of lactogenic activity in the circulation which was immunologically distinct from caprine pituitary prolactin. Co-culture of placental and mammary tissue indicated the likely placental origin of this activity which Buttfe et al. (1972) referred to as caprine placental lactogen (caprine PL).

The recent development of relatively sensitive assay methods for protein hormones, using membrane fragments from known target organs, in particular the use of mammary receptors for lactogenic activity (Shiu, Kelly & Friesen, 1973), has enabled the detection in
several additional non-primate species of hitherto unknown or poorly characterized placental lactogens (Shiu et al. 1973; Kelly, Robertson & Friesen, 1974; Kelly, Tsushima, Shiu & Friesen, 1976).

The present interest in caprine placental lactogen is an extension of earlier efforts (Thorburn, Nicol, Bassett, Shutt & Cox, 1972; Currie, Wong, Cox & Thorburn, 1973) to provide detailed comparative hormonal data on the goat and sheep during pregnancy and at parturition. Hormonal similarities in closely related species such as these, considered together with the distinct specializations already described for these species, may help assess the relative importance of the various factors in the regulatory mechanisms of pregnancy.

Kelly et al. (1974) suggested that the temporal correlation between changes in levels of placental lactogen and progesterone in the circulation of sheep might reflect a regulatory role for PL in placental steroidogenesis in that species. Since pregnancy in goats is critically dependent upon continued luteal secretion of progesterone (Meites, Webster, Young, Thorp & Hatch, 1951; Thorburn & Schneider, 1972; Irving, Jones & Knifton, 1972), interest in the goat has focused on the nature of luteotrophic influences present during pregnancy. An earlier study (Currie & Thorburn, 1974) prompted the suggestion that the goat placenta may produce a luteotrophic hormone.

As a preliminary step in the investigation of the possible identity of this putative luteotrophin with caprine PL, this paper presents detailed information on the changes during pregnancy in levels of lactogenic activity and progesterone in the circulation. Since preliminary studies indicated that both lactogenic and growth hormone (GH)-like activities were present in goat plasma and placental tissue, assays were extended to include a description of changes in the latter. Both receptor assays used in this study fail to discriminate between the placental hormone and the respective pituitary hormones so that grossly increased levels of lactogenic or GH-like activities can be equated only tentatively with caprine PL.

**METHODS**

*Animals and sampling*

Four Saanen goats, two of which subsequently delivered twin kids and two which delivered single kids, were kept at pasture throughout pregnancy at the Ian Clunies Ross Animal Research Laboratory, Prospect, Australia. The day of mating was recorded by the use of a marking crayon (Radford, Watson & Wood, 1960) fitted to the buck. Blood was obtained by jugular venipuncture and samples were transferred to heparinized tubes in melting ice for no longer than 30 min before separating the plasma by centrifugation at 4 °C. Samples were obtained in the same manner from four additional Saanen goats during late pregnancy; these were housed indoors for intensive sampling as part of another experiment.

Plasma was stored at −20 °C until analysed for progesterone. Aliquots (1 ml) were lyophilized for transport and subsequently reconstituted with 1 ml distilled water for measurement of lactogenic and GH-like activities in Winnipeg.

*Plasma hormone assays*

Progesterone concentrations were measured on n-hexane extracts of plasma using the protein-binding assay described and validated in a previous paper (Thorburn & Schneider, 1972). The progesterone data presented here for individual animals have been published with data from other animals in the form of means for monotocous and ditocous goats in the paper cited above.

