Seasonal variation in the reproductive hormones of male goats

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The aim of this work was to study the circannual plasma profiles of LH, PRL, testosterone (T) and estrone sulfate (E1S) in different periods of the year and to characterize the possible variations in LH and PRL release patterns. We also tried to verify a possible relationship between plasma PRL fluctuations and ambient temperature, as well as the influence of an acute stress condition on levels of plasma PRL. Six adult male goats of the Ionica and Alpine breed reared in Southern Italy (40°N lat.) were subjected to frequent samplings (every 15 min for 6 h) once a month for a whole year. The blood samples were assayed for plasma concentrations of E1S, LH and PRL by radioimmunoassay, and for T by enzyme-immunoassay. The ambient temperature was recorded on each day of bleeding. Sex steroids and PRL showed marked circannual variations, with the highest levels during the summer (July) and the lowest during the winter–early spring (March). The concentrations of plasma LH did not indicate significant seasonal variations. A positive relationship was observed between plasma levels of PRL and E1S and ambient temperature. The patterns of LH release seemed to change depending on season, but this was not an obvious and common feature in all bucks. The hormone concentrations for plasma PRL profiles seemed to be influenced by an acute stress condition (associated with cannula insertion and handling procedures), with the variations more marked when the plasma levels of PRL were low.

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The seasonal profiles of reproductive hormones, closely related to the variations in breeding activity, have been extensively studied in the female (1); no conclusive data are currently available on the adult male goat, however. To our knowledge, the few reports (2, 3) published on seasonal secretory patterns and plasma levels of the sex hormones (gonadal steroids, LH, FSH and PRL) are mainly partial and limited to one or a few aspects only. The circannual variations of the reproductive hormones in males of other seasonal-breeding species have received closer attention. In the ram, for example, LH and testosterone (T) seasonal profiles are well documented (4, 5) and it is now clear that the increased T secretion observed in the breeding season results from high-frequency, low-amplitude LH pulses (6). Irvine et al.'s (7) study of the relationship between LH and sex steroids in the stallion indicates a seasonal variation in the feedback mechanism(s) by which gonadal hormones regulate LH serum levels. Conjugated estrogens such as estradiol 17 β sulfate (E2S) and estrone sulfate (E1S) have been found in the testicular tissue of the stallion (8). The biological significance of these hormones is still not clear, but it has been hypothesized (9) that E1S could be an index of the testicular function in this species. Moreover, many authors (9–11) have observed circannual variations of conjugated estrogens in both the blood and seminal plasma of this species.

PRL is related to reproductive function in both ewes and goats (12, 13) and reflects changes in photoperiod. In both species, PRL secretion is high during long-days and low during short-days (4, 14–17), and seems to be secreted in a pulsatile manner (18), which could be season-dependent (19); furthermore, a relationship has been found between plasma PRL levels and temperature (17).

This work was undertaken to study the plasma profiles of LH, T, E1S and PRL in the male goat and to characterize the possible seasonal changes in LH and PRL release patterns; the relationship between hormone blood levels and ambient temperature was also examined. Furthermore, we tried to assess whether handling connected with bleeding procedures is effective in influencing PRL concentrations, as already reported for other species (20–22).

Materials and methods

Animals, management and blood sampling

The experiment was carried out during the course of one year in Southern Italy at 40°N latitude. Bucks are scarcely photoperiodic at these latitudes. In fact, they usually initiate mating activity during early summer (even if goats are not in cycle) and continue until January–February. Six adult male goats (aged 2–5 years) of the Ionica (N = 3) and Alpine (N = 3) breed were
used. The animals were housed outdoors and subjected to the natural photoperiod; they were fed a standard ration of hay and concentrates and had free access to water.

For the whole experimental period, care was taken to avoid any kind of interference with management of the flock and the animals’ physiological reproductive activity. Throughout the year, neither hormonal substances nor drugs were administered to the animals. Once a month, frequent blood samples (every 15 min for 6 h) were collected in heparinized tubes through a cannula inserted into the jugular vein under local anesthetic. To subject the animals to an acute “stress” condition, they were confined to the bleeding pen on the same day of bleeding and the vascular cannulation was performed immediately before the beginning of the blood samplings. The blood was immediately centrifuged at 2300 × g for 10 min and the plasma thus obtained stored at −20°C until assayed. The ambient temperature was recorded on each day of sampling.

**Hormone assays**

Plasma LH, PRL, and EIS concentrations were determined by RIA, according to the methods described by Bono et al. (23), Tamanini et al. (24) and Tamanini et al. (25), respectively: oLH (LER-1374A) and oPRL (LER-860-2) were used as standards and for iodination. Plasma testosterone levels were determined using a commercial enzyme-immunoassay kit (Poli Industria Chimica S.p.A., Milano, Italy). The validation data for the assays are reported in Table 1. All the samples were assayed for LH and PRL, concentrations, while plasma T and EIS levels were determined only on the three samples collected at 1 h intervals during the last hours of each bleeding.

