Effect of Nutrition on Plasma Progesterone Levels, Metabolic Parameters and Small Follicles Development in Unstimulated Goats Reared Under Constant Photoperiod Regimen

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Contents

Sixteen local adult goats were submitted for 9 weeks to 2.09 (high group) and 0.54 (low group) × dietary maintenance respectively. During the experimental period, goats were weighed, oestrus was detected and plasma insulin, urea, non-esterified fatty acids and progesterone concentrations were assessed. At the end of the experiment, ovarian small follicles population was studied by histological analysis. Final weight loss in low group was 18.37 ± 2.02%, whereas weight gain of high group was 13.84 ± 2.70%. Insulin and urea were lower in low group, while non-esterified fatty acids were significantly higher. A lower number of fasted goats was in oestrus or ovulated and an extended length of oestrus (p < 0.05) and a higher frequency of short or long cycles (p < 0.05) were also observed. Fed animals showed heavier ovaries (p < 0.01) and a lower number of primordial follicles (p < 0.05). In restricted goats a significant qualitative alteration of follicle classes involved in the initiation process of primordial pool was found. In this phase, granulosa thickness and oocyte size were the most affected (p < 0.01). However in small follicles beyond the primary stage no differences were found between the groups in either number or qualitative characteristics (p > 0.05). Collectively, these results indicate that opposite dietary intakes for a prolonged period of feeding. However the experiment received elephant grass plus concentrate to provide, respectively, 2.09 and 0.54 times of the energetic requirement for maintenance of live weight (Agricultural and Food Research Council 1998). All females were multiparous and not pregnant with a live weight of 31.66 ± 1.06 kg. (mean ± SEM) The goats were kept in individual shaded pens with free water and salt access and submitted to 20 days of housing adaptation. During this period, parasite treatment and control of ovary function by laparoscopy was performed. A buck was introduced three times daily in each pen to detect oestrus (09:00, 12:00, 16:00 hours), and the oestrous cycles were classified according to Chemineau et al. (1992).

Materials and Methods

Animals and experimental design

The experiment was carried out at the Ceará State University located at 3°43'S and 38°30'W. The mean ambient temperature recorded during the experimental period was 28.9 ± 0.08°C (mean ± SEM). For 9 weeks, two groups of eight local goats were submitted to high (high group) or low (low group) nutritional treatment and received elephant grass plus concentrate to provide, respectively, 2.09 and 0.54 times of the energetic requirement for maintenance of live weight (Agricultural and Food Research Council 1998). All females were multiparous and not pregnant with a live weight of 31.66 ± 1.06 kg. (mean ± SEM) The goats were kept in individual shaded pens with free water and salt access and submitted to 20 days of housing adaptation. During this period, parasite treatment and control of ovary function by laparoscopy was performed. A buck was introduced three times daily in each pen to detect oestrus (09:00, 12:00, 16:00 hours), and the oestrous cycles were classified according to Chemineau et al. (1992).

Progesterone, insulin and metabolite assays

Once a day (9:00 hours), before feeding, blood samples were collected in heparinized tubes by jugular venipuncture. For high group the sampling was performed from the second oestrus detected, whereas in the low group it was performed for 21 days starting from the 30th day of the experiment. Plasma progesterone was analysed by radioimmunoassay as described previously (Tamanini et al. 1985). The sensitivity of the assay was 3.7 pg/tube. The intra- and interassay coefficients of variation were 6.3% and 8.6% respectively.

Insulin was determined using a RIA kit (Insulin; Medical Systems, Genova, Italy). The sensitivity was 0.4 IU/ml, the intra- and interassay CVs were <3% and 10% respectively. Parallelism with standard curves and scalar dilution of goat plasma performed for all assays did not show any significant difference. Enzymatic-colorimetric methods were used to determine plasma concentrations of urea and NEFA (non-esterified fatty

acids, Boehringer Mannheim, Milan, Italy). The intra- and interassay CVs were 2.5% and 7.6% for urea and 4% and 6.2% for NEFA respectively.

**Histological techniques and follicle classification**

Ovaries were collected on the fourth day of cycle (oestrus = day 0) after the end of the nutritional treatment or in ninth week in the low animals that did not show oestrus. Immediately after recovery, ovaries, after corpora lutea removal, were weighed, fixed in Carnoy’s solution, dehydrated in ethanol and embedded in paraffin wax. Every 10th section sliced (8 µm thickness) was mounted and stained with haematoxylin–eosin. The preantral follicles were classified as described by Braw-Tal and Yossefi (1997) namely: primordial, transitory, primary, small preantral, large preantral and small antral follicles.