Concentrations of lactogenic and GH-like activities were measured by radioreceptor assays using particulate membrane preparations of mammary gland (Shiu et al. 1973; Shiu & Friesen, 1974) and liver (Tsushima & Friesen, 1973), both obtained from rabbits in
late pregnancy. Ovine prolactin (NIH-P-S-10, 26 i.u./mg) was used to prepare $^{125}$I-labelled tracer hormone as described by Shiue & Friesen (1974) and for preparing standards for the lactogenic assay: lactogenic activity is therefore expressed in ovine prolactin equivalents throughout this paper. Human growth hormone (NIH-HS-1648E, 2 i.u./mg) was used to prepare $^{125}$I-labelled tracer hormone and standards for the assay of GH-like activity which is therefore expressed in human GH-equivalents. Human GH was used because it was the highest potency GH available when this study was conducted. Iodinated human GH was a relatively stable tracer preparation and retained its high capacity to bind to liver membranes without repurification. The binding sites in liver membranes of pregnant rabbits appeared to interact primarily with the GH determinants rather than the lactogenic regions of $^{125}$I-labelled human GH. Various GH preparations competed with $^{125}$I-labelled human GH with relative binding directly related to their bioassayed potencies whereas prolactin offered only minimal competition (Tsushima & Friesen, 1973).

When performing preliminary radioreceptor assays on plasma samples, it became evident that fibrin clots occasionally formed in the assay tubes, possibly as a result of minimal heparinization of the samples and the use of 10 mM-MgCl$_2$ in the assay buffer. Clot formation was prevented by the addition of 1.25 i.u. heparin/ml to the buffer and this was used throughout. Other preliminary studies indicated a depressant effect of increasing concentrations of male goat plasma on the binding of tracer hormones and while this was minimal when 25 µl plasma was incubated in a final volume of 500 µl (5%, v/v), 25 µl male goat plasma was substituted for 25 µl assay buffer in the tubes containing standards.

The recovery of caprine PL from goat plasma was assessed using the two radioreceptor assays by enriching 900 µl aliquots of male goat plasma with 100 µl assay buffer containing various amounts of caprine PL, isolated by procedures to be described in a forthcoming paper. The caprine PL contained equal amounts of lactogenic and GH-like activities when assayed in the radioreceptor assays using plasma-free incubation systems. After incubation of the enriched plasma samples for 60 min at room temperature, they were assayed in quadruplicate in the standard plasma assays described in the preceding paragraph.

**Gel filtration studies**

A 7.5 ml sample of peripheral serum collected from a ditocous goat on day 126 of pregnancy was fractionated on a column (1.7 cm internal diameter, 93 cm long) packed with Sephadex G-100 using 50 mM-ammonium bicarbonate, adjusted with ammonium hydroxide to pH 8.5, as the eluant. The column was calibrated with Blue Dextran 2000 (void volume) and Na$^{125}$I (bed volume). Fractions of 2 ml were collected, protein was estimated by absorbance at 280 nm and lactogenic and GH-like activities were determined by radioreceptor assays. Assays were performed using the systems described above but with 100 µl sample size. In addition, to minimize spurious buffer effects, 100 µl of a pool of fractions eluting before the void volume ($K_{av} = 0$) was substituted for 100 µl of assay buffer in the tubes containing standards.

A crude extract of placental cotyledons was similarly fractionated. Foetal cotyledonal tissue was dissected from a goat placenta delivered vaginally at term, homogenized briefly in 5 vol. 100 mM-ammonium bicarbonate, adjusted with ammonium hydroxide to pH 8.7, and extracted overnight at 4 °C. After centrifugation for 90 min at 100000 g, 7.5 ml of the supernatant (equivalent to 1.3 g wet tissue) was fractionated on the column packed with Sephadex G-100 and the fractions were assayed for protein, lactogenic and GH-like activities as described above.
RESULTS

A preliminary assessment of the pattern of displacement of $^{125}$I-labelled tracer hormones from particulate preparations of rabbit liver and mammary gland in the presence of increasing amounts of male goat plasma was performed. Regressions fitted to the data on the portion of the displacement curves where c.p.m. bound and log-concentration of unlabelled hormone were linearly related indicated that 20% plasma resulted in a significant depression of binding whereas no differences were detected in either slope or position of curves fitted to data from standard curves when the tubes contained 0, 5 or 10% plasma. Notwithstanding these observations, all plasma samples in this study were assayed using 25 µl (5%, v/v) plasma against standards containing an equal proportion of male goat plasma. Interpolations of concentrations were made only in the linear region of the standard curves.