**Statistical analysis**

Hormonal variations throughout the year were tested by ANOVA. The correlation between the plasma profile of a single hormone and the profile of each other was evaluated by simple linear regression, as well as the correlation between hormonal variations and temperature. The data on LH concentrations in plasma were analyzed for episodic pattern of hormone release according to the criteria defined by Dodson et al. (26).

**Table 1.** Characteristics of the LH, PRL, EIS and T assays.

<table>
<thead>
<tr>
<th></th>
<th>LH</th>
<th>PRL</th>
<th>EIS</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (SI/l)</td>
<td>0.09 μg</td>
<td>0.33 μg</td>
<td>0.13 nmol</td>
<td>1.72 nmol</td>
</tr>
<tr>
<td>Intra-assays cv (%)</td>
<td>9.3</td>
<td>8.0</td>
<td>5.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Inter-assays cv (%)</td>
<td>10.9</td>
<td>12.2</td>
<td>6.5</td>
<td>9.0</td>
</tr>
<tr>
<td>Blank (nmol/l)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&lt;0.007</td>
</tr>
</tbody>
</table>

**Results**

**Circannual profiles**

Since no significant differences were observed between the two breeds of male goats, all the data were pooled and a single profile of each hormone plasma concentration was drawn.

In order to minimize the effects of the plasma LH and PRL fluctuations observed during the 6 h bleeding
periods, the circannual profiles of these hormones were drawn by pooling the values of the samples collected at 3, 4, 5 and 6 h of each bleeding period.

The seasonal variations in plasma EIS, T, LH and PRL levels and those of the environmental temperature and photoperiod are reported in Fig. 1.

During the winter, plasma EIS levels ranged between 0.13 and 0.29 nmol/l and remained below 0.19 nmol/l until the end of June. In July, we observed a sudden increase to 1.09 nmol/l: plasma EIS concentrations were high (>0.9 nmol/l) in August, gradually decreasing to lower values in November.

Throughout the whole experimental period, plasma LH concentrations ranged between the lowest values (0.99 μg/l) observed in March and the highest (1.41 μg/l) registered in July, without exhibiting any significant variation. The plasma testosterone profile was similar to that of LH, but with more marked variations: the lowest plasma concentrations were observed in March (1.9 nmol/l), the highest during the summer (4.5 and 10.9 nmol/l in July and August, respectively). Plasma T levels then gradually dropped to 5.5 nmol/l (November) and increased again to 6.2 nmol/l in December; this increase, however, is not significant.

Plasma PRL concentrations were about 1 μg/l in January, increasing and reaching the highest levels in July (76.6 μg/l), then dropping 0.5 μg/l by late autumn (November and December).

With the exception of LH, all the plasma hormone levels exhibited significant variations (p < 0.001) during the year. The ambient temperature averaged between the lowest values (about 11°C) registered during the winter months and the highest (37°C) observed in July.

The relationship among the plasma hormonal profiles and those between the hormones and the environmental temperature variations are reported in Table 2.

**Table 2.** Correlation coefficients (r) and significant differences between the different parameters (NS = not significant: * = p < 0.05; ** = p < 0.01; *** = p < 0.001).

<table>
<thead>
<tr>
<th></th>
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<th>LH</th>
<th>T</th>
<th>EIS</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRL</td>
<td>-</td>
<td>NS</td>
<td>NS</td>
<td>0.69*</td>
<td>0.90***</td>
</tr>
<tr>
<td>LH</td>
<td>NS</td>
<td>-</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>T</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>0.87***</td>
<td>0.65*</td>
</tr>
<tr>
<td>EIS</td>
<td>0.69*</td>
<td>NS</td>
<td>0.87***</td>
<td>—</td>
<td>0.74**</td>
</tr>
<tr>
<td>Temp.</td>
<td>0.90***</td>
<td>NS</td>
<td>0.65*</td>
<td>—</td>
<td>0.74**</td>
</tr>
</tbody>
</table>

**Fig. 2.** Patterns of LH release observed in samples collected at 15 min intervals for 6 h from two representative bucks in two different periods of the year (July, dash line: January, solid line).

As far as the plasma PRL profiles observed in 6 h frequent sampling periods are concerned, the hormone concentrations tended to be high at the beginning of bleeding gradually decreasing to lower values; however, these data varied depending on the month of bleeding.

**Fig. 3.** Plasma PRL variations observed in two representative bucks during two 6 h frequent sampling periods during which PRL levels were either high (August, top panel) or low (October, bottom panel).