**Histological analysis**

Only non-atretic follicles (Silva et al. 2002) with visible oocyte nucleus were used. Estimates of the number of follicles counted in all sections were performed by the Fractionator method (Gundersen et al. 1988). Follicular images from classes and nutritional group were randomly captured at x60 magnification with a digital camera (TK-C1381: JVC American Corp., Wayne, NJ, USA) mounted on a microscope (Eclipse 400; Kurobane Nikon Co. Ltd, Tochigi, Japan). Follicle, oocyte and antrum diameters were determined by a computer program (Scion Image for Windows 2000; Scion Corporation, Frederick, MD, USA). The thickness of granulosa cell layer was determined by the difference between follicle and oocyte diameters. The number of granulosa cells was counted in all follicular images.

**Statistical analysis**

All data were analysed using SAS (SAS Inc., Cary, NC, USA). The effect of nutritional treatment (high and low groups) was analysed by GLM procedure. Comparison between means of nutritional treatments was performed by the Student’s t-test. Differences among proportions were performed by chi square analysis. Before statistical analysis, number of follicles was transformed to log. Values were represented as mean ± SEM.

**Results**

At the end of the experimental period the mean live weight recorded was significantly superior for high than for low group (36.50 ± 1.59 kg vs 25.94 ± 1.10 kg; p < 0.01). The weight loss achieved in restricted animals was 18.37 ± 2.02%, whereas supplied goats increased the initial weight (13.84 ± 2.70%).

Figure 1 shows the changes in plasma progesterone, insulin, NEFA and urea in both groups. Insulin and urea were significantly higher in high than in the low group (p < 0.01), while NEFA were significantly (p < 0.01) lower in high animals.

During the sampling period, progesterone levels of high group rose gradually to a maximum on day 10 and remained relatively constant thereafter defining the luteal phase (Fig. 1). All goats in high group showed oestrus during the experiment (Table 1).

In undernourished group, only four of eight goats exhibited oestrus by the seventh week of the experiment. The number of cycles was different between high and low groups (18 vs. 4; p < 0.05). In low group animals the oestrus length was longer than high group (p < 0.05) and a high frequency of abnormal (short and long) cycles (Table 1) was observed.

Two of these goats showed an extended rise in progesterone after oestrus, indicating the occurrence of ovulation and corpus luteum formation. The remaining two animals that exhibited an oestrus showed a fall of progesterone to levels <1 ng/ml shortly after the initial rise, probably because of premature regression of the corpus luteum.

In the high group, the mean number of corpora lutea counted at ovary recovery was 1.13 ± 0.44 (Table 1).
Moreover these animals showed heavier ovaries than low group (p < 0.01) (Table 1). In underfeeding group the inspection of ovarian surface performed at recovery did not identify the presence of corpora lutea (Table 1) and the number of primordial follicles was significantly higher (p < 0.05) than supplied goats. No significant differences between high and low group in the number of follicles belonging to the categories from primary to small antral were found.

Table 2 shows the morphological characteristics of follicle populations from high group and from goats of low group with verified ovary failure (absence of oestrus and ovulation). The number of granulosa cells of both groups was similar in all classes of follicles considered. Likewise follicular and oocyte size from the small preantral to small antral follicles was not influenced by nutritional treatments.

Fasted goats exhibited a significant reduction (p < 0.01) in oocyte diameter in primary follicles (Table 2) and a smaller follicle size in both primordial and primary classes (p < 0.01). During the transition phase between primordial and primary follicles, a slower increase of both granulosa thickness and oocyte size was observed (Fig. 2).

By the end of experiment, however, dietary restriction provoked the arrest of oestrus activity in the females of low group. Undernutrition for short (Mani et al. 1992, 1996), medium (Tanaka et al. 2004) or long period (Paula et al. 2005) has been extensively reported to exert a negative influence on ovarian activity in goats. Usually the timing of the onset of nutritional anoestrous is subordinate to the level of body reserves before undernutrition (Diskin et al. 2003) and thus may generate considerable individual variation (Tanaka et al. 2003). In low group, no relationship with body mass or weight loss was established and the ample susceptibility to fasting verified during the experiment is probably because of the ability of this type of goat to survive in a harsh environment.

**Table 1. Oestrus response, ovary weight and ovarian response in goats with different feeding level**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>High</th>
<th>Low</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrus response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of animals exhibiting oestrus (n)</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Length of oestrus (h)</td>
<td>32.08 ± 3.87</td>
<td>58.30 ± 18.08</td>
<td>*</td>
</tr>
<tr>
<td>Number of short + long cycles (n)</td>
<td>50% (10/18)</td>
<td>75% (3/4)</td>
<td>NS</td>
</tr>
<tr>
<td>Ovary weight*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight of ovaries (g)</td>
<td>2.94 ± 0.23</td>
<td>1.97 ± 0.21</td>
<td>**</td>
</tr>
<tr>
<td>Weight of left ovary (g)</td>
<td>1.48 ± 0.14</td>
<td>1.03 ± 0.10</td>
<td>*</td>
</tr>
<tr>
<td>Weight of right ovary (g)</td>
<td>1.45 ± 0.10</td>
<td>0.94 ± 0.12</td>
<td>**</td>
</tr>
<tr>
<td>Ovarian response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of corpora lutea counted at ovary recovery* (n)</td>
<td>1.13 ± 0.44</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Primordial follicles* (n)</td>
<td>9.3 ± 2.51</td>
<td>27.1 ± 8.30</td>
<td>*</td>
</tr>
<tr>
<td>Primary follicles* (n)</td>
<td>0.6 ± 0.18</td>
<td>0.5 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Growing follicles* (n)</td>
<td>0.4 ± 0.06</td>
<td>0.5 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Follicular concentration in the ovary (n.mg -1 )</td>
<td>8.06 ± 2.30</td>
<td>32.07 ± 9.15</td>
<td>*</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM. NS, not significant; *p < 0.05, **p < 0.01.