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Measurement of purified caprine placental lactogen added to male goat plasma. The quantity of caprine placental lactogen added is expressed in prolactin- and growth hormone-equivalents since these estimates were identical. The measured concentrations of lactogenic (●) and growth hormone-like (○) activities are shown relative to the line of equivalence (…….).

When highly purified caprine PL was added to male goat plasma and subsequently assayed by the above procedures, recoveries (means ± S.E.M.) of 86.2 ± 6.3% lactogenic activity and 94.4 ± 7.2% GH-like activity were obtained (Fig. 1). Increased variation amongst replicate determinations was evident at the highest concentration of lactogenic activity but such measurements were obtained from the upper limit of the standard curve for the mammary gland radioreceptor assay.

The pattern of change in levels of lactogenic and GH-like activities in the plasma of goats during pregnancy is shown along with progesterone concentrations in Fig. 2a, b. Concentrations of the protein hormone activities were at or near the limit of sensitivity of the par-
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particular assay until day 50 of pregnancy: the levels are therefore shown as being < 50 ng/ml. However, concentrations of one or both activities were detectably increased by 65-70 days and perhaps even a little earlier in ditocous goats. Further increases occurred in a reasonably consistent fashion for both lactogenic and GH-like activities and maximal concentrations were reached during the last third of pregnancy. With the exception of goat 87, changes in levels of the two activities were closely correlated and highly significant linear relationships existed between the measurements. Differences between regression slopes (P < 0.001) precluded using the overall regression so that the ratio of GH-like activity to lactogenic activity was computed independently (Table 1). The overall ratio was 1.06 but there were clearly differences among animals as to which of the two activities was greater.

Changes during the last 25 days of pregnancy are shown in Fig. 3 as a composite for a group of up to eight animals. There were no significant differences between measured levels of lactogenic and GH-like activities until the last 3 days but a divergence in mean concentrations was apparent during the last 12 days of pregnancy. Prolactin-like activity exceeded GH-like activity throughout this time and did not exhibit a general decline except 3 and 2 days before parturition. During the last 36 h, levels of lactogenic activity in the circulation actually increased despite a continuing decline in GH-like activity.

With the exception of goat 158, changes in the concentrations of progesterone in the circulation during pregnancy bore no obvious relation to changes in lactogenic or GH-like activities. Some increase in progesterone concentration occurred about the time when the increase in these activities was first detected but insufficient samples were collected to assess the significance of such changes. In goat 158, there was a distinct augmentation of progesterone concentrations during the second half of pregnancy and the levels appeared to increase in a similar fashion to those of lactogenic and GH-like activities. Although not readily apparent in the data presented here, progesterone concentrations fell abruptly about 24 h before foetal delivery (see Discussion) and this change occurred some 2 days after the less marked changes in GH-like and lactogenic activities as noted above.

The pattern of elution of lactogenic and GH-like activities upon gel filtration of serum of late pregnancy using Sephadex G-100 is shown in Fig. 4a. The activities co-eluted with a distribution coefficient (Kav) of approximately 0.5. Measured levels of GH-like activity were approximately twice those of lactogenic activity in the eluted fractions but the pattern of elution was essentially identical.

When a crude placental extract was fractionated using identical operating conditions, the two activities again co-eluted but with almost identical measured concentrations (Fig. 4b). Fractions containing the highest concentrations of lactogenic and GH-like activity eluted with Kav = 0.54-0.58.

DISCUSSION

The data presented here provide confirmation and extension of the demonstration by Buttle et al. (1972) of the existence in goats of a prolactin-like hormone derived from the placenta. Radio-receptor assays for lactogenic and GH-like activities have greatly facilitated studies of this kind since they are easier to perform than bioassays in vivo or in vitro, and sensitivities of the order of 30-50 ng/ml (ovine prolactin- or human GH-equivalents) are readily attained. A further increase in sensitivity of some 20-fold might be expected should a radio-immunoassay for caprine PL be available, and such a development appears necessary to define precisely the changes in levels of caprine PL in the circulation during the first third of pregnancy.