**Plasma LH and PRL variations in 6 h frequent sampling periods**

The patterns of LH release seemed to vary depending on the month, but a statistical analysis of the data showed neither significant difference among months nor univocal characteristics among males. LH was not released in a pulsatile manner on any day of bleeding. Plasma LH fluctuations observed in frequent samples collected from two representative male goats in different periods of the year are depicted in Fig. 2.
and were not constant in all the animals. The variations in plasma PRL levels observed in frequent samples collected from two representative male goats in periods during which PRL is either low or high are reported in Fig. 3. In the first case, an at first marked and then gradual decrease in PRL blood levels was observed during the first 2 h of bleeding with low concentrations thereafter; in the second case the hormone profile shows a trend towards a decrease during the first hours, the differences between high and low levels being less pronounced.

Discussion

The activity of the hypothalamic-pituitary-testicular axis in the male goat is seasonal and closely related to variations in daylength. Corteel (2) documented the seasonal changes in sexual behaviour, testis size and sperm production in the Alpine male goat, as well as the circannual fluctuations in plasma T levels. All these parameters are higher during the breeding season than during the anestrous season; a similar situation as far as T is concerned has also been reported by Miyamoto et al. (3). Our data confirm the seasonal profile of T, even though plasma concentrations of this hormone are different from those reported by the above-mentioned authors, perhaps because of the different assay methods. Also plasma E1S concentrations show a circannual profile similar to that of T; this observation is quite original, at least for this species, because, to our knowledge, no data are available in the literature on this topic. Our results on the circannual variations of plasma E1S levels confirm the data obtained by Raeside (9) in the stallion, in which E1S concentrations are highest during the breeding season; similar data in the same species were also reported by Marusi et al. (11), who observed seasonal variations in E1S levels in seminal plasma too. Since the mating activity of bucks at the latitude at which our experiment was performed is coincident with the periods of high E1S (and T) levels, the concentrations of this steroid may be proposed as a suitable index of sexual activity.

It is surprising that the circannual profile of sex steroids such as T and E1S is superimposable on that of PRL; an inverse relationship between PRL and T was in fact observed by Sanford et al. (27) and D’Occhio et al. (4) in the ram.

Our data confirm our previous hypothesis (17), namely that PRL does not play a precise and direct role in gonadal activity, but is possibly a “sign” of reproductive seasonality, rather than a cause of it: alternatively, PRL levels may simply be related to the variations in photoperiod and involved in the mechanism(s) of transduction of the photic stimulus. A third possible explanation for the plasma PRL profile is that this hormone is strictly influenced by environmental factors such as temperature, as it can be assessed by the strong parallelism between these two parameters; a direct effect of temperature on PRL levels has been observed in both goats (17) and cattle (28). Furthermore, Mori et al. (12) observed that temperatures higher than 27°C markedly increase plasma PRL levels in goats, irrespective of photoperiodic variations. The findings about the patterns of PRL release observed in the frequent samplings are also quite interesting: in fact, the high levels at the beginning of the 6 h periods and the lower concentrations thereafter lead us to the assumption that the initially high levels are due to the stress associated with the restraint of the animals, cannula insertions and handling procedures. These results agree well with those reported by De Silva et al. (21) in sheep, Gaiani et al. (20) in cattle and Robert et al. (22) in sows, and suggest that care must be taken when monitoring this hormone to avoid misunderstandings in interpreting the data. The responses (in terms of PRL variations) observed in the bucks are not constant throughout the experimental period, however, in that they are less pronounced (on a percentage basis) when the hormone concentrations are highest (i.e. summer period); this may be due to the fact that the high PRL levels associated with the season can mask the variations induced by the handling procedures.

The protocol we adopted to define the mean plasma LH variations throughout the experimental period (i.e. pooling the data of four plasma samples collected from each buck) did not show any significant difference related to season, unlike Miyamoto et al. (3), who observed a peak in plasma LH levels at the end of July. In the ram, too, many authors (4, 5, 18) have observed different concentrations, albeit fairly insignificant in plasma LH levels, depending on the changing (natural or artificial) photoperiod. The sampling protocol we adopted (frequent samplings for 6 h once a month) was ineffective for characterizing the expected different patterns of LH release related to seasonality; a pulsatile LH release was observed only occasionally and not in all the bucks. Different data have been reported in rams (4, 6), in which both frequency and amplitude of LH pulses change depending on the period of the year. Examination of the data of the present study allow us to conclude that: plasma levels of T and E1S change during the year and are highest in summer periods; their profiles present a strong parallelism with that of PRL. This latter hormone is markedly influenced by environmental temperature and by the stressing conditions to which male goats may be exposed. Plasma LH variations throughout the year are slight and not significant; other studies should be conducted to establish the variations in pulsatility (if any) related to seasonality.

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