**Table 2. Morphological characteristics of small follicles in goats with different feeding level**

<table>
<thead>
<tr>
<th>Follicle</th>
<th>Granulosa cells (n)</th>
<th>Follicle diameter (μm)</th>
<th>Oocyte diameter (μm)</th>
<th>Antrum diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(range)</td>
<td>(range)</td>
<td>(mean ± SEM)</td>
<td>(range)</td>
</tr>
<tr>
<td>Primordial</td>
<td>208</td>
<td>3–8</td>
<td>3–7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>8–30</td>
<td>9–27</td>
<td>NS</td>
</tr>
<tr>
<td>Small preantral</td>
<td>93</td>
<td>16–98</td>
<td>16–67</td>
<td>NS</td>
</tr>
<tr>
<td>Large preantral</td>
<td>29</td>
<td>90–149</td>
<td>98–145</td>
<td>NS</td>
</tr>
<tr>
<td>Small antral</td>
<td>53</td>
<td>155–273</td>
<td>117–278</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM. NS, not significant; *p < 0.05, **p < 0.01.
Our data showed that feeding restriction induced a considerable mobilization from lipasic and proteic reserves. In our experiments, undernourishment not only affected body weight but also the ovarian tissue, as its mass was mainly characterized with high group ovaries. A less evident decrease of body weight (12% of initial weight) was reported by Kusina et al. (2001) in Mashona goats submitted to 50% of energetic requirement for 60 days, while greater mass loss (25%) was achieved by Tanaka et al. (2004) in Shiba goats fasted 7 weeks at 30% of energetic requirement. Chillard et al. (1998) reported that during mid-term restriction the decrease of live weight involves tissue and organ mass in contrast to short-term adaptation when it occurs mainly in the gut. A mid-term decrease in intake from maintenance to 0.5x maintenance leads to a reduction of detrimental effects on portal, hepatic blood flows and oxygen consumption (Chillard et al. 1998).

Feeding planes for some weeks alter the circulating metabolites and insulin. It is well known that these changes may serve as signs of the animal’s nutritional status. Moreover, glycaemia and insulinaemia play an important role in regulating ovarian follicle responsiveness to gonadotrophins (Selvaraju et al. 2003).

Our results demonstrated that the small follicle population during initial growth was sensitive to the energy balance. In undernourished goats, the greater number of primordial follicles compared with high group suggest a lower depletion process.

Borwick et al. (1997) demonstrated that foetal oogonial degradation can be delayed, when pregnant ewes were fasted at 50% of energetic requirement for 9 weeks. Several authors, analysing the effect of negative energy status on ovulation rate in ewes (Nottle et al. 1999) or during cattle postpartum (Britt 1991), strongly suggest that a low nutrient flow to ovary for prolonged period impairs the earliest stages of folliculogenesis.

It is recognized that the activation mechanism in primordial follicles is a crucial point in folliculogenesis and occurs through the transition of the follicle to a further development stage (Braw-Tal 2002). Our data demonstrate that in fasted goats the development from inactive-to active follicular stage is significantly distorted. Oocyte size of primordial follicle increases less in underfed group, while the transformation of flattened granulosa cells to cuboidal shape was not accompanied by an equal follicle enlargement.

Apart from the initial follicular classes our results show that nutrient deficiency did not interfere in the number of growing follicles and did not prevent growth beyond the primary stage, in agreement with data by Lintern-Moore and Everitt (1978) in fasted prepuberal rats. The lack of influence of energy balance in ultimate development of primary follicles suggests that growing follicles are controlled by additional factors or others ovarian/extraovarian stimuli.

Follicle growth from activated to mid–late–preantral stage is mainly characterized by increases of oocyte size, proliferation of granulosa cells arranged in additional layers and formation of antral cavity (Webb et al. 1999). Some authors (Braw-Tal 2002; Fortune 2003), who reviewed the complexity of mechanisms regulating preantral follicles development, suggested separating follicle differentiation in consecutive stages, each characterized by a distinct growth environment.

In conclusion, our findings support the hypothesis that nutrition during medium period can exert a qualitative and quantitative influence on small follicle population in goats. The study provides useful information about nutritional influence on ovary function in goats and reveals aspects to be considered in the reproductive management of tropical semiarid areas where food availability is a fundamental prerequisite for reproduction control in goat herds.

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