There were clearly two distinct hormone-like activities in the samples investigated with the concentrations of lactogenic and GH-like activities in the circulation being closely correlated during most of pregnancy. Subsequent attempts to isolate and purify caprine PL
Fig. 2. Changes in caprine placental lactogenic and growth hormone-like activities and progesterone concentration (---) during monotocous pregnancy in goats 138 and 158 and ditocous pregnancy in goats 87 and 151. Lactogenic activity is expressed in ovine prolactin-equivalents (●) and growth hormone-like activity in human growth hormone-equivalents (○). The last samples were collected on the day of parturition.
Fig. 2. For legend see opposite.
Table 1. Relationship between measured concentrations of GH-like and lactogenic activities in plasma samples collected from goats after day 60 of pregnancy

<table>
<thead>
<tr>
<th>Goat</th>
<th>n</th>
<th>( \hat{r} )</th>
<th>( \ddot{\text{GH}} ).Prolactin</th>
<th><strong>GH-like activity</strong></th>
<th>Lactogenic activity (mean ± s.e.m.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>87</td>
<td>30</td>
<td>0.5423</td>
<td>0.4225 ( P &lt; 0.01 )</td>
<td>0.88 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>41</td>
<td>0.8453</td>
<td>0.5779 ( P &lt; 0.001 )</td>
<td>0.57 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>151</td>
<td>34</td>
<td>0.9222</td>
<td>1.4065 ( P &lt; 0.001 )</td>
<td>1.32 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>158</td>
<td>36</td>
<td>0.9015</td>
<td>1.0724 ( P &lt; 0.001 )</td>
<td>1.32 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>All data</td>
<td>141</td>
<td>0.655</td>
<td>0.8751 ( P &lt; 0.001 )</td>
<td>1.06 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Differences between individual regressions \( F_{3.128} = 28.01, P < 0.001 \)
* Ratio of activities in individual samples.

Fig. 3. Changes in plasma levels of lactogenic activity expressed in ovine prolactin-equivalents (●) and growth hormone-like activity expressed in human growth hormone-equivalents (○) before spontaneous parturition at term. Means and standard errors are shown along with the number of paired assays. The data at 36 h before parturition are means of two observations. The last samples were collected during the 6 h before foetal delivery.
Fig. 4. Gel-exclusion chromatography on Sephadex G-100 of serum from a goat in late pregnancy (a) and a crude placental extract (b). The column was operated as described under Methods and fractions (2 ml) were monitored for u.v. absorbance at 280 nm (——) and caprine placental lactogen. Lactogenic activity expressed in ovine prolactin-equivalents (●) and growth hormone-like activity expressed in human growth hormone-equivalents (○) were measured by radioreceptor assays.
using a variety of preparative techniques have also indicated that the two activities are not separable (W. B. Currie, unpublished observations). All of the above observations on the apparent identity of lactogenic and GH-like activities in caprine PL are consistent with what is known of the nature of ovine placental lactogen (Chan, Robertson & Friesen, 1976; Kelly et al. 1976). Both ovine PL and caprine PL display similar distribution coefficients to pituitary prolactin and GH upon gel-filtration using Sephadex G-100 and thus behave as having molecular weights of approximately 20000. The pattern of change in levels in the blood during pregnancy is similar for the two species, both in the increase observed during the second third of pregnancy and in the decrease occurring shortly before term. Some variation has been encountered in the relative levels of lactogenic and GH-like activities, both in the goats studied here and in similar measurements in sheep. The mean ratio for all data shown in Fig. 2 was approximately 1:1 but there were clearly significant differences among animals. Data obtained in sheep (Kelly et al. 1976) indicate higher levels of lactogenic than GH-like activities in the circulation yet ovine PL, purified from placental tissue, has consistently demonstrated near equivalent amounts of the two activities (Chan et al. 1976).

The disparate pattern of change in lactogenic and GH-like activities during the last 15 days before parturition may reflect the increased secretion of pituitary prolactin known to occur in goats during this time (Buttle et al. 1972; Hart, 1972). The mammary radioreceptor assay for lactogenic hormones does not discriminate between pituitary prolactin and the placental hormone. The radioreceptor assay would thus provide estimates of lactogenic activity at variance with measured concentrations of GH-like activity since, if there is increased pre-partum secretion of GH in the goat such as has been described in sheep (< 20 ng/ml; Bassett, Thorburn & Wallace, 1970) and cattle (Olsen, Trenkle, Witzel & McDonald, 1974), it is likely to be of far less magnitude than that of prolactin. These considerations make it likely that concentrations of GH-like activity provide a more reliable index of caprine PL levels during such times of heightened pituitary prolactin secretion.

The general lack of correlation between caprine PL levels and progesterone concentrations during most of pregnancy (with the exception of goat 158) lend little support to the notion that caprine PL may serve as a direct regulator of luteal function during pregnancy. A previous study (Currie & Thorburn, 1974) demonstrated a striking reduction in luteal function after goats were hysterectomized at a time corresponding approximately to the first detected increases in caprine PL levels shown here. It was speculated earlier that hysterectomy had effected a withdrawal of a placental luteotrophin but it remains unknown if the putative luteotrophin bears any relation to the caprine PL described here. It seems likely that the placental lactogen present in rodents (reviewed by Matthes, 1974) provides the necessary luteotrophic influence during late pregnancy in the rat and other rodents, but there is at present no direct evidence for such a role for prolactin or other lactogenic hormones in the goat. A definitive test of this function for caprine PL would be to examine the luteal response in progesterone secretion when caprine PL is administered to hysterectomized goats. Alternatively, the demonstration of specific binding sites for caprine PL on the corpus luteum of pregnant goats would provide strong supportive evidence for this suggested action of the hormone.

The sudden withdrawal of progesterone from the circulation of goats about 24 h before foetal delivery (Thorburn & Schneider, 1972; Currie, 1974) is critical for the initiation of labour in this species (Meites et al. 1951; Rawlings & Ward, 1973) and appears to result from a definite luteolytic signal (Currie & Thorburn, 1973; Currie & Thorburn, 1977). If caprine PL is shown to be the pregnancy luteotrophin in goats, then the gradual decline in caprine PL levels during the last 15 days, reflected in decreasing GH-like activity as discussed above, is unlikely to provide for precisely timed luteolysis simply as a result of inadequate
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trophic stimulation. Rather, the pattern of change in caprine PL may reflect changes occurring in the placenta during the last 15 days when increasing concentrations of corticosteroids are detected in the foetal circulation (Currie & Thorburn, 1977). A number of endocrine and physical changes in the placenta have been observed after experimentally stimulating adrenocortical secretory activity in foetal goats (Thorburn et al. 1972; Currie & Thorburn, 1977) and preliminary data (W. B. Currie, unpublished observations) indicate that decreased caprine PL secretion results after administration of corticotrophin to foetal goats.

The increase in caprine PL concentration during the second third of pregnancy corresponds reasonably to what is known of the pattern of mammogenesis (i.e. expansion of mammary parenchymal mass with ductal and alveolar proliferation) in goats (see Cowie, 1971). If the quantities of lactogenic and GH-like activities measured by the receptor assays possess the biological activities of equivalent amounts of the pituitary hormones, both of which inter alia comprise the mammogenic complex of goats (Cowie, Tindal & Yokoyama, 1966), then caprine PL may provide a powerful trophic stimulus to the mammae during pregnancy.

The possible roles of caprine PL alluded to above may be clarified when the hormone is isolated and more specific immunoassays have been developed. The increased sensitivity that might be expected should enable a more precise description of caprine PL changes in early pregnancy than was possible in this work.